ISSN 2096-7071
CN 10-1629/R1

CHINA CDC WEEKLY

Vol. 3 No. 44 Oct. 29, 2021

中国疾病预防控制中心周报

WORLD FLU DAY ISSUE

ISSN 2096-7071
9 772096 707217 44>

China CDC Weekly

This week's issue was organized by Guest Editor Yuelong Shu.

Editorial

Let's Get Vaccinated for Both Flu and COVID-19: On the World Flu Day 2021

George F. Gao[1,#]; William J. Liu[1]

This year marks the fourth World Flu Day on November 1. In 2018, on the occasion of centenary of the 1918 flu pandemic, the first World Flu Day was formally initiated and developed by leading influenza specialists in China and all over the world at a symposium in Shenzhen, China, with the theme to "Commemorate the Centenary of the 1918–19 Influenza Pandemic" (*1*). Scientists and representatives from different international associations and organizations, including the five World Health Organization (WHO) Collaborating Centers for Reference and Research on Influenza came together and advocated for raising public awareness of influenza, accelerating scientific innovation and international cooperation on influenza surveillance and basic research, and pushing for stronger global support on influenza prevention and control (*2*). The second and the third World Flu Day Symposia were held in Beijing with the themes as "Know Flu/Prevent Flu/Beat Flu" in 2019 and "Influenza Control and COVID-19 Pandemic Response" in 2020.

In the past century, humans had endured 5 influenza pandemics: "Spanish flu" (H1N1) in 1918, "Asian flu" (H2N2) in 1957, "Hong Kong flu" (H3N2) in 1968, and "Russian flu" (H1N1, "pseudo-pandemic") in 1977, and "Mexico Swine flu" (H1N1) in 2009 (*3*). With the emerging events of diverse human-infecting avian influenza viruses, i.e., H5N1, H5N6, H6N1, H7N9, H9N2, H10N8, H7N4, etc. in the recent decades, scientists estimated that the next pandemic may be caused by the influenza virus and the world seems to not be prepared for it. Meanwhile, a new respiratory tract infectious disease coronavirus disease 2019 (COVID-19) emerged in the end of 2019 and developed rapidly in the early months of 2020 as the first recognized pandemic caused by the coronavirus (*4*). Beyond all doubt, humans were not prepared for the pandemic and COVID-19 affected and even changed virtually everyone's lives (*5*). The relevant scientists and health workers all over the world have tried their best to understand and control the new disease (*6*). However, the etiology of COVID-19, severe acute respiratory syndrome (SARS-CoV-2; initially named 2019-NCIP and hCoV-19) is still wreaking havoc with the continuously emerging variants (*7*). Thus, in the coming seasons of influenza, we are facing the complexity and challenge of influenza and COVID-19 cocirculation. Currently, promoting vaccination coverage is still the prioritized strategy to efficiently control the current COVID-19 pandemic and to prevent seasonal influenza. Thus, for the World Flu Day 2021, we advocate the theme as "Flu and COVID-19, Let's Get Vaccinated" this year.

From early 2020, the seasonal influenza activity has had a dramatic drop with the implementation of non-pharmaceutical interventions (NPIs) and behavioral changes to mitigate COVID-19 (*8–10*). The 2019–2020 seasonal influenza activity was significantly reduced compared with the previous seasons in China, the United States, and other countries, and the decreases in influenza virus infection were also associated with the timing of NPIs (*9*). In actuality, this relief situation of influenza was sustained in the following seasons and one influenza lineage, i.e. B/Yamagata, has not been isolated from April 2020 to August 2021 and seems to have possibly become extinct (*11*). NPIs implemented against COVID-19 played an unexpected extra role in this process, including use of individual protection, e.g., wearing masks, watching distance, washing hands (3-W), and restriction of personal movement, e.g., canceling mass gatherings, closing public entertainment venues, closing schools, restricting domestic and international travel, and issuing stay-at-home orders (*12*).

In spite of the low prevalence of seasonal influenza viruses among humans, an undercurrent of avian influenza viruses still whirls among poultry populations. Since the end of 2019, highly pathogenic H5Ny avian influenza has frequently occurred in birds in Eurasia and Africa (*13*). Analyses of highly pathogenic avian influenza H5 viruses from poultry outbreaks and recent detections in migratory waterfowl across a wide Eurasian region revealed maintenance of A/H5 clade 2.3.4.4b, i.e., H5N8, H5N5, and H5N1,

etc. (*14*), which raised a highly alarming threat to poultry production, veterinary disease control, and human public health. Especially in the spring of 2020, the 2.3.4.4b branch H5N8 avian influenza virus caused multiple outbreaks in Central and Eastern Europe, implying a new wave of highly pathogenic H5Ny avian influenza in the broad area (*15*). A detailed genetic and biological analysis of avian influenza outbreaks in wild birds in China over the decade before 2019 found that 5 out of 10 highly pathogenic H5Ny avian influenza could bind human-type receptors, all of which were from clade 2.3.4.4 (*16*). Human infection of clade 2.3.4.4b influenza A(H5N8) virus with 7 cases was reported at a poultry farm in Astrakhan, Russia in December 2020 (*17*). Before the end of 2020, a total of 26 laboratory-confirmed cases of human infection with influenza A(H5N6) virus have been reported (*18*). However, in 2021 thus far, more than 20 sporadic human infections have been recorded in different provincial-level administrative divisions (*19*). All this evidence of human H5Ny infections indicate a sustained spillover of avian influenza virus and deserve close attention.

The pandemic of COVID-19 brings unprecedented pressure and challenges to global public health and changes the landscape of influenza prevalence. In front of the complexity and unpredictability of the influenza epidemic and its co-circulation with COVID-19, the concerns for seasonal influenza among humans and the avian influenza among birds should not be reduced. Herein, on the occasion of the fourth World Flu Day, we would like to lift up our voice to bring the attention of the public to influenza. Public understanding of science plays a pivotal role in the implementation of disease control and prevention strategies (*20–21*). Historical lessons learnt from influenza and current experience obtained from COVID-19 should remain at the core of global efforts for pandemic preparedness. Commemorating World Flu Day together is not just a timely call for raising global awareness about this common and easily ignored disease, especially with the COVID-19 pandemic under the global spotlight, but it is also an important opportunity to advocate for the vaccination for the two pandemic-igniting viruses.

In this issue of the *China CDC Weekly*, we have reported the surveillance of influenza activity in the mainland of China in 2020–2021, with an intensity of activity lower than before COVID-19, but gradually increasing compared to 2020 (*22*); this issue also reported two human cases of H5N6 infection in 2021 in Guangxi Zhuang Autonomous Region, China, which provides evidence of a continuous threat to humans from H5Ny avian influenza viruses (*23*). We also included a review article on continuous circulation, frequent gene reassortment, and mammalian adaptation of H3 avian influenza viruses in the past two decades in the mainland of China (*24*). A review on the Global Influenza Surveillance and Response System (GISRS) was also included (*25*), which summarized the existing WHO influenza surveillance systems that have been leveraged for global responses to COVID-19 since the beginning of the pandemic and addressed future challenges and plans.

doi: 10.46234/ccdcw2021.227

[#] Corresponding author: George F. Gao, gaofu@chinacdc.cn.

[1] Chinese Center for Disease Control and Prevention, Beijing, China.

Submitted: October 28, 2021; Accepted: October 29, 2021

REFERENCES

1. The Lancet. World Flu Day: momentum from China for influenza control. Lancet 2018;392(10158):1600. http://dx.doi.org/10.1016/S0140-6736(18)32770-3.
2. Liu WJ, Bi YH, Wang DY, Gao GF. On the centenary of the spanish flu: being prepared for the next pandemic. Virol Sin 2018;33(6):463 – 6. http://dx.doi.org/10.1007/s12250-018-0079-1.
3. Liu WJ, Wu Y, Bi YH, Shi WF, Wang DY, Shi Y, et al. Emerging HxNy influenza A viruses. Cold Spring Harb Perspect Med 2020a038406. http://dx.doi.org/10.1101/cshperspect.a038406.
4. Tan WJ, Zhao X, Ma XJ, Wang WL, Niu PH, Xu WB, et al. A novel coronavirus genome identified in a cluster of pneumonia cases - Wuhan, China 2019-2020. China CDC Wkly 2020;2(4):61 – 2. http://dx.doi.org/10.1016/j.bsheal.2020.01.003.
5. Daszak P, Olival KJ, Li HY. A strategy to prevent future epidemics similar to the 2019-nCoV outbreak. Biosaf Health 2020;2(1):6 – 8. http://dx.doi.org/10.1016/j.bsheal.2020.01.003.
6. Liu WJ, Wu GZ. Convincing the confidence to conquer COVID-19: from epidemiological intervention to laboratory investigation. Biosaf Health 2020;2(4):185 – 6. http://dx.doi.org/10.1016/j.bsheal.2020.11.005.
7. Huang BY, Tan WJ. SARS-CoV-2 variants: surveillance, evaluation, and challenges. Infect Dis Immun 2021;1(3):121 – 4. http://dx.doi.org/10.1097/ID9.0000000000000019.
8. Sakamoto H, Ishikane M, Ueda P. Seasonal influenza activity during the SARS-CoV-2 outbreak in Japan. JAMA 2020;323(19):1969 – 71. http://dx.doi.org/10.1001/jama.2020.6173.
9. Feng LZ, Zhang T, Wang Q, Xie YR, Peng ZB, Zheng JD, et al. Impact of COVID-19 outbreaks and interventions on influenza in China and the United States. Nat Commun 2021;12(1):3249. http://dx.doi.org/10.1038/s41467-021-23440-1.
10. Soo RJJ, Chiew CJ, Ma S, Pung R, Lee V. Decreased influenza incidence under COVID-19 control measures, singapore. Emerg Infect Dis 2020;26(8):1933 – 5. http://dx.doi.org/10.3201/eid2608.201229.
11. Koutsakos M, Wheatley AK, Laurie K, Kent SJ, Rockman S. Influenza lineage extinction during the COVID-19 pandemic? Nat Rev Microbiol 2021. http://dx.doi.org/10.1038/s41579-021-00642-4.
12. Lai SJ, Ruktanonchai NW, Zhou LC, Prosper O, Luo W, Floyd JR, et al. Effect of non-pharmaceutical interventions for containing the COVID-19 outbreak in China. medRxiv 2020. http://dx.doi.org/10.1101/2020.03.03.20029843.

13. World Organization for Animal Health (OIE). Update on avian influenza in animals (types H5 and H7). http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/. [2021-10-29].
14. Lewis NS, Banyard AC, Whittard E, Karibayev T, Al Kafagi T, Chvala I, et al. Emergence and spread of novel H5N8, H5N5 and H5N1 clade 2.3.4.4 highly pathogenic avian influenza in 2020. Emerg Microbes Infect 2021;10(1):148-51. http://dx.doi.org/10.1080/22221751.2021.1872355.
15. Shi WF, Gao GF. Emerging H5N8 avian influenza viruses. Science 2021;372(6544):784 – 6. http://dx.doi.org/10.1126/science.abg6302.
16. Cui Y, Li Y, Li M, Zhao L, Wang D, Tian J, et al. Evolution and extensive reassortment of H5 influenza viruses isolated from wild birds in China over the past decade. Emerg Microbes Infect. 2020;9(1):1793-803. http://dx.doi.org/10.1080/22221751.2020.1797542.
17. Pyankova OG, Susloparov IM, Moiseeva AA, Kolosova NP, Onkhonova GS, Danilenko AV, et al. Isolation of clade 2.3.4.4b A(H5N8), a highly pathogenic avian influenza virus, from a worker during an outbreak on a poultry farm, Russia, December 2020. Euro Surveill 2021;26(24):2100439. http://dx.doi.org/10.2807/1560-7917.ES.2021.26.24.2100439.
18. World Health Organization. Avian influenza weekly update number 815. 2021. https://www.who.int/docs/default-source/wpro---documents/emergency/surveillance/avian-influenza/ai-20211022.pdf?sfvrsn=30d65594_176. [2021-10-29].
19. Xiao CK, Xu JN, Lan Y, Huang ZP, Zhou LJ, Guo YX, et al. Five independent cases of human infection with avian influenza H5N6 — Sichuan province, China, 2021. China CDC Wkly 2021;3(36):751 – 6. http://dx.doi.org/10.46234/ccdcw2021.187.
20. Wang QH, Chen H, Shi Y, Hughes AC, Liu WJ, Jiang JK, et al. Tracing the origins of SARS-CoV-2: lessons learned from the past. Cell Res 2021. http://dx.doi.org/10.1038/s41422-021-00575-w.
21. Tong YG, Liu WL, Liu PP, Liu WJ, Wang QH, Gao GF. The origins of viruses: discovery takes time, international resources, and cooperation. Lancet 2021;398(10309):1401 – 2. http://dx.doi.org/10.1016/S0140-6736(21)02180-2.
22. Huang WJ, Li XY, Tan MJ, Cheng YH, Chen T, Wei HJ, et al. Epidemiological and virological surveillance of seasonal influenza viruses — China, 2020–2021. China CDC Wkly 2021;3(44):918 – 22. http://dx.doi.org/10.46234/ccdcw2021.224.
23. Bi FY, Jiang LL, Huang LH, Wei JG, Pan XW, Ju Y, et al. Genetic characterization of two human cases infected with the avian influenza A (H5N6) viruses — Guangxi Zhuang Autonomous Region, China, 2021. China CDC Wkly 2021;3(44):923 – 8. http://dx.doi.org/10.46234/ccdcw2021.199.
24. Yang JY, Yang L, Zhu WF, Wang DY, Shu YL. Epidemiological and genetic characteristics of the H3 subtype avian influenza viruses in China. China CDC Wkly 2021;3(44):929 – 36. http://dx.doi.org/10.46234/ccdcw2021.225.
25. Hammond A, Cozza V, Hirve S, Medina MJ, Pereyaslov D, Zhang WQ. Leveraging global influenza surveillance and response system for the COVID-19 pandemic response and beyond. China CDC Wkly 2021;3(44):937 – 40. http://dx.doi.org/10.46234/ccdcw2021.226.

Vital Surveillances

Epidemiological and Virological Surveillance of Seasonal Influenza Viruses — China, 2020–2021

Weijuan Huang[1]; Xiyan Li[1]; Minju Tan[1]; Yanhui Cheng[1]; Tao Chen[1]; Hejiang Wei[1]; Xiaoxu Zeng[1]; Yiran Xie[1]; Jia Liu[1]; Ning Xiao[1]; Lei Yang[1]; Dayan Wang[1,#]

ABSTRACT

Introduction: During the coronavirus disease 2019 (COVID-19) pandemic, the circulation of seasonal influenza virus declined globally and remained below previous seasonal levels. We analyzed the results of the epidemiology, antigenic, and genetic characteristics, and antiviral susceptibilities of seasonal influenza viruses isolated from the mainland of China during October 5, 2020 through September 5, 2021, to better assess the risk of influenza during subsequent influenza season in 2021–2022.

Methods: Positive rates of influenza virus detection during this period were based on real-time polymerase chain reaction (PCR) detection by the Chinese National Influenza Surveillance Network laboratories, and isolated viruses from influenza positive samples were submitted to the Chinese National Influenza Center. Antigenic analyses for influenza viruses were conducted using the hemagglutination inhibition assay. Next-generation sequencing was used for genetic analyses. Viruses were tested for resistance to antiviral medications using a phenotypic assay and next-generation sequencing.

Results: In southern China, the influenza positivity rate was elevated especially after March 2021 and was higher than the same period the previous year with the COVID-19 pandemic. In northern China, influenza positive rate peaked at Week 18 in 2021 and has declined since then. Nearly all isolated viruses were B/Victoria lineage viruses during the study period, and 37.3% of these viruses are antigenically similar to the reference viruses representing the vaccine components for the 2020–2021 and 2021–2022 Northern Hemisphere influenza season. All seasonal influenza viruses were susceptible to neuraminidase inhibitors and endonuclease inhibitors.

Conclusions: Influenza activity has gradually increased in the mainland of China in 2021, although the intensity of activity is still lower than before the COVID-19 pandemic. The diversity of circulating influenza types/subtypes decreased, with the vast majority being B/Victoria lineage viruses. The surveillance data from this study suggest that we should strengthen influenza surveillance during the upcoming traditional influenza season. It also provided evidence for vaccine recommendations and prevention and control of influenza and clinical use of antiviral drugs.

INTRODUCTION

Influenza is a respiratory illness that infects between 5%–15% of the global population annually during normal seasonal epidemics, and the World Health Organization (WHO) estimates that these infections result in 3–5 million cases of severe illness and about 290,000 to 650,000 respiratory deaths every year (*1*). Rapid evolution of influenza viruses creates difficulties in recognizing and predicting current and future epidemic threats (*2*).

Since the March 2020 co-incident with the exponential global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a dramatic decrease in influenza detection has been observed, despite the testing for influenza continuing at similar levels in many countries (*3*–*4*). Influenza is traditionally prevalent in the Southern Hemisphere during April–July 2020, but the positive rate for influenza decreased dramatically from 13.7% in 2017–2019 to 0.06% in 2020 (*5*). The Northern Hemisphere has also experienced almost no influenza during the typical timing of the 2020–2021 season, compared to around 18%–23% in 2019–2020, the US and EU reporting only 0.15%–0.20% of samples as positive for influenza (*6*).

Influenza A(H1N1)pdm09, A(H3N2), and influenza B viruses circulated in low numbers and the predominant circulating viruses varied among reporting countries. Since September 2020 influenza activity was mostly reported from countries in the tropics and subtropics and some countries in the

temperate zone of the Northern Hemisphere and Southern Hemisphere (*7*). It is possible that this continuous low-level virus circulation may allow influenza transmission following significant restarting of international travel. Now that we are approaching the influenza season in the mainland of China, it is important that we understand last year's influenza activity and surveillance results so that we can be better prepared for future influenza outbreaks. Therefore, we analyzed the antigenic and genetic characteristics and antiviral susceptibilities of influenza viruses isolated from the mainland of China in 2020–2021.

METHODS

The Chinese National Influenza Surveillance Network currently includes 410 laboratories and 554 sentinel hospitals. Influenza-like illness (ILI) cases were reported by sentinel hospitals to the Chinese National Influenza Surveillance Information System (CNISIS), and respiratory specimens were collected. Network laboratories determined whether the sample was influenza virus positive using real-time reverse transcription polymerase chain reaction (RT-PCR). Influenza positive specimens were propagated in Madin-Darby canine kidney (MDCK) cells and/or embryonated chicken eggs. The Chinese National Influenza Center (CNIC) received the viruses for further analysis.

Antigenic characterization was based primarily from results of hemagglutination inhibition (HI) assays, and genetic characterization was carried out based on next-generation sequencing. An HI titer less than or equal to 4 times that for the homologous virus was considered to be antigenically similar. Testing of seasonal influenza viruses for resistance to the neuraminidase and endonuclease inhibitors was performed at CNIC using next-generation sequencing analysis, a functional assay, or both. The viruses evaluated were isolated from specimens collected between Week 41 in 2020 (October 5, 2020) and Week 35 in 2021 (September 5, 2021).

RESULTS

Influenza activity in the mainland of China was below pre-pandemic COVID-19 levels during the period October 5, 2020 to September 5, 2021 (surveillance Week 41 in 2020 to Week 35 in 2021). The percentage of specimens testing positive for influenza each week ranged from 0.1% to 6.2% in southern China and ranged from 0% to 10.6% in northern China. The positivity rate of specimens collected from ILI cases slightly increased starting at Week 49 in 2020 and had been increasing in 2021 in southern China. The positivity rate in northern China began to increase in Week 10 of 2021, peaking at Week 18 of 2021, and declined since then (Figures 1 and 2).

In southern China, the network laboratories tested 308,698 specimens between October 5, 2020 and September 5, 2021; among these specimens, 7,593 (2.5%) tested positive including 79 (1.0%) for influenza A and 7,514 (99.0%) for influenza B. Most of the positive samples (7,299, 96.1%) were collected since 2021. Among the 58 seasonal influenza A positive specimens that were subtyped, 39 (67.2%) were influenza A(H1N1)pdm09 and 19 (32.8%) were

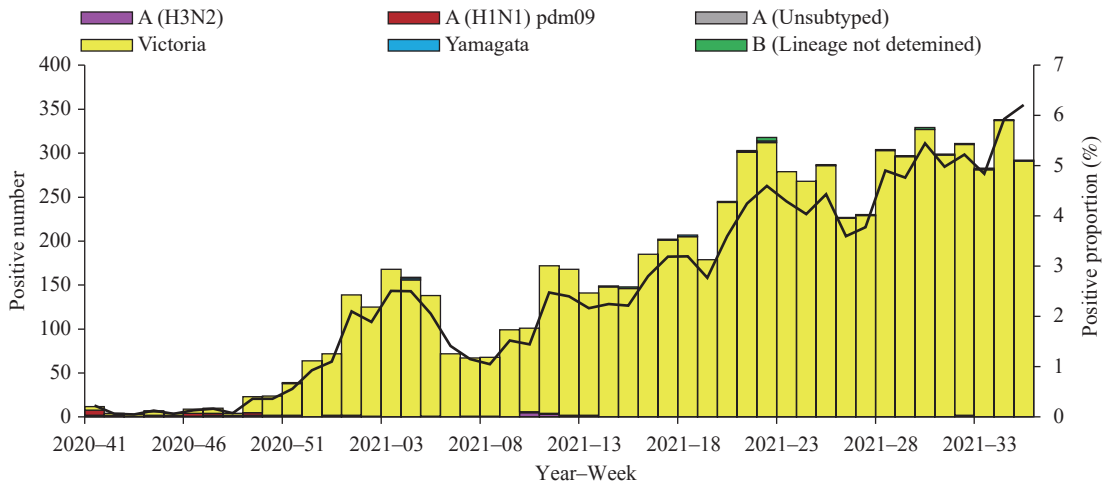

FIGURE 1. Influenza positive tests reported by network laboratories in southern China.

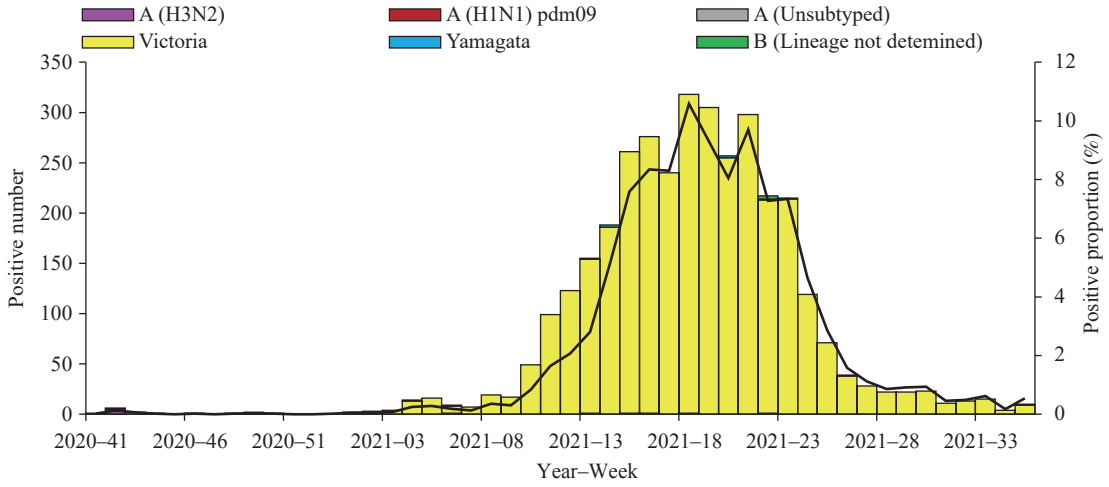

FIGURE 2. Influenza positive tests reported by network laboratories in northern China.

influenza A(H3N2). Among the 7,497 influenza B viruses for which lineage was determined, 7,481 (99.8%) belonged to the B/Victoria lineage and 16 (0.2%) belonged to the B/Yamagata lineage (Figure 1).

During the study period, network laboratories in northern China tested 209,219 specimens for influenza; among these, 3,489 (1.7%) were positive for an influenza virus: 19 (0.5%) for influenza A and 3,470 (99.5%) for influenza B. Among the 14 seasonal influenza A viruses subtyped, 7 (50.0%) were influenza A(H1N1)pdm09 and 7 (50.0%) were influenza A(H3N2). Of the 3,465 Influenza B viruses with B lineage results, 3,456 (99.7%) were B/Victoria and 9 (0.3%) were B/Yamagata (Figure 2).

Two A(H1N1)pdm09 viruses were antigenically characterized by HI tests, and one virus was well inhibited by ferret antisera raised against A/Guangdong-Maonan/SWL1536/2019, the egg-propagated reference virus representing the A(H1N1)pdm09 component of the 2020–2021 Northern Hemisphere influenza vaccines (*7*). One of the viruses was well inhibited by ferret antisera raised against A/Victoria/2570/2019, the egg-propagated reference virus representing the A(H1N1)pdm09 component for the upcoming 2021–2022 Northern Hemisphere influenza vaccines (*8*). Phylogenetic analysis of hemagglutinin (HA) gene segments determined that 1 belonged to genetic subclade 6B.1A5A with HA amino acid substitutions N129D, T185I, and N260D; the other one belonged to group 6B.1A5A2 with HA amino acid substitutions K130N, N156K, L161I, V250A, and E506D, which evolved from subclade 6B.1A5A.

Antigenic characterization of the 1,819 B/Victoria lineage viruses were conducted using HI tests. A total of 1,141 (62.7%) B/Victoria lineage viruses were not recognized well by the ferret antisera raised against B/Washington/02/2019, the egg-propagated reference virus representing the B/Victoria lineage component of the 2020–2021 and 2021–2022 Northern Hemisphere influenza vaccines (*7*–*8*). Among the 797 viruses HA gene segments sequenced and phylogenetically analyzed, all belonged to genetic clade 1A, nearly all belonged to subclade 1A.3, which shared a three amino acid deletion in HA (positions 162–164) and the substitution K136E, and in which, 96.7% belong to group 1A.3a characterized by HA amino acid substitutions N150K, G184E, N197D, and R279K, and was further divided into subgroup 1A.3a1 (520, 65.2%), which had additional HA amino acid substitutions V220M and P241Q, and 1A.3a2 (251, 31.5%), which had HA amino acid substitutions A127T, P144L, and K203R.

No A(H3N2) and B/Yamagata lineage viruses were isolated and available for characterization during the study period.

Of the 2 A(H1N1)pdm09 viruses collected in the mainland of China, neither showed evidence of reduced inhibition by oseltamivir and zanamivir, nor to baloxavir. Of the 1,258 influenza B/Victoria lineage viruses screened for neuraminidase inhibitors susceptibility, all showed normal inhibition by oseltamivir and zanamivir. A total of 797 B/Victoria lineage viruses were screened for susceptibility to the endonuclease inhibitor, baloxavir, by genetic analysis, and none showed evidence of reduced susceptibility.

DISCUSSION

In the mainland of China, circulation of influenza

virus was disrupted during the COVID-19 pandemic. During 2020, influenza virus circulation showed historically low levels (*9*). In 2021, influenza continues to circulate at low levels but higher than the equivalent period since March 2020 (*9*).

The WHO meets twice a year to recommend influenza vaccines. In late February 2021, the WHO issued its recommendations for the 2021–2022 Northern Hemisphere influenza vaccines. Compared to the 2020–2021 Northern Hemisphere influenza vaccine components, the A(H1N1)pdm09 and A(H3N2) vaccine components were updated, except for the B/Yamagata and B/Victoria lineage (*7*–*8*).

Influenza B/Victoria lineage viruses were predominant nationwide during the study period. Significant genetic diversity was seen in circulating viruses of the B/Victoria lineage. The viruses in the 1A.3a group were the most prevalent in the mainland of China. This 1A.3a HA group further diversified into 2 subgroups that 1A.3a1 had substitutions of either V220M and P241Q, which were predominant and seen almost exclusively in China, or 1A.3a2 with additional amino acid substitutions A127T, P144L, and K203R, for which the proportion has been increasing in recent months. Subgroup 1A.3a2 shows further genetic differentiation, with additional HA amino acid substitutions found in viruses from other global regions (*10*). The majority of group 1A.3a viruses showed reductions in inhibition by post-infection ferret antisera raised against egg-propagated 1A.3 viruses, such as B/Washington/02/2019 (2020–2021 and 2021–2022 Northern Hemisphere influenza vaccine components).

Only 2 A(H1N1)pdm09 viruses were available for antigenic analysis during this period. Ferret antisera raised against egg-propagated A/Victoria/2570/2019 (2021–2022 Northern Hemisphere influenza vaccine components), which belonged to phylogenetic group 6B.1A5A2, recognized the virus within 6B.1A5A2 virus well but showed poor recognition of 6B.1A5A virus. The global 6B.1A5A2 viruses increased rapidly in the early 2020s until it reached similar proportions to 6B.1A5A1, but 6B.1A5A1 viruses dominant from September 2020 and 6B.1A5A2 viruses were recently detected (*10*).

No A(H3N2) and B/Yamagata lineage viruses were available for characterization during the study period. Influenza A(H3N2) viruses were reported in most regions, including Africa, Asia, North America, Oceania and Europe, but the clades of A(H3N2) viruses circulating varied among reporting countries. The largest proportion of 3C.2a1b2a2 viruses with HA amino acid substitutions K83E, Y94N, T131K, Y159N, T160I, L164Q, G186D, D190N, F193S, and Y195F in this period were found in South Asia, Southeast Asia, the Middle East, East Africa, Oceania, North America, and Europe (*10*). There are no B/Yamagata lineage viruses isolated globally with dates after March 2020 (*10*).

Based on our analysis, all tested viruses for susceptibility to therapeutics were susceptible to neuraminidase and endonuclease inhibitors. Anti-influenza drugs were used as an effective means of post-exposure prophylaxis and treatment of influenza virus infection. Continuous monitoring of circulating influenza viruses for antiviral resistance is still important.

Because of the common transmission route between SARS-CoV-2 and influenza, the same protective behaviors can greatly limit the spread of influenza (*11*–*13*). In the mainland of China, B/Victoria lineage viruses continued to be identified, but the diversity of types/subtypes co-circulating has decreased compared to recent seasons. Globally, few B/Yamagata lineage viruses have been found during the COVID-19 pandemic. The surveillance data presented here are a reminder that the low level of influenza circulation since early 2020 may not necessarily persist into the upcoming influenza season space. Given the long absence of sustained natural exposure to influenza viruses, the reduction in influenza virus transmission over the past year may affect the severity of the upcoming influenza season. Lower levels of population immunity, especially in young children and the elderly, may portend more widespread illness and potentially more severe epidemics when the influenza viruses re-emerge (*14*–*15*). The lack of influenza circulation in the previous season will reduce our confidence in the strains that may circulate in the next season and may result in mismatch between the vaccine and circulating strains. Maintaining surveillance and outbreak response is essential to track geographic spread of the virus and its variation characteristics so that the vaccine remains optimized for circulating influenza viruses, and influenza surveillance should continue to be strengthened.

doi: 10.46234/ccdcw2021.224

[#] Corresponding author: Dayan Wang, wangdayan@ivdc.chinacdc.cn.

[1] National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention; WHO Collaborating Centre for Reference and Research on Influenza; Key Laboratory for Medical Virology, National Health Commission, Beijing, China.

Submitted: October 08, 2021; Accepted: October 26, 2021

REFERENCES

1. World Health Organization. Influenza (seasonal). https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal). [2018-11-6].
2. Blackburne BP, Hay AJ, Goldstein RA. Changing selective pressure during antigenic changes in human influenza H3. PLoS Pathog 2008;4(5):e1000058. http://dx.doi.org/10.1371/journal.ppat.1000058.
3. Huang QS, Wood T, Jelley L, Jennings T, Jefferies S, Daniells K, et al. Impact of the COVID-19 nonpharmaceutical interventions on influenza and other respiratory viral infections in New Zealand. Nat Commun 2021;12(1):1001. http://dx.doi.org/10.1038/S41467-021-21157-9.
4. Laurie KL, Rockman S. Which influenza viruses will emerge following the SARS-CoV-2 pandemic? Influenza Other Respir Viruses 2021;15(5):573-6. http://dx. doi.org/10.1111/irv.12866.
5. Olsen SJ, Azziz-Baumgartner E, Budd AP, Brammer L, Sullivan S, Pineda RF, et al. Decreased influenza activity during the COVID-19 pandemic — united states, Australia, Chile, and south Africa, 2020. MMWR Morb Mortal Wkly Rep 2020;69(37):1305 – 9. http://dx.doi.org/10.15585/mmwr.mm6937a6externalicon.
6. Zipfel CM, Colizza V, Bansal S. The missing season: the impacts of the COVID-19 pandemic on influenza. Vaccine 2021;39(28):3645 – 8. http://dx.doi.org/10.1016/j.vaccine.2021.05.049.
7. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2020–2021 northern hemisphere influenza season. https://www.who.int/publications/m/item/recommended-composition-of-influenza-virus-vaccines-for-use-in-the-2020-2021-northern-hemisphere-influenza-season. [2020-2-28].
8. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2021-2022 northern hemisphere influenza season. https://www.who.int/publications/m/item/recommended-composition-of-influenza-virus-vaccines-for-use-in-the-2021-2022-northern-hemisphere-influenza-season. [2021-2-26].
9. Huang WJ, Cheng YH, Chen T, Li XY, Tan MJ, Wei HJ, et al. Characterization of influenza viruses — China, 2019–2020. China CDC Wkly 2020;2(44):856 – 61. http://dx.doi.org/10.46234/ccdcw2020.228.
10. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2022 southern hemisphere influenza season. https://www.who.int/publications/m/item/recommended-composition-of-influenza-virus-vaccines-for-use-in-the-2021-southern-hemisphere-influenza-season. [2021-9-24].
11. Chiu NC, Chi H, Tai YL, Peng CC, Tseng CY, Chen CC, et al. Impact of wearing masks, hand hygiene, and social distancing on influenza, enterovirus, and all-cause pneumonia during the coronavirus pandemic: retrospective national epidemiological surveillance study. J Med Internet Res 2020;22(8):e21257. http://dx.doi.org/10.2196/21257.
12. Cowling BJ, Ali ST, Ng TWY, Tsang TK, Li JCM, Fong MW, et al. Impact assessment of non-pharmaceutical interventions against coronavirus disease 2019 and influenza in Hong Kong: an observational study. Lancet Public Health 2020;5(5):E279 – 88. http://dx.doi.org/10.1016/S2468-2667(20)30090-6.
13. Soo RJJ, Chiew CJ, Ma S, Pung R, Lee V. Decreased influenza incidence under COVID-19 control measures, Singapore. Emerg Infect Dis 2020;26(8):1933 – 5. http://dx.doi.org/10.3201/eid2608.201229.
14. Baker RE, Park SW, Yang WC, Vecchi GA, Metcalf CJE, Grenfell BT. The impact of COVID-19 nonpharmaceutical interventions on the future dynamics of endemic infections. Proc Natl Acad Sci USA 2020;117(48):30547 – 53. http://dx.doi.org/10.1073/pnas.2013182117.
15. Olsen SJ, Winn AK, Budd AP, Prill MM, Steel J, Midgley CM, et al. Changes in influenza and other respiratory virus activity during the COVID-19 pandemic — United States, 2020–2021. MMWR Morb Mortal Wkly Rep 2021;70(29):1013 – 9. http://dx.doi.org/10.15585/mmwr.mm7029a1.

Outbreak Reports

Genetic Characterization of Two Human Cases Infected with the Avian Influenza A (H5N6) Viruses — Guangxi Zhuang Autonomous Region, China, 2021

Fuyin Bi[1,&]; Lili Jiang[2,&]; Lihua Huang[3]; Jingguang Wei[3]; Xiaowen Pan[2]; Yu Ju[1]; Jianjun Mo[1]; Minmei Chen[1]; Ning Kang[1]; Yi Tan[1]; Yonghong Li[1]; Jing Wang[1,#]

Summary

What is known about this topic?

H5N6 has replaced H5N1 as a dominant avian influenza virus (AIV) subtype in southern China. The increasing genetic diversity and geographical distribution of H5N6 pose a serious threat to the poultry industry and human health.

What is added by this report?

A total of 2 cases of H5N6 that occurred from February 2021 to July 2021 in Guangxi, China were reported in this study. Phylogenetic analysis of gene was constructed, and some mutations of HA gene, PB2 gene, PA gene, M1 gene, NS1 gene, the receptor-binding site were detected. The evolutionary origins of the internal genes were different.

What are the implications for public health practice?

As a multi-source reassortant virus, the H5N6 highly pathogenic AIV is continuously evolving. There is an urgent need to strengthen the surveillance of drug-resistant strains and novel variants.

Since the first human infections with highly pathogenic avian influenza (HPAI) H5N6 virus was detected in Sichuan Province of China in April 2014, a total of 38 human cases and 21 deaths due to H5N6 infection have been reported as of August 6, 2021 in the Western Pacific Region (*1*). However, the newly emergent HPAI H5N6 virus belonging to the genetic clade 2.3.4.4 of H5 virus subtypes has possessed the capability for binding human-origin SA-α, 6 Gal-linked receptor and has demonstrated more transmissibility than H5N1 virus in a ferret model (*2*), suggesting that this subtype virus may be of high public health risk.

Guangxi Zhuang Autonomous Region in southern China has a history of human infection with avian influenza virus (AIV). H7N9 virus emerged here (*3*) and HPAI H7N9 virus hit this region in 2017, with 27 infections and 14 deaths (*4*).

INVESTIGATION AND RESULTS

We reported 2 cases infected with the H5N6 virus belonging to genetic clade 2.3.4.4b and clade 2.3.4.4h in Guangxi, China in 2021. These 2 cases occurred in two different cities. A case was admitted to the intensive care unit (ICU) due to severe clinical symptoms and was subsequently tested positive for H5N6. The other case was detected from the influenza-like illness (ILI) surveillance system. When a case is found, the local CDC immediately conducts epidemiological and environmental investigations.

On February 16, 2021, a 50-year-old male (Patient A) developed a fever with chest tightness, tightness of breath, headache, cough, sputum, and pneumonia, and was transferred to the Respiratory Department of Hechi People's Hospital for hospitalization on February 21. Patient A had suffered from rheumatoid arthritis for more than 10 years, and he was found to be H5N6 influenza virus positive on February 26. He had a history of exposure to infected poultry 9 days before the onset of illness and died on March 2.

On July 6, 2021, a 61-year-old female (patient B) developed a fever with a maximum temperature of 38.5 °C and was admitted to the Second People's Hospital of Guangxi for treatment on July 9. After 6 days, she was confirmed to be positive for H5N6 by Guangxi CDC. She had a history of chronic gastritis and denied having contact with live poultry.

Viral RNA from the throat swabs of the two patients was extracted using the QIAamp® Viral RNA Mini Kit (Qiagen, Germany), according to the manufacturer's instructions. Specific real-time reverse transcriptase polymerase chain reaction (RT-PCR) assays with specific primer and probe sets for detecting avian influenza A (H5N6). The viral genomes were reverse-transcribed and amplified using Easy-Fast FluA whole

genome amplification one-step kit (Xinlihechuang Technology Co., Ltd.). The sequencing libraries were prepared using the Illumina Nextera® XT Library Prep Kit. The final viral-enriched libraries were sequenced using the Illumina MiSeq platform (Illumina, San Diego, USA). The whole genome sequences of the 2 avian influenza A (H5N6) strains were assembled and obtained using CLC Genomics Workbench 9.5.2, and the 2 strains were designated A/GX-hechi/01/2021(H5N6) (GX01) from Patient A and A/GX-guilin/11151/2021(H5N6) (GX11151) from Patient B. The genomes were aligned with the reference genomes downloaded from GISAID (www.gisaid.org) using MAFFT v7.037b (https://mafft.cbrc.jp/alignment/software/). The genetic and evolutionary analyses were conducted in MEGA 7.0.14 (https://www.megasoftware.net/).

Through sequencing and splicing, 8 gene fragments of the virus were successfully extracted. Some mutations related to viral replication, receptor-binding, mammalian virulence-related markers and drug-resistance related markers were detected. The PB2 and MP genes of GX01 virus showed high homology with H9N2 viruses, and the following genes showed high homology with H5N6 viruses. The HA and NP genes of GX11151 virus showed high homology with H5N8 and H9N2 viruses, respectively, and the following genes showed high homology with H5N6 viruses (Table 1).

Phylogenetic analysis of HA gene was constructed according to the World Health Organization (WHO) reference sequence. Their nucleotide sequence showed a similarity of 90.7% between each other. The HA gene of GX01 falls into clade 2.3.4.4h, while GX11151falls into clade2.3.4.4b (Figure 1).

The HA cleavage site of two viruses possessed a multiple basic amino acids motif, indicating potentially high pathogenicity in chickens. The receptor-binding site at the 222–224 motif was QGG of GX01 virus and QRG of GX11151 virus, respectively, suggesting that these viruses preferred binding to avian-like receptors (α 2,3 SA) (*5*). However, D94N (*6*), S133A (*7*), and T156A (*8*) mutations in GX01 HA gene, S133A, D155N (*8*), T156A, and T188I (*7*) mutations in the GX11151 HA gene increased virus binding to human-like receptors (α 2-6 SA). GX01 virus exhibited 8 potential glycosylation sites at 27, 39, 70, 140, 180, 301, 498, and 557 (H5 numbering). GX11151 virus exhibited seven potential glycosylation sites at 27, 39, 180, 209, 301, 498, and 557 (Table 2).

A263T mutation in HA gene was detected in both strains, suggesting that the virulence was enhanced. However, there was no mutation associated with resistance to NA inhibitors in the NA gene of the two strains. K389R, V598T/I mutations of PB2 gene, and N409S mutation of PA gene, which could increase virus replicative ability in mammals, were observed in both strains. Some mutations increasing virulence in

TABLE 1. Similarity analysis of H5N6 virus sequences from the two cases in Guangxi zhuang autonomous region, China, 2021.

Virus	Segment	Length (bp)	Strain with the highest similarity	GISAD ID	Similarity (%)
GX01	PB2	2348	A/goose/Fujian/3.15_FZHX0011-O/2018 (H9N2)	EPI1816470	92.21
	PB1	2341	A/Guangxi/31906/2018 (H5N6)	EPI1352803	100.00
	PA	2229	A/chicken/Miyazaki/2-4C/2017 (H5N6)	EPI1891595	96.55
	HA	1773	A/chicken/Anhui/8.28_YHZGS017-O/2018 (H5N6)	EPI1825343	97.68
	NP	1565	A/Guangxi/31906/2018 (A/H5N6)	EPI1352798	98.98
	NA	1431	A/Env/Guangdong/Qingyuan/C18285099/2018 (H5N6)	EPI1366600	98.25
	MP	1027	A/chicken/Shanxi/06.28_TGRL001-O/2018 (H9N2)	EPI1833450	98.64
	NS	875	A/Env/Guangdong/Dongguan/C172863577/2017 (H5N6)	EPI1366948	98.86
GX11151	PB2	2342	A/Env/Guangdong/zhanjiang/C17277335/2017 (H5N6)	EPI1366759	96.63
	PB1	2344	A/Env/Guangdong/zhanjiang/C18277136/2018 (H5N6)	EPI1366684	97.78
	PA	2233	A/chicken/Miyazaki/2-4C/2017 (H5N6)	EPI891595	97.08
	HA	1775	A/chicken/Omsk/0112/2020 (H5N8)	EPI1813345	99.44
	NP	1565	A/duck/Hunan/5.29_YYGK90P3-OC/2018 (H9N2)	EPI1835016	98.72
	NA	1433	A/Env/Guangdong/Qingyuan/C18285099/2018 (H5N6)	EPI1366600	97.77
	MP	1027	A/Sichuan/06681/2021 (A/H5N6)	EPI1883262	99.51
	NS	875	A/Env/Guangdong/Huizhou/C17280804/2017 (A/H5N6)	EPI1366935	98.86

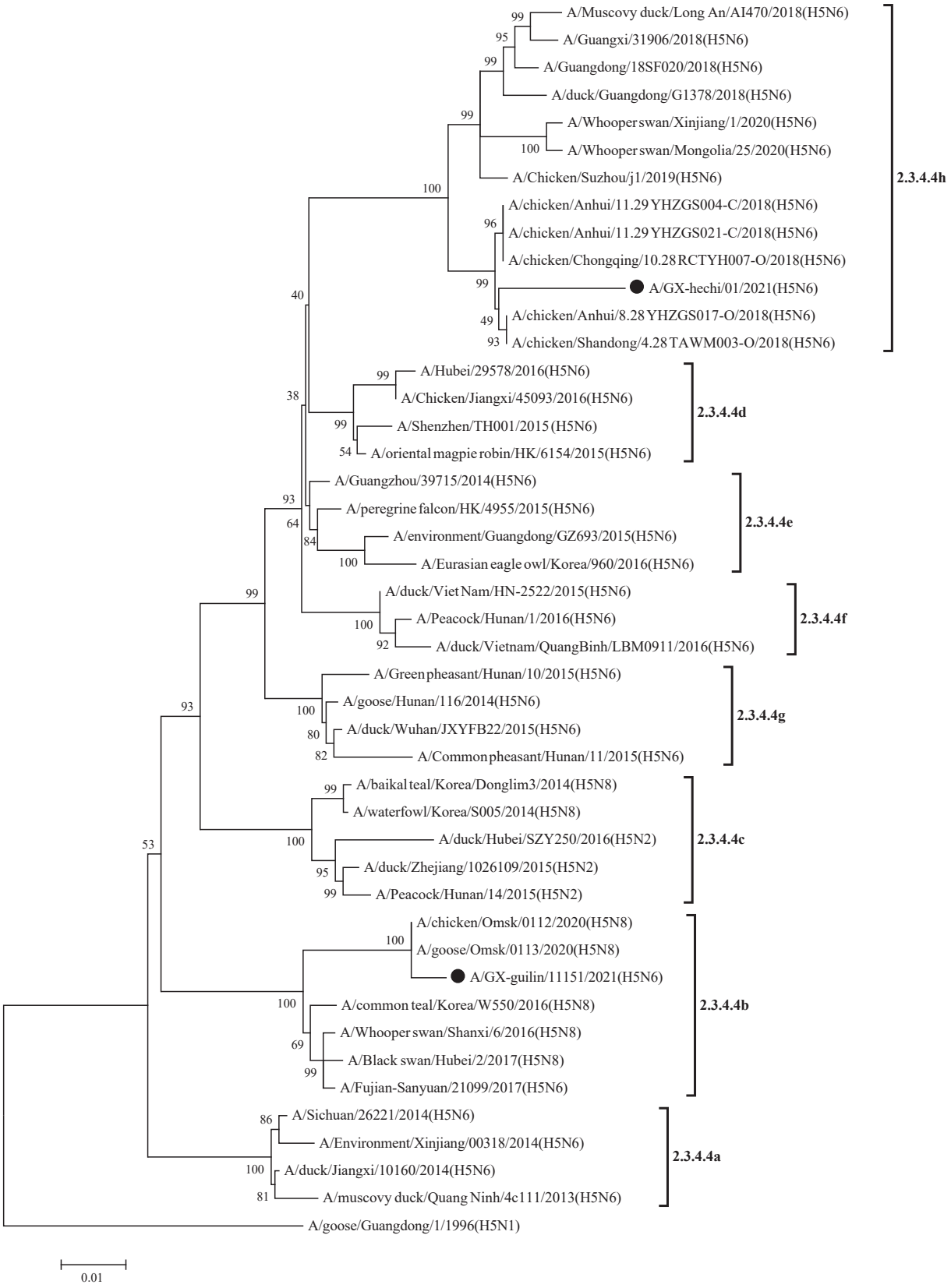

FIGURE 1. Phylogenetic relationships of A (H5) clade 2.3.4.4 HA genes using the maximum likelihood method with 1,000 bootstrap.
Note: The two Guangxi strains were indicated by black dots.

mice were detected in our strains, such as N30D, T139A, and T215A of M1 gene and P42S and 80–84 deletion of NS1 gene. The M2 gene of GX01 had the mutations of D21G and S31N, suggesting that the strain was resistant to amantadine, but GX11151 was not observed (Table 2).

DISCUSSION

The previous study showed H5N6 has replaced H5N1 as one of the dominant AV subtypes in southern China (*9*). The avian influenza A (H5N6) virus continues to threaten human life and health.

TABLE 2. Molecular features of the genes of H5N6 viruses isolated from humans.

Gene	GX01	GX11151	SC26221	GX31906	GZ39715	HB29578	Phenotypic characteristics
HA (H5 no.)							
D94N	N	S	N	N	N	N	Increased virus binding to α2-6 SA
S133A	A	A	A	A	A	A	Increased pseudovirus binding to α2-6 SA
D155N	D	N	D	D	D	D	Increased virus binding to α2-6 SA
T156A	A	A	A	A	T	A	
T188I	T	I	T	A	T	T	Increased pseudovirus binding to α2-6 SA
A263T	T	T	T	T	T	T	The residue is related to virulence.
222-224	QGG	QRG	QRG	QRG	QRG	QSG	222–224 QS(R)G avian-like α2–3 receptor binding preference
Cleavage peptides	RERRRKR	REKRRKR	REKRRKR	RERRRKR	RERRRKR	RERRRKR	Highly pathogenic avian influenza
NA (N6 no.)							
E119D/V	E	E	E	E	E	E	Antiviral oseltamivir resistance
A247V	A	A	A	A	A	A	
H274Y	H	H	H	H	H	H	
R293K	R	R	R	R	R	R	
R372K	R	R	R	R	R	R	
PB2							
K389R	R	R	R	R	R	K	Enhanced growth capacity in human and mammalian cells
V598T/I	T	T	T	T	T	V	
PB1							
I368V	I	I	I	I	I	V	Transmissible among ferrets
PA							
N409S	S	S	S	S	S	N	Increased virus replicative ability in mammalian systems
M1							
N30D	D	D	D	D	D	D	Increased virulence in mice
T139A	A	A	T	T	T	A	
T215A	A	A	A	A	A	A	
M2							
D21G	G	D	D	D	D	G	Antiviral amantadine resistance
L26F/I	L	L	L	L	L	L	
A30T	A	A	A	A	A	A	
S31N	N	S	S	S	S	N	
G34E	G	G	G	G	G	G	
NS1							
P42S	S	S	S	S	S	S	Increased virulence in mice
80-84Del	Yes	Yes	Yes	Yes	Yes	No	
L98F	M	M	M	M	M	M	

From January to July 2021, 2 cases were reported in Guangxi zhuang autonomous region. Compared with 4 H5N6 infections reported from 2016 to 2019, and the number of infections cases was slightly higher than that in previous years. It is necessary to sequence and analyze the virus. The case infected with GX01 had obvious clinical symptoms and a history of exposure to dead poultry. Homology analysis showed that GX01 virus was a recombinant virus of H5N6 and H9N2, while GX11151 was a recombinant virus of H5N8, H5N6, and H9N2. Previous study showed H5N6 lineage has been co-circulating in different regions in China (*10*–*11*). The mutations in the important sites of proteins of avian influenza virus may change the adaptivity, virulence, tissue tropism, and infectivity. The cleavage between HA1 and HA2 proteins of HA gene of these two viruses were multiple continuous basic amino acids motif (RERRRKR), indicating that they possessed potentially high pathogenicity in chickens. The 222–224 receptor binding site of HA gene suggested that the viruses were avian-like receptors (α 2,3 SA). Studies have shown that the sensitivity of viruses carrying H274Y mutation in NA protein to oseltamivir decreased 1,000 times. In addition, when the NA protein of the virus carries E119A/D/V, A247V, R293K, and R372K mutations, it will cause different degrees of resistance to oseltamivir and zanamivir (*12*–*13*). Fortunately, these mutations were not found in our virus. Therefore, using NA inhibitors to treat the 2 cases infected with avian influenza A (H5N6) was still a good choice.

The median age of the two cases was over 50 years old. Like other studies previously reported, the elderly people may be more vulnerable to avian influenza (*3*). One case was reported through ILI surveillance system (*14*), indicating that the ILI system was beneficial for the detection of avian influenza cases to a certain extent. However, this may be just the tip of the iceberg, and perhaps many mild cases have not been detected.

This study has certain limitations. Sampling of the environment exposed by the cases, and poultry around the living environment were missing, resulting in lacking of laboratory tests. One reason was that the period from onset to reporting was too long for sampling, in spite of dead poultry found in epidemiology survey, and another reason was that patient B had no clear history of exposure to poultry.

At present, coronavirus disease (COVID-19) has caused a worldwide pandemic (*15*), and over 200 million people have been infected. Meanwhile, the increasing trend of human infection with avian influenza virus has become an important public health issue that cannot be ignored, alerting us that COVID-19 and avian influenza may be simultaneously prevalent in some regions.

Acknowledgments: Dr. Yu Lan and Dr. William J. Liu.

Funding: Supported by the Self-Funded Scientific Research Project of Guangxi Health and Family Planning Commission (Z2015457, Z2016433).

doi: 10.46234/ccdcw2021.199

[#] Corresponding author: Jing Wang, wangjing0630@126.com.

[1] Guangxi Key Laboratory of Major Infectious Disease Prevention and Control and Biosafety Emergency Response, Guangxi Center for Disease Control and Prevention, Nanning, Guangxi Zhuang Autonomous Region, China; [2] Guilin Center for Disease Control and Prevention, Guilin, Guangxi Zhuang Autonomous Region, China; [3] Hechi Center for Disease Control and Prevention, Hechi, Guangxi Zhuang Autonomous Region, China.
[&] Joint first authors.

Submitted: August 25, 2021; Accepted: September 02, 2021

REFERENCES

1. World Health Organization. Avian influenza weekly update number 804. https://www.who.int/docs/default-source/wpro---documents/emergency/surveillance/avian-influenza/ai_20210806.pdf?Status=Master&sfvrsn=5f006f99_42. [2021-8-6].
2. Sun HL, Pu J, Wei YD, Sun YP, Hu J, Liu LT, et al. Highly pathogenic avian influenza H5N6 viruses exhibit enhanced affinity for human type sialic acid receptor and in-contact transmission in model ferrets. J Virol 2016;90(14):6235 – 43. http://dx.doi.org/10.1128/JVI.00127-16.
3. Wang XL, Jiang H, Wu P, Uyeki TM, Feng LZ, Lai SJ, et al. Epidemiology of avian influenza a H7N9 virus in human beings across five epidemics in mainland China, 2013-17: an epidemiological study of laboratory-confirmed case series. Lancet Infect Dis 2017;17(8):822 – 32. http://dx.doi.org/10.1016/S1473-3099(17)30323-7.
4. Wang J, Jiang LN, Ning CY, Yang YP, Chen M, Zhang C, et al. First outbreak of human infection with avian influenza A(H7N9) virus in Guangxi, China, 2016 to 2017. Chin Med J (Engl) 2019;132(16):1995 – 7. http://dx.doi.org/10.1097/CM9.0000000000000376.
5. Chutinimitkul S, van Riel D, Munster VJ, van den Brand JMA, Rimmelzwaan GF, Kuiken T, et al. *In vitro* assessment of attachment pattern and replication efficiency of H5N1 influenza a viruses with altered receptor specificity. J Virol 2010;84(13):6825 – 33. http://dx.doi.org/10.1128/JVI.02737-09.
6. Su Y, Yang HY, Zhang BJ, Jia HL, Tien P. Analysis of a point mutation in H5N1 avian influenza virus hemagglutinin in relation to virus entry into live mammalian cells. Arch Virol 2008;153(12):2253 – 61. http://dx.doi.org/10.1007/s00705-008-0255-y.
7. Yang ZY, Wei CJ, Kong WP, Wu L, Xu L, Smith DF, et al. Immunization by avian H5 influenza hemagglutinin mutants with altered receptor binding specificity. Science 2007;317(5839):825 – 8. http://dx.doi.org/10.1126/science.1135165.
8. Wang WJ, Lu B, Zhou HL, Suguitan AL Jr, Cheng X, Subbarao K, et al. Glycosylation at 158N of the hemagglutinin protein and receptor binding specificity synergistically affect the antigenicity and immunogenicity of a live attenuated H5N1 A/Vietnam/1203/2004 vaccine virus in ferrets. J Virol 2010;84(13):6570 – 7. http://dx.doi.org/10.1128/JVI.00221-10.

9. Bi YH, Chen QJ, Wang QL, Chen JJ, Jin T, Wong G, et al. Genesis, evolution and prevalence of H5N6 avian influenza viruses in China. Cell Host Microbe 2016;20(6):810 – 21. http://dx.doi.org/10.1016/j.chom.2016.10.022.
10. Bi YH, Mei K, Shi WF, Liu D, Yu XL, Gao ZM, et al. Two novel reassortants of avian influenza A (H5N6) virus in China. J Gen Virol 2015;96(Pt 5):975 – 81. http://dx.doi.org/10.1099/vir.0.000056.
11. Xiao CK, Xu JA, Lan Y, Huang ZP, Zhou LJ, Guo YX, et al. Five independent cases of human infection with avian influenza H5N6 — Sichuan Province, China, 2021. China CDC wkly 2021;3(36):751 – 6. http://dx.doi.org/10.46234/ccdcw2021.187.
12. Liu WJ, Wu Y, Bi YH, Shi WF, Wang DY, Shi Y, et al. Emerging HxNy influenza A viruses. Cold Spring Harb Perspect Med 2020;a038406. https://doi.org/10.1101/cshperspect.a038406
13. Fan SF, Deng GH, Song JS, Tian GB, Suo YB, Jiang YP, et al. Two amino acid residues in the matrix protein M1 contribute to the virulence difference of H5N1 avian influenza viruses in mice. Virology 2009;384(1):28 – 32. http://dx.doi.org/10.1016/j.virol.2008.11.044.
14. Ip DKM, Liao QH, Wu P, Gao ZC, Cao B, Feng LZ, et al. Detection of mild to moderate influenza A/H7N9 infection by China's national sentinel surveillance system for influenza-like illness: case series. BMJ 2013;346:f3693. http://dx.doi.org/10.1136/bmj.f3693.
15. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/. [2021-9-2].

Review

Epidemiological and Genetic Characteristics of the H3 Subtype Avian Influenza Viruses in China

Jiaying Yang[1,2]; Lei Yang[2]; Wenfei Zhu[2]; Dayan Wang[2]; Yuelong Shu[1,2,#]

Avian influenza viruses (AIVs) are naturally preserved in waterfowls and sometimes spill over to infect humans and mammalian animals. The H3 subtype is one of the most prevalent influenza virus subtypes in waterfowls. Since 2000, H3 subtype AIVs have been continuously isolated from poultry and wild birds in the mainland of China, which implied a dynamic spread in large-scale geography and multiple species. Combinations of H3 with N1–N8 subtypes were reported, among which H3N2 and H3N8 subtypes predominated. Frequent mutations and reassortments increased the genetic diversity associated with altered virus virulence, transmission, and mammalian adaptation of H3 AIVs, posing a potential threat to animal and human health. This study systematically analyzed the epidemiology, genetic characteristics, and mammalian adaptation-related mutations of H3 subtype AIVs in the mainland of China, facilitating the H3 subtype AIVs research and risk assessment.

Avian influenza virus (AIV) belongs to the *Orthomyxoviridae* family *Influenza virus A* genus, packaged with 8 negative-sense and single-strand RNA segments, which encode a total of 14 proteins (*1*). Based on the antigenic diversity of surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), AIV can be classified into 16 HA subtypes (H1–H16) and 9 NA subtypes (N1–N9) (*2–3*). Except for the H17N10 and H18N11 subtypes found in bats in recent years, all other subtypes of influenza A virus can be found in wild waterfowls. Therefore, wild waterfowls are known as the natural reservoir and gene pool of influenza A virus, and AIV is considered to be the source of other animal influenza viruses (*4*). According to the pathogenicity of AIV to chickens, it can be divided into highly pathogenic AIV (HPAIV) and lowly pathogenic AIV (LPAIV) (*5*). Most avian influenza viruses exhibit low pathogenicity. To date, only partial proportions of H5 and H7 subtype AIVs have developed high pathogenicity, causing high mortality in wild birds, poultry, and even humans.

Among influenza A virus subtypes, the H3 subtype has a wide range of hosts. In addition to circulating in wild birds and poultry, it can infect multiple species of mammals such as humans, pigs, dogs, cats, horses, and even seals (*6*). The H3 subtype AIV is LPAIV and one of the influenza virus subtypes with the highest isolation rate among ducks (proportion up to 91.76%) (*7*). Surveillance data showed that the H3 subtype AIV was widely prevalent in domestic ducks in the live poultry markets (LPMs) in China and reassortants were continuously being detected (*8–9*). Genetically, the HA genes of H3 subtype AIVs could be divided into Eurasian and North American lineages. Both H3 lineages were able to cross the species barrier to infect swine, while Eurasian lineage was identified to infect a wider range of hosts (swine, equine, canine, and human) than North American lineage (swine and seals) (*10*). In China, only the Eurasian lineage has been detected in domestic poultry (*11*). The interspecies transmissibility of H3 AIVs historically caused the H3N2 influenza pandemic in 1968 (*12*), which has posed a threat to human health (*13*).

Here, we systematically reviewed the current situation of the H3 subtype AIVs in China using surveillance data from 82 published studies and summarized the epidemiological and genetic characteristics and the mammalian adaptation of H3 AIVs in the first 20 years of 21st century in the mainland of China.

EPIDEMIOLOGY OF H3 AIVS IN CHINA

Since the 1970s, China has reported isolation of AIVs (*14*). After Hong Kong Special Administrative Region (SAR) reported 18 human infections and 6 deaths caused by HPAIV (H5N1) in 1997 (*15*), avian influenza surveillance has received global attention for pandemic preparedness. During 2000–2019, AIV surveillance had been conducted in LPMs, poultry farms, and wild birds habitats in the mainland of China. Extensive and continuous active surveillance work included collecting throat and cloacal samples from birds and bird-related environmental specimens

such as feces, water, and cage surfaces (*11*,*16*–*21*). Since 2009, surveillance has been intensified in all of 31 provincial-level administrative divisions (PLADs), with a sampling frequency from 2–4 weeks a time to once a year. H3 AIVs were subtyped by RT-PCR or sequencing directly from the samples or AIV isolates.

H3 combinations with multiple NA (N1–N8) subtypes were detected in the mainland of China, dynamically circulating in poultry and wild birds, usually with no apparent illness (*22*). H3Nx could affect domestic ducks, chickens, geese, pigeons, and quails and mainly circulated in LPMs in China (*23*–*25*). From January 2000 to June 2021, 50 studies have reported the detection of H3 subtype AIVs in domestic ducks, including 6 H3 subtype AIVs such as H3N1, H3N2, H3N3, H3N6, H3N7, and H3N8. Overall, 23 articles reported the prevalence of H3 subtype AIVs in domestic chickens. Furthermore, 8 subtypes, H3 combined with N1-N8, have circulated in wild birds, and H3N8 was predominant, with a higher prevalence rate in Anseriformes (*20*–*27*). A 4-year surveillance study (2015–2019), including more than 28,000 samples of wild birds from the Qinghai-Tibet plateau, found that H3N8 was the most abundantly detected H3 virus subtype (*20*).

To summarize the activities of H3 AIVs in the mainland of China, we have counted each H3Nx subtype at the provincial scale each year. For example, if one or more studies reported that H3N2 AIV was found in Heilongjiang Province in 2010, it would be an activity of H3N2 AIV. Since 2000, 214 activities of H3 AIVs have been detected in 26 PLADs in the mainland of China except for the PLADs of Xizang (Tibet), Gansu, Shaanxi, Shanxi, and Hainan (Figure 1A). Surveillance indicated that H3 viruses have become enzootic in domestic ducks in southern China*, where LPMs bring together numerous host species in a high-density setting, creating an ideal environment for viral reassortment and interspecies transmission (*23*). In eastern China† and northeastern China§, H3 AIVs could affect both domestic poultry and wild birds, except for Fujian, Shandong and Zhejiang provinces where H3 viruses were only detected in domestic poultry (Figure 1A). Previous studies have reported that H3N8 circulated in the Poyang Lake area around Jiangxi Province (*18*) and the wetlands of Jiangsu Province (*28*), which are along the wild fowl migratory flyway. In northeastern China, H3 AIVs with multiple NA subtypes (including N2–N8) were detected in wild birds in Heilongjiang Province (Figure 1A), where the complicated bird migration network lies (*29*). Researchers have continuously detected novel H3N8 subtype AIVs in wild ducks in their habitats such as the Heilongjiang Sanjiang Nature Reserve (*30*). H3 subtype AIVs were mainly detected in wild birds in northwestern China¶, while in southwestern China** they were mainly found in domestic poultry (Figure 1A).

In the last decade, increased detection of H3 AIVs was reported throughout the country. Notably, H3N2 and H3N8 have been consistently detected (Figure 1B). An epidemiological survey showed that poultry infection with H3N2 AIV occurred throughout the year in southern China, with higher detection rates in winter and spring and a lower detection rate in summer (*31*). In the wetlands of northeastern China, virus isolation rate of H3 subtype AIVs varied among seasons, and the highest rate was in autumn, which might due to the migration of wild birds (*32*).

In summary, H3N2 subtype AIV was the main prevalent subtype in poultry farms and LPMs from China, while H3N8 subtype AIV was widespread among wild birds in this region. Therefore, the following focuses on the H3N2 subtype and H3N8 subtype AIVs.

GENETIC EVOLUTION OF H3 AIVS IN CHINA

H3N2 Subtype AIV

Phylogenetic analysis showed that all of the H3N2 subtype AIVs in China belonged to the Eurasian lineage (*11*). The HA gene may be derived from viruses circulating in different regions of China and neighbouring countries in East and Southeast Asia (*7*,*17*,*33*), while the NA gene may be derived from other AIVs such as H5 (*34*–*35*), H7 (*9*,*36*), H9 (*23*,*36*), and H11 (*34*) subtype AIVs in birds in East and Southeast Asia. The prevalence of H3N2 AIVs in birds and frequent reassortments with other AIVs also led to the emergence of various genotypes. Most of the

* Including Guangdong Province and Guangxi Zhuang Autonomous Region.
† Including Anhui Province, Jiangsu Province, Jiangxi Province, Fujian Province, Shandong Province, Zhejiang Province, and Shanghai Municipality.
§ Including Heilongjiang Province, Jilin Province and Liaoning Province.
¶ Including Qinghai Province, Ningxia Hui Autonomous Region, and Xinjiang Uygur Autonomous Region.
** Including Sichuan Province and Chongqing Municipality.

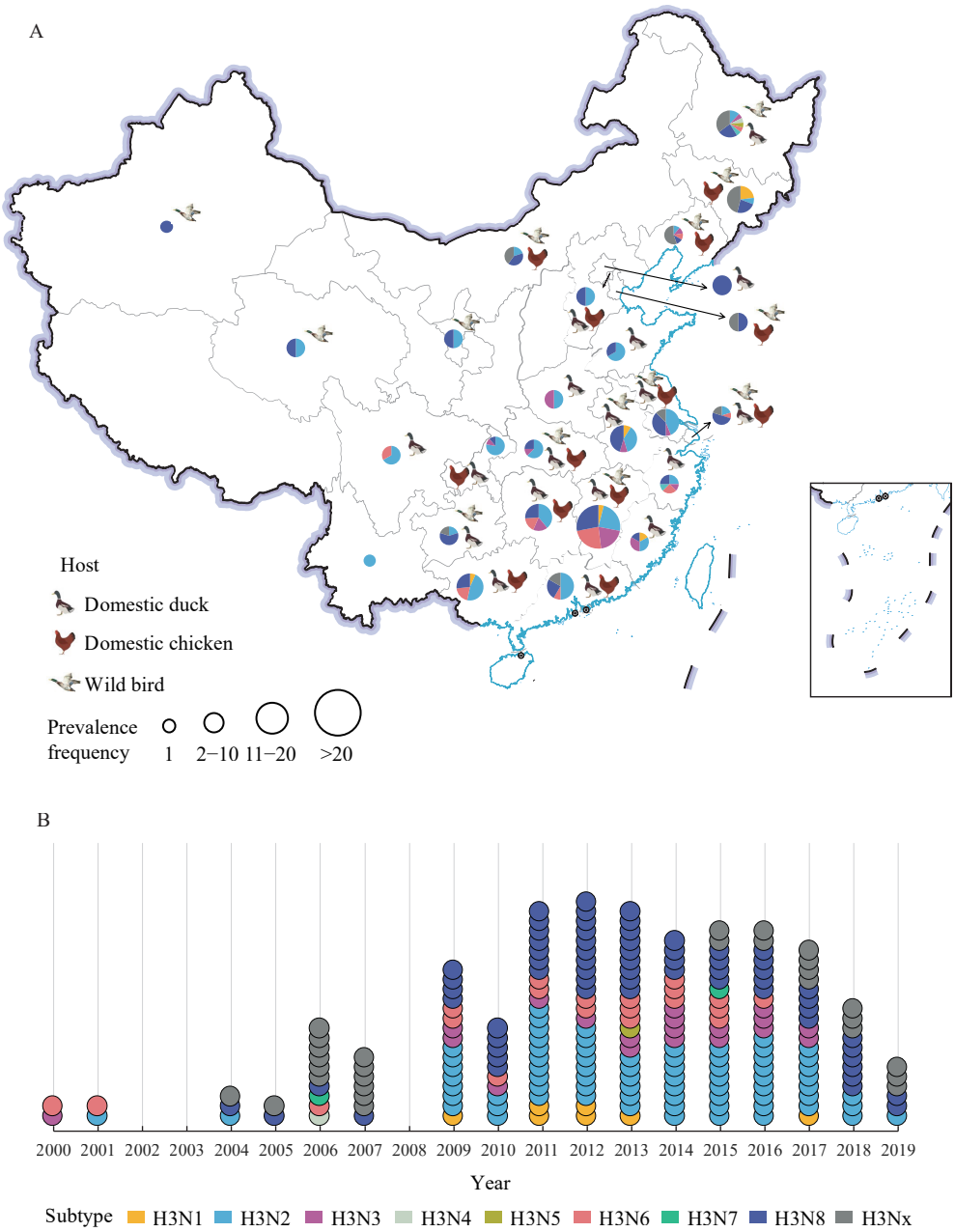

FIGURE 1. The spatiotemporal distribution and bird species of H3 subtype avian influenza viruses (AIVs) reported in the mainland of China from 2000 to 2019. (A) The spatial distribution and bird species of H3 subtype AIVs. (B) The temporal distribution of H3 subtype AIVs (according to collection year).
Note: As of June 6, 2021, 214 activities* of H3 subtype AIV have been counted in 26 provincial-level administrative divisions (PLADs) in China since 2000. Each H3Nx subtype is shown as specific color. The diameter of the pie chart (A) represents the number of H3Nx subtype AIV activities. Each circle in (B) represents an activity. Data were accessed from 82 published literatures on PubMed, CNKI, and Wanfang databases, searched up to June 6, 2021.
* Each H3Nx subtype at the provincial scale each year has been counted. For example, if one or more studies reported that H3N2 AIV was found in Heilongjiang Province in 2010, it would be an activity of H3N2 AIV.

reassortants came from LPMs along the bird migration routes, which suggests that wild bird migration might have a great impact on poultry AIV infection (*8*,*18*,*23*,*37*). Studies have found that the PA gene of H3N2 subtype AIV of Chinese ducks was highly homologous to the HPAIV H7N9 in Korean wild ducks (*33*). The H9N2 viruses that were prevalent in poultry in eastern China [Jiangxi (*23*), Jiangsu (*38*), Zhejiang (*39*)] also provided internal genes for H3N2 AIVs. The internal genes of some H3N2 AIVs were

closely related to HPAI H5 AIVs, which may suggest reassortment with H5N1, H5N2, H5N3, H5N6, and H5N8 subtype AIVs (*8*,*32*,*34*,*40*).

H3N8 Subtype AIV

Molecular epidemiological analysis revealed that the H3N8 subtype AIVs had complex sources. Studies have shown that the HA gene of H3N8 subtype AIV might come from the H3 subtype AIV that is prevalent in poultry in East Asia, Southeast Asia, and Europe (*41*–*42*), and the NA gene might come from East Asia, North America, and Europe (*43*–*44*). The NA gene of H3N8 subtype AIV could be divided into Eurasian lineage and North American lineage (*45*). The Eurasian lineage was widely detected in the whole country, while the North American lineage was mainly detected in eastern China (*8*,*11*,*45*), indicating that gene reassortments have occurred between AIVs from the Eurasian lineage and the North American lineage (*8*,*45*–*47*). These events most likely occurred in LPMs and could be transmitted from ducks to chickens (*48*). The internal genes of H3N8 AIVs were derived from a variety of AIVs from wild birds or domestic ducks (*24*,*49*–*50*), including HPAI H5 viruses (*28*,*43*,*51*–*52*).

The H3N8 subtype AIVs might be potential gene sources of AIVs causing interspecies transmission. During 2004–2005, researchers detected an H3N8 virus in domestic ducks in Beijing LPMs, of which HA gene was highly similar to the H3N8 virus that caused the 1989–1990 outbreaks in equine populations in northern China (*43*). In 2018, researchers found that the NA gene of the H3N8 virus isolated from a Guangdong LPM was highly homologous to H10N8 AIV and speculated that the N8 gene originated from the same lineage that caused human infections in 2014 (*48*).

Other H3 Subtypes AIV

Except for the AIVs of H3N2 and H3N8 subtypes, other AIVs of H3 subtypes were scattered in various regions of China (Figure 1) and had a high degree of genetic diversity and origin. A study indicated that the H5N1 subtype AIV in domestic ducks in Anhui Province and Fujian Province might be involved in the gene reassortment of H3N1 subtype AIV (*53*). The internal genes of H3N3 might be derived from viruses of waterfowl origin (mainly ducks) in North America (Alaska) and East Asia (Japan and the Republic of Korea) (*21*–*23*,*39*). The HA gene of H3N5 detected in wild birds in Khanka Lake was closely related to poultry-origin AIVs isolated from southern China (*21*). Studies have shown that H3N6 frequently reassorted with H5N6 subtype AIVs (*18*,*54*–*55*), while PB2 and NP genes were also derived from H9N2 AIV that circulated in the same period (*23*). Experimental results showed that the novel H3N6 reassortant could effectively replicate in mammalian cells (*54*). The gene of H3N7 subtype AIV came from a variety of LPAIVs and had greater antigenic differences compared with the H3 subtype AIV strains isolated from previous studies (*56*).

MAMMALIAN ADAPTIONS OF H3 SUBTYPE AIV

In 1968, a novel H3N2 subtype virus contained genes from human-derived H2N2 influenza virus and avian-derived H3 subtype influenza virus was firstly reported in Hong Kong SAR and caused the pandemic globally (*12*). It highlighted the pandemic potential of H3 subtype AIVs. Mutations that increased replicative ability and transmissibility of the virus in mammals may facilitate the interspecies transmission (*57*–*59*). Previous studies have experimentally demonstrated the relation between some molecular mutations and receptor binding, transmission, replication, pathogenicity, and drug resistance of the H3 subtype AIV in mammals. Here, we have summarized the mammalian adaptation molecular markers in Table 1.

Receptor binding is the initial process of virus life circle. AIVs usually show higher binding preference for α 2,3-Gal sialic acid receptors (avian-type), and increased binding capacity of α 2,6-Gal sialic acid receptors (human-type) indicates increased adaptability in mammals. Studies have shown that H3N2 and H3N8 subtype AIVs could simultaneously bind to both avian-type and human-type receptors (*17*,*22*,*60*). Overall, 2 residues, 226 and 228, of HA of the H3 subtype were well known to be important for the host range restriction (*61*). Recently, researchers found that residue 155T in the HA protein could enhance the ability of H3N2 subtype AIV to bind to human-type receptors, and the virus could replicate effectively in the lungs and turbinates of mice (*9*). The G225D mutation of HA could increase the thermal stability of H3N2 AIV, which could probably lead to increased virus replication on Madin-Darby canine kidney (MDCK) cells (*7*). The H3N2 subtype AIV with Q226R and G228S mutations in HA could enhance the replicative ability and mammalian adaptability in

TABLE 1. Key amino acid substitutions of H3 subtype avian influenza viruses associated with mammalian adaptation and their biological effects.

Protein	Subtype	Mutation	Biological effects	References
PB2	H3N8	K574E	Decreased replicative ability in ferrets and transmission ability in mice, decreased RNA polymerase activity in 293T cells	(*69*)
		T588A	Decreased replicative ability in ferrets and transmission ability in mice, decreased RNA polymerase activity in 293T cells	(*69*)
		E627K,D701N	Increased replicative ability on mammalian cells and transmission ability among mammals	(*51*)
PB1	H3N8	S524G	Enhanced RNA polymerase activity in human cells and MDCK cells, increased replicative ability and pathogenicity in mice, enhanced airborne transmissibility between ferrets	(*45*)
HA	H3N2	G225D	Increased HA thermostability and replicative ability on MDCK cells	(*7*)
		Q226R	Increased replicative ability and virulence in ferrets	(*17*)
		G228S	Increased replicative ability and virulence in ferrets	(*17*)
	H3N8	155T	Increased binding capacity of human α2,6-Gal sialic acid receptors	(*9*)
NA	H3N6	D198N	Increased probability of drug resistance, which might reduce the sensitivity of NA inhibitor drugs (such as oseltamivir)	(*53*,*70*)
	H3N8	G119P	Increased probability of drug resistance, which might reduce the sensitivity of NA inhibitor drugs (such as oseltamivir)	(*28*,*70*)
M1	H3N8	N30D	Increased pathogenicity in mice	(*51*)
		T215A	Increased pathogenicity in mice	(*51*)
M2	H3N2	V27I	Reduce the sensitivity to M2 ion channel blockers (amantadine)	(*11*)
		S31N	Reduce the sensitivity to M2 ion channel blockers (amantadine)	(*8*,*11*,*32*,*71*)
NS1	H3N2	D92E	Increase probability of viral resistance to interferon, and further experimental verification is needed	(*67*,*72*)
		P42S	Increased replicative ability and virulence in mice	(*7*)

Abbreviation: MDCK=Madin-Darby canine kidney.

ferrets (*17*). The Q226L mutation of HA could promote the airborne transmission of the H3N8 virus in ferrets (*45*).

The RNA polymerase of the influenza virus is composed of three subunits including PB2, PB1, and PA proteins, which are related to the virus's host-specificity and replication. Dong et al. found that E627K and D701N mutations in the PB2 protein of H3N8 AIV (*51*) might enhance the adaptability of the virus in mammalian cells and enable AIV to replicate efficiently in mammals (*62*–*65*). Another study found that the PB1 S524G mutation of wild bird-origin H3N8 virus could enhance the virulence and fitness for transmission in mammals (*45*).

Mutations that increase pathogenicity were also found. The N30D and T215A mutations found in the M1 protein could increase the pathogenicity of the H3N8 subtype AIV in mice (*51*). The residue at 42 of the NS1 protein in the H3N2 subtype AIV changed from P to S, which might increase virus replication and virulence in mice (*7*).

For drug resistance, the D198N and G119P mutations of NA protein have been identified in the natural isolates of H3N6 and H3N8, respectively. These mutations were likely to cause the H3 subtype AIV with reduced sensitivity of NA inhibitors (*28*,*53*). In addition, many studies have found that the V27I and S31N mutations of the matrix protein (M) of H3N2 subtype AIV caused the virus to be resistant to amantadine (*8*,*11*,*18*,*32*).

DISCUSSION

The H3 subtype AIVs have continuously circulated in wild birds and poultry in the mainland of China. Wild bird migration and live poultry trade play important roles for virus transmission. Frequent gene reassortments were observed between viruses from poultry and wild birds (*52*,*55*–*66*), which increased the genetic diversity of H3 AIVs and contributed genes to other subtype AIVs such as HPAIV H5 (*67*–*68*). Despite the relatively improved surveillance studies having been performed in recent years in China, our understanding of the H3 AIVs transmission and evolution is still limited, especially in intercontinental migratory birds. The H3 viruses could acquire mammalian adaptation mutations during replication. Considering the pandemic history and the potential threat to public health, a long-term systematic

surveillance of H3 AIV is imperative.

Funding: The National Natural Science Foundation of China (No: 81961128002).

doi: 10.46234/ccdcw2021.225

[#] Corresponding author: Yuelong Shu, shuylong@mail.sysu.edu.cn.

[1] School of Public Health (Shenzhen), Shenzhen Campus of Sun Yat-sen University, Shenzhen, Guangdong, China; [2] Chinese National Influenza Center, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China.

Submitted: October 09, 2021; Accepted: October 27, 2021

REFERENCES

1. Eisfeld AJ, Neumann G, Kawaoka Y. At the centre: influenza A virus ribonucleoproteins. Nat Rev Microbiol 2015;13(1):28 – 41. http://dx.doi.org/10.1038/nrmicro3367.
2. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev 1992;56(1):152 – 79. http://dx.doi.org/10.1128/mr.56.1.152-179.
3. Fouchier RAM, Munster V, Wallensten A, Bestebroer TM, Herfst S, Smith D, et al. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J Virol 2005;79(5):2814 – 22. http://dx.doi.org/10.1128/JVI.79.5.2814-2822.2005.
4. Tong SX, Zhu XY, Li Y, Shi M, Zhang J, Bourgeois M, et al. New world bats harbor diverse influenza A viruses. PLoS Pathog 2013;9(10):e1003657. http://dx.doi.org/10.1371/journal.ppat.1003657.
5. Mahy BWJ, Brown CC. Emerging zoonoses: crossing the species barrier. Rev Sci Tech 2000;19(1):33 – 40. http://dx.doi.org/10.20506/rst.19.1.1212.
6. Bean WJ, Schell M, Katz J, Kawaoka Y, Naeve C, Gorman O, et al. Evolution of the H3 influenza virus hemagglutinin from human and nonhuman hosts. J Virol 1992;66(2):1129 – 38. http://dx.doi.org/10.1128/jvi.66.2.1129-1138.1992.
7. Cai TY. Epidemiological analysis and biological characterization of H3 subtype avian influenza virus in Eastern China from 2017 to 2019. Yangzhou: Yangzhou University; 2020. http://dx.doi.org/10.27441/d.cnki.gyzdu.2020.000350. (In Chinese).
8. Cui HR, Shi Y, Ruan T, Li XS, Teng QY, Chen HJ, et al. Phylogenetic analysis and pathogenicity of H3 subtype avian influenza viruses isolated from live poultry markets in China. Sci Rep 2016;6:27360. http://dx.doi.org/10.1038/srep27360.
9. Li C, Yu M, Liu LT, Sun HL. Characterization of a novel H3N2 influenza virus isolated from domestic ducks in China. Virus Genes 2016;52(4):568 – 72. http://dx.doi.org/10.1007/s11262-016-1323-0.
10. Liu S, Ji K, Chen JM, Tai D, Jiang WM, Hou GY, et al. Panorama phylogenetic diversity and distribution of type A influenza virus. PLoS One 2009;4(3):e5022. http://dx.doi.org/10.1371/journal.pone.0005022.
11. Zou SM, Tang J, Zhang Y, Liu LJ, Li XY, Meng Y, et al. Molecular characterization of H3 subtype avian influenza viruses based on poultry-related environmental surveillance in China between 2014 and 2017. Virology 2020;542:8 – 19. http://dx.doi.org/10.1016/j.virol.2020.01.003.
12. Cockburn WC, Delon PJ, Ferreira W. Origin and progress of the 1968-69 Hong Kong influenza epidemic. Bull World Health Organ 1969;41(3):345–8. https://pubmed.ncbi.nlm.nih.gov/5309437/.
13. Zhang YP, Zhao CH, Hou YJ, Chen Y, Meng F, Zhuang YC, et al. Pandemic threat posed by H3N2 avian influenza virus. Sci Chin Life Sci 2021;64:1984 – 7. http://dx.doi.org/10.1007/s11427-021-1916-4.
14. Guo YJ, Gao SQ, Wang YX, Zhang ZQ, Meng FY, Wang M, et al. Isolation from wild ducks influenza group of viruses: two type A viruses with new hemagglutinin. Acta Microbiol Sin 1981;21(2):166 – 3. http://dx.doi.org/10.13343/j.cnki.wsxb.1981.02.006. (In Chinese).
15. Chan PKS. Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. Clin Infect Dis 2002;34 Suppl 2:S58-64. http://dx. doi.org/10.1086/338820.
16. Pepin KM, Wang J, Webb CT, Smith GJD, Poss M, Hudson PJ, et al. Multiannual patterns of influenza A transmission in Chinese live bird market systems. Influenza Other Respir Viruses 2013;7(1):97 – 107. http://dx.doi.org/10.1111/j.1750-2659.2012.00354.x.
17. Guan LZ, Shi JZ, Kong XT, Ma SJ, Zhang YP, Yin X, et al. H3N2 avian influenza viruses detected in live poultry markets in China bind to human-type receptors and transmit in guinea pigs and ferrets. Emerg Microbes Infect 2019;8(1):1280 – 90. http://dx.doi.org/10.1080/22221751.2019.1660590.
18. Jiao M, Liu XQ, Chen T, Zhang H, Fu WJ, Xie Y, et al. Epidemiological and phylogenetic analysis of H3 subtypes avian influenza viruses isolate from Poyang lake region, China, 2014–2017. Chin J Virol 2018;34(1):1–8. https://d.wanfangdata.com.cn/periodical/bdxb201801001. (In Chinese).
19. Qiu BF, Liu WJ, Peng DX, Hu SL, Liu XF. Distribution of avian influenza virus subtypes among domestic ducks in eastern China. Acta Microbiol Sin 2008;48(10):1290 – 4. http://dx.doi.org/10.3321/j.issn:0001-6209.2008.10.003. (In Chinese).
20. Sun J. Study on temporal and spatial relationship between bar-headed goose and avian influenza virus on Qinghai-Tibet plateau. Harbin: Northeast Forestry University. 2020. http://dx.doi.org/10.27009/d.cnki.gdblu.2020.000068. (In Chinese).
21. Li X. Surveillance and evolution analysis of avian influenza virus in wild birds in Khanka Lake, Heilongjiang, 2013-2018. Harbin: Northeast Forestry University. 2019. http://dx.doi.org/10.27009/d.cnki.gdblu.2019.000332. (In Chinese).
22. Guan LJ. Characterization of H3 subtype avian influenza virus in China from 2009 to 2013. Harbin: Northeast Forestry University; 2014. http://dx.doi.org/10.7666/d.Y2620838. (In Chinese).
23. Liu M, He SQ, Walker D, Zhou NN, Perez DR, Mo B, et al. The influenza virus gene pool in a poultry market in South Central China. Virology 2003;305(2):267 – 75. http://dx.doi.org/10.1006/viro.2002.1762.
24. Liu TT, Xie ZX, Song DG, Luo SS, Xie LJ, Li M, et al. Genetic characterization of a natural reassortant H3N8 avian influenza virus isolated from domestic geese in Guangxi, Southern China. Genome Announc 2014;2(4):e00747 – 14. http://dx.doi.org/10.1128/genomeA.00747-14.
25. Hu JK, Zha YF, Jin XJ, Wen XH, Li ZX, Wang X, et al. Continuous adaptation of the HA and NA gene of H3N2 subtypes of avian influenza virus in South China, 2017-2018. J Infect 2019;79(1):61 – 74. http://dx.doi.org/10.1016/j.jinf.2019.01.009.
26. Tang L, Tang WJ, Li XF, Hu CX, Wu D, Wang TH, et al. Avian influenza virus prevalence and subtype diversity in wild birds in Shanghai, China, 2016-2018. Viruses 2020;12(9):1031. http://dx.doi.org/10.3390/v12091031.
27. Yang XY. Surveillance of wild bird influenza combined with sentinels in Hubei Province. Harbin: Northeast Forestry University. 2020. http://dx.doi.org/10.27009/d.cnki.gdblu.2020.000661. (In Chinese).
28. Wang C, Fei RM. Genome sequencing and genetic analysis of H3N8 subtype avian influenza virus isolated from mallard. Chin Vet Sci 2016;46(12):1489 – 96. http://dx.doi.org/10.16656/j.issn.1673-4696.2016.12.003. (In Chinese).
29. Tian HY, Zhou S, Dong L, Van Boeckel TP, Cui YJ, Newman SH, et al. Correction for Tian et al. , Avian influenza H5N1 viral and bird migration networks in Asia. Proc Natl Acad Sci USA 2015;112(22):E2980.
30. Zeng XW, Hua YP, Li XB, Zhang ZM. Monitoring Influenza A virus and Newcastle disease virus in migratory waterfowls in Sanjiang natural reserve of Heilongjiang Province. Acta Microbiol Sin 2008;48(10):1403 – 7. http://dx.doi.org/10.3321/j.issn:0001-6209.2008.10.023. (In Chinese).

31. Peng Y, Xie ZX, Liu JB, Pang YS, Deng XW, Xie ZQ, et al. Epidemiological surveillance of low pathogenic avian influenza virus (LPAIV) from poultry in Guangxi Province, Southern China. PLoS One 2013;8(10):e77132. http://dx.doi.org/10.1371/journal.pone.0077132.
32. Yang DQ, Liu J, Ju HB, Ge FF, Wang J, Li X, et al. Genetic analysis of H3N2 avian influenza viruses isolated from live poultry markets and poultry slaughterhouses in Shanghai, China in 2013. Virus Genes 2015;51(1):25 – 32. http://dx.doi.org/10.1007/s11262-015-1198-5.
33. Liu TT, Xie ZX, Song DG, Luo SS, Xie LJ, Deng XW, et al. Whole genome sequencing and analysis of eleven strains of avian influenza virus subtype H3N2 isolated in Guangxi province. Chin J Vet Sci 2017;37(5):859 – 65. http://dx.doi.org/10.16303/j.cnki.1005-4545.2017.05.18. (In Chinese).
34. Li QH, Zhao QQ, Gu M, Zhu J, Gu XB, Zhao G, et al. Genome sequence of a novel reassortant H3N2 avian influenza virus from domestic mallard ducks in Eastern China. Genome Announc 2013;1(2):e0022112. http://dx.doi.org/10.1128/genomeA.00221-12.
35. Li J, Liu F, Liu DF, Luo Q, Li YJ, Liu CG. Isolation, identification and sequence analysis of a H3N2 subtype influenza virus originated from wild duck. Mod J Anim Husb Vet Med 2015(8):1 – 6. http://dx.doi.org/10.3969/j.issn.1672-9692.2015.08.001. (In Chinese).
36. Zhao G, Zhong L, Zhao KK, Lu XL, Peng DX, Liu XF. Isolation and characterization of H3N2 influenza viruses from ducks. Acta Vet Zootech Sin 2010;41(10):1354–8. https://d.wanfangdata.com.cn/periodical/ChlQZXJpb2RpY2FsQ0hJTmV3UzIwMjExMDI2Eg94bXN5eGIyMDEwMTAwMjMaCDdvNjl2aDRl. (In Chinese).
37. Deng GH, Tan D, Shi JZ, Cui PF, Jiang YP, Liu LL, et al. Complex reassortment of multiple subtypes of avian influenza viruses in domestic ducks at the Dongting Lake Region of China. J Virol 2013;87(17):9452 – 62. http://dx.doi.org/10.1128/JVI.00776-13.
38. Hu T. Full-length genes sequence and pathogenicity analysis of two H3N2 avian influenza viruses isolate from ducks [dissertation]. Changchun: Jilin Agricultural University; 2013. http://cdmd.cnki.com.cn/Article/CDMD-10193-1013309621.htm. (In Chinese).
39. Wu HB, Wu NP, Peng XR, Jin CZ, Lu XY, Cheng LF, et al. Molecular characterization and phylogenetic analysis of H3 subtype avian influenza viruses isolated from domestic ducks in Zhejiang Province in China. Virus Genes 2014;49(1):80 – 8. http://dx.doi.org/10.1007/s11262-014-1065-9.
40. Li CR, Bai R, Deng JL, Che RF, Liang D, Zhao W, et al. Reassortant H3N2 avian influenza virus isolated outside live poultry markets in Guangdong province. J Pathog Biol 2020;15(6):685 – 91,697. http://dx.doi.org/10.13350/j.cjpb.200613. (In Chinese).
41. Cui HR. Phylogenetic analysis and pathogenicity of H3 subtype avian influenza viruses isolated in live poultry market of southern China. Beijing: Chinese Academy of Agricultural Sciences; 2016. http://cdmd.cnki.com.cn/Article/CDMD-82101-1016169857.htm. (In Chinese).
42. Cui PF, Lu KP, Chen S, Xiao L, Guan LZ, Deng GH, et al. Biological characteristic of two H3N8 subtype avian influenza viruses (AIV). J Agric Biotechnol 2016;24(7):980–6. http://www.cnki.com.cn/Article/CJFDTotal-NYSB201607005.htm. (In Chinese).
43. Pu J, Liu QF, Xia YJ, Fan YL, Brown EG, Tian FL, et al. Genetic analysis of H3 subtype influenza viruses isolated from domestic ducks in northern China during 2004-2005. Virus Genes 2009;38(1):136 – 42. http://dx.doi.org/10.1007/s11262-008-0300-7.
44. Wang YL. HA and NA genes phylogenic analysis of AIV isolates from wild birds and the distribution/transmission pattern of AIV in wild birds. Harbin: Northeast Forestry University; 2009. http://cdmd.cnki.com.cn/Article/CDMD-10225-2009132374.htm. (In Chinese).
45. Zhang XH, Li YG, Jin S, Zhang YM, Sun LY, Hu XY, et al. PB1 S524G mutation of wild bird-origin H3N8 influenza A virus enhances virulence and fitness for transmission in mammals. Emerg Microbes Infect 2021;10(1):1038 – 51. http://dx.doi.org/10.1080/22221751.2021.1912644.
46. Cheng SJ, Li JP, Hou GY, Peng C, Wang SC, Jiang WM, et al. Whole genome sequence analysis of 14 strains of H3 subtype avian influenza virus. Chin J Vet Med 2018;54(10):30–5. https://kns.cnki.net/KCMS/detail/detail.aspx?dbcode=CJFD&filename=ZSYZ201810007.
47. Zhao T. Epidemiological surveilence study on avian influenza virus and its infection risk [dissertation]. Beijing: Academy of Military Sciences; 2018. http://cdmd.cnki.com.cn/Article/CDMD-90101-1018094574.htm. (In Chinese).
48. Li X, Chen JL, Jin XJ, Hu JK, Xie SM, Dai YX, et al. Characterization of three H3N2 and one new reassortant H3N8 avian influenza virus in South China. Infect Genet Evol 2019;75:104016. http://dx.doi.org/10.1016/j.meegid.2019.104016.
49. Wu HB, Yang F, Liu FM, Peng XM, Chen B, Cheng LF, et al. Molecular characterization of H10 subtype avian influenza viruses isolated from poultry in Eastern China. Arch Virol 2019;164(1):159 – 79. http://dx.doi.org/10.1007/s00705-018-4019-z.
50. Wu HB, Peng XM, Peng XR, Wu NP. Molecular characterization of a reassortant H11N9 subtype avian influenza virus isolated from a domestic duck in Eastern China. Arch Virol 2015;160(10):2595 – 601. http://dx.doi.org/10.1007/s00705-015-2528-6.
51. Dong BB, Xu CL, Dong LB, Cheng HJ, Yang L, Zou SM, et al. A novel reassortant H3N8 influenza virus isolated from drinking water for duck in a domestic duck farm in Poyang Lake area. Biomed Environ Sci 2013;26(7):546 – 51. http://dx.doi.org/10.3967/0895-3988.2013.07.005.
52. Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EP, Chen LM, et al. Transmission of equine influenza virus to dogs. Science 2005;310(5747):482 – 5. http://dx.doi.org/10.1126/science.1117950.
53. Guan LZ, Wang J, Lu KP, Zhang F, Xiao L, Cui PF, et al. Biological characteristics of two H3N1 subtype avian influenza viruses (AIV). J Agric Biotechnol 2017;25(7):1162–9. https://d.wanfangdata.com.cn/periodical/nyswjsxb201707014. (In Chinese).
54. Li XY, Yang JY, Liu B, Jia YN, Guo J, Gao X, et al. Co-circulation of H5N6, H3N2, H3N8 and emergence of novel reassortant H3N6 in a local community in Hunan province in China. Sci Rep 2016;6:25549. http://dx.doi.org/10.1038/srep25549.
55. Li RY, Zhang T, Xu J, Chang JX, Xu B. Isolation of two novel reassortant H3N6 avian influenza viruses from long-distance migratory birds in Jiangxi Province, China. Microbiologyopen 2020;9(8):e1060. http://dx.doi.org/10.1002/mbo3.1060.
56. Gu WL, Cui PF, Cui JQ, Han SY, Xing X, Deng GH, et al. The complete genomic sequence and infectivity analysis of a H3N7 subtype avian influenza virus. Chin J Prev Vet Med 2017;40(7):565-8, 574. https://d.wanfangdata.com.cn/periodical/ChlQZXJpb2RpY2FsQ0hJTmV3UzIwMjExMDI2EhF6Z3lmc3l4YjIwMTgwNzAwMRoIcW9saHkyMjU%3D. (In Chinese).
57. Herfst S, Schrauwen Eefje J, Linster M, Linster M, Chutinimitkul S, de Wit E, et al. Airborne transmission of influenza A/H5N1 virus between ferrets. Science 2012;336(6088):1534 – 41. http://dx.doi.org/10.1126/science.1213362.
58. Wan HQ, Perez DR. Amino acid 226 in the hemagglutinin of H9N2 influenza viruses determines cell tropism and replication in human airway epithelial cells. J Virol 2007;81(10):5181 – 91. http://dx.doi.org/10.1128/JVI.02827-06.
59. Wang D, Yang L, Gao R, Zhang X, Tan Y, Wu A, et al. Genetic tuning of the novel avian influenza A(H7N9) virus during interspecies transmission, China, 2013. Euro Surveill 2014;19(25):20836. http://dx.doi.org/10.2807/1560-7917.es2014.19.25.20836.
60. Guan LZ. Biological characterization of H3N2 (from 2009 to 2014) and H5 (from 2015 to 2016) avian influenza viruses in China. Lanzhou: Gansu Agricultural University. 2017. http://cdmd.cnki.com.cn/Article/CDMD-10733-1017824803.htm. (In Chinese).
61. Galvan G. Hypothalamic peptide hormones. Acta Med Austriaca 1978;5(3):55-61. https://pubmed.ncbi.nlm.nih.gov/110023/.
62. Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. Science 2001;293(5536):1840 – 2. http://dx.doi.org/10.1126/science.1062882.
63. Subbarao EK, London W, Murphy BR. A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. J Virol 1993;67(4):1761 – 4. http://dx.doi.org/10.1128/jvi.67.4.1761-1764.

1993.
64. Zhang LS, Zhang ZG, Weng ZP. Rapid reassortment of internal genes in avian influenza A(H7N9) virus. Clin Infect Dis 2013;57(7):1059 – 61. http://dx.doi.org/10.1093/cid/cit414.
65. Yu ZJ, Sun WY, Zhang XH, Cheng KH, Zhao CQ, Gao YW, et al. Multiple amino acid substitutions involved in the virulence enhancement of an H3N2 avian influenza A virus isolated from wild waterfowl in mice. Vet Microbiol 2017;207:36 – 43. http://dx.doi.org/10.1016/j.vetmic.2017.05.020.
66. Yang SY. Surveillance of influenza virus in wild birds in Qin Huangdao in Hebei and Xianghai in Jilin. Harbin: Northeast Forestry University. 2015. http://cdmd.cnki.com.cn/article/cdmd-10225-1015655577.htm. (In Chinese).
67. Hu CX, Li XF, Zhu CH, Zhou F, Tang WJ, Wu D, et al. Co-circulation of multiple reassortant H6 subtype avian influenza viruses in wild birds in eastern China, 2016–2017. Virol J 2020;17(1):62. http://dx.doi.org/10.1186/s12985-020-01331-z.
68. Zhang Y, Chen MM, Huang YW, Zhu WF, Yang L, Gao LD, et al. Human infections with novel reassortant H5N6 avian influenza viruses in China. Emerg Microbes Infect 2017;6(6):e50. http://dx.doi.org/10.1038/emi.2017.38.
69. Cui PF. Biological characterization of H3N8 avian influenza viruses isolated from 2009 to 2013 in China. Lanzhou: Gansu Agricultural University; 2016. http://cdmd.cnki.com.cn/Article/CDMD-10733-1016902421.htm. (In Chinese).
70. Richard M, Deléage C, Barthélémy M, Lin YP, Hay A, Lina B, et al. Impact of influenza A virus neuraminidase mutations on the stability, activity, and sensibility of the neuraminidase to neuraminidase inhibitors. J Clin Virol 2008;41(1):20 – 4. http://dx.doi.org/10.1016/j.jcv.2007.10.021.
71. Choi JG, Kang HM, Kim MC, Paek MR, Kim HR, Kim BS, et al. Genetic relationship of H3 subtype avian influenza viruses isolated from domestic ducks and wild birds in Korea and their pathogenic potential in chickens and ducks. Vet Microbiol 2012;155(2 – 4):147 – 57. http://dx.doi.org/10.1016/j.vetmic.2011.08.028.
72. Li WZ, Wang GF, Zhang H, Xin G, Zhang DG, Zeng J, et al. Effects of NS1 variants of H5N1 influenza virus on interferon induction, TNFα response and p53 activity. Cell Mol Immunol 2010;7(3):235 – 42. http://dx.doi.org/10.1038/cmi.2010.6.

Review

Leveraging Global Influenza Surveillance and Response System for the COVID-19 Pandemic Response and Beyond

Aspen Hammond[1,#]; Vanessa Cozza[1]; Siddhi Hirve[1]; Marie-Jo Medina[1]; Dmitriy Pereyaslov[1]; Wenqing Zhang[1]

INTRODUCTION

Since the beginning of the coronavirus disease 2019 (COVID-19) pandemic, existing influenza surveillance systems, platforms, and capacities have been leveraged for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus detection, reporting, risk assessment, and sharing of virus materials and data. This article reviews the contributions made and addresses some of the future challenges and plans for the Global Influenza Surveillance and Response System (GISRS) and associated influenza surveillance systems.

DESCRIPTION

Since 1952, the world has benefited from an international collaboration of laboratories, public health institutions, and other partners to protect people from influenza. Beginning with 26 laboratories, the World Health Organization (WHO) Global Influenza Surveillance Network (GISN), renamed as the "Global Influenza Surveillance and Response System (GISRS)" in 2011, has expanded in number and scope to now 158 institutions in 127 countries. The network has adapted to the needs and changing capacities in countries as well as events and scientific advancements, with trust and country ownership key to its success (*1*–*2*).

SCOPE

From the early years of the network, there has been an emphasis on the sharing of influenza virus specimens and related information from Member States within the network and on the development and dissemination of technical guidance and standardized protocols. Member States continue to regularly share virus materials, alongside virologic and epidemiologic influenza-related information year-round. Through web-based data platforms such as FluNet (*3*) and FluID (*4*), there has been a steady increase in the number of samples tested for influenza since 2011. Since 2015, surveillance of respiratory syncytial virus (RSV) (*5*) based on GISRS has been piloted for integration into the influenza surveillance system. WHO, through the Global Influenza Programme (*6*), regularly updates technical guidance on laboratory techniques, influenza surveillance standards, influenza vaccine virus strains, and other pharmaceutical and non-pharmaceutical measures to mitigate influenza-related morbidity and mortality.

HISTORICAL PERSPECTIVE

Ever since its inception, GISRS has played key roles in the response to 3 influenza pandemics (in 1957, 1968, and 2009), outbreaks of animal influenza viruses, and the occasional spill-over of these viruses into humans, also including other non-influenza events such as the SARS outbreak in 2002 and the current SARS-CoV-2 pandemic. These events have highlighted the important international coordinating role of WHO and GISRS in pandemic preparedness and response (*7*).

GISRS continues to monitor the influenza viruses circulating in animal populations that may be a risk to humans. Working closely with international animal health partners, such as the Food and Agriculture Organization (FAO) (http://www.fao.org) and the World Organisation for Animal Health (OIE) (*8*), GISRS works to detect and respond to the sporadic spill-over of animal influenza viruses into humans (zoonotic transmission events).

In 2011, WHO's Member States and Executive Board called upon WHO's Global Influenza Programme and GISRS to expand its work on pandemic preparedness and response with the adoption of the Pandemic Influenza Preparedness Framework for the Sharing of Influenza Viruses and Access to Vaccines and Other Benefits (PIP Framework). The objective of the framework was to improve influenza pandemic preparedness and response by improving and

strengthening GISRS (*9*). With partners globally, the PIP Framework assists Member States with building national and international capacities to better detect and respond to inevitable future pandemics of influenza and beyond.

ACHIEVEMENTS

The achievements of GISRS and WHO's Global Influenza Programme since its inception, notably the timely response to the 2009 influenza pandemic, have been demonstrated and documented elsewhere (*7*, *10*–*11*). However, it is worth outlining how GISRS and associated influenza surveillance systems have also significantly contributed to the response to COVID-19 pandemic, caused by a non-influenza respiratory virus.

National Influenza Centres were Crucial to Initial Laboratory Response During COVID-19

National Influenza Centres (NICs) are the backbone of GISRS, collecting clinical specimens from sentinel and non-sentinel sources in their country, testing them for influenza and other respiratory viruses, performing preliminary analyses on influenza viruses detected, and sharing representative specimens or viruses with WHO Collaborating Centres (CCs) for advanced antigenic and genetic analyses. NICs are designated by national Ministries of Health and recognized by WHO. Given the capacities and expertise in these laboratories of the GISRS network, they became COVID-19 testing centres in many countries early in the SARS-CoV-2 pandemic. As of July 2020, approximately 85% of over 220 national COVID-19 reference or testing laboratories globally were either NICs or closely associated with GISRS (*12*).

National Influenza Centres have Demonstrated Expertise of Virus Detection

The first External Quality Assessment Project (EQAP) for SARS-CoV-2, which was critical to ensure national capacity of laboratory detection of the novel virus, was undertaken through the GISRS annual influenza EQAP mechanism, coordinated by WHO's GIP (*13*). A total of 233 laboratories (including 130 NICs) in 164 countries, areas, and territories participated, with the survey dispatched to labs from April to June 2020, despite disruptions caused by the pandemic. Overall, 94% of laboratories received a 100% pass rate. Of the 233 laboratories that participated in the SARS-CoV-2 EQAP, 68% (159/233) also participated in the influenza EQAP in 2019, indicating the substantial capacity for influenza testing that was utilized for testing during the COVID-19 response. Among laboratories that participated in the 2019 influenza EQAP, 94% of the labs scored 100% on the 2020 SARS-CoV-2 EQA.

Surveillance Data Generating and Sharing to Global Platforms

Data is key to understanding the dynamics of and informing the response to seasonal epidemics and pandemics. A key component of GISRS is the global data platform FluMart, in place since 1997, where countries report data on samples processed for influenza, such as the number of positive samples and subtype, on a weekly basis to a dataset called FluNet. Outputs of the data are visible to the public on WHO's website (*14*). Since the emergence of SARS-CoV-2, WHO has requested that Member States regularly report counts of COVID-19 cases and deaths at different levels of aggregation for global situational awareness. FluMart formed the basis of the data collection platform for COVID-19 data (COVMart) since early in the pandemic. Globally, as of September 10, 2021, there have been 223,022,538 confirmed cases of COVID-19, including 4,602,882 deaths, reported to WHO through COVMart and displayed on the WHO COVID-19 dashboard (*15*).

Genomic Sequencing and Genetic Sequence Data Sharing

The sharing of virus genetic sequence data is essential, especially in the early stages of the response to an outbreak of a novel virus. Sequence data allows for the development of laboratory protocols to enable countries to detect and monitor novel viruses. GISAID (available at: https://www.gisaid.org/), which was launched in 2008 to enable rapid and open access to epidemic and pandemic influenza virus genetic data and associated clinical and geographical information through its EpiFlu platform, quickly became the host of the genetic sequence data of SARS-CoV-2. In fact, it published the first SARS-CoV-2 sequence data hours after it became available. GISAID has been an important partner of GISRS with confidence from

countries for sharing their data through its data sharing mechanism. The development of the EpiCoV database and associated tools has been instrumental in the COVID-19 pandemic response. As of September 12, 2021, since the emergence of SARS-CoV-2, nearly 3.5 million complete genome sequences of SARS-CoV-2 have been uploaded to GISAID. About 80% of GISRS laboratories uploaded complete genome sequence data of SARS-CoV-2 to GISAID EpiCoV. In 2021, GISAID has also expanded to collect and share information on RSV, aside from influenza and SARS-CoV-2, though its EpiRSV data platform.

LEVERAGING EXISTING SURVEILLANCE SYSTEMS FOR MONITORING COVID-19

Sentinel surveillance is an efficient way to collect high-quality data in a timely manner systematically (using a consistent case definition and sampling strategy) and routinely from representatives of the population under surveillance so that the information gathered can be applied to the population or among subpopulations at higher risk of developing severe disease. Sentinel surveillance for COVID-19 using GISRS complements COVID-19 surveillance activities under the overall COVID-19 pandemic response. It is a cost-effective way to meet pandemic response objectives, including monitoring the geographic spread, intensity of transmission, and severity trends of community transmission of COVID-19 over time; understanding the risk factors for disease; systematically monitoring the genetic evolution of the COVID-19 virus; and assessing the impact on health systems. In addition, understanding the relative contribution of SARS-CoV-2 to the data captured in syndromic disease surveillance systems is critical to guide national responses and interpret the burden of disease caused by SARS-CoV-2 and influenza viruses. The data gathered can be used as a baseline for future evaluations of COVID-19 interventions such as vaccination, as has been already undertaken for influenza (*12*).

Since March of 2020, GISRS has incorporated SARS-CoV-2 into laboratory algorithms for testing specimens obtained from sentinel surveillance to monitor trends in co-circulation of the influenza and SARS-CoV-2 viruses and has provided guidance on the reporting of this information to FluNet, the global dataset managed by GIP (*16*). This integrated surveillance has been enhanced by the development, validation, and procurement of multiplex assays to test samples for influenza and SARS-CoV-2 simultaneously. In response to the recommendations of the 6th International Health Regulations (2005) (IHR) Emergency Committee for COVID-19, WHO encourages GISRS laboratories to sequence SARS-CoV-2-positive samples from its sentinel surveillance in a timely manner and share genetic sequence data with accompanying metadata through publicly accessible databases such as the GISAID EpiCoV database, with a focus on data quality over quantity (*17*).

CURRENT CHALLENGES AND THREATS

Much progress has been made on building capacities globally, regionally, and nationally to protect the world from influenza. However, in addition to the disruptions caused by the COVID-19 pandemic to livelihoods and health care systems, and the threat of misinformation, there are some challenges unique to influenza surveillance worth including here.

Although in most parts of the world, influenza activity has been lower than usual during the COVID-19 pandemic, an unusual epidemic can break out anytime. On the other hand, animal influenza viruses continue to sporadically infect humans, highlighting the continuing threat of zoonotic transmission and the need to remain vigilant for the unexpected and work with the One Health concept in mind (*18*). We also need to remain open to the possibility of another respiratory virus emerging and causing the next pandemic.

It is not clear when seasonal influenza activity will resume, and at what levels globally, but it is clear the surveillance systems need to be in place and functioning to quickly detect the return of influenza activity, alongside monitoring the trends post-COVID-19. In many countries, the current priority is understandably the response to the COVID-19 pandemic and exhaustive case finding and management efforts to control transmission. Context-specific solutions to achieving the pandemic response objectives and the ongoing influenza surveillance objectives need to be put in place. The collecting and sharing of influenza data and viruses need to continue to ensure the world is as prepared as possible against influenza epidemic and pandemic threats.

FUTURE DEVELOPMENT

With these challenges in mind, WHO has been developing a roadmap to further build GISRS into GISRS+: an enhanced network, built upon existing influenza infrastructure, to achieve integrated surveillance and response systems to influenza and a range of other respiratory viruses with epidemic or pandemic potential. The roadmap will address two main areas of work: 1) developing technical capacities; and 2) exploring coordination mechanism options for a GISRS+ system. The GISRS+, as envisaged with extensive input from GISRS members, countries and international agencies, will be a natural extension given the strength and successes the system had demonstrated over the past 70 years.

Acknowledgments: The GISRS network and all national and institutional public health professionals and all the generations of scientists throughout the world who have dedicated themselves to influenza surveillance, preparedness, and response, collaborating, generating and sharing of data; current and past WHO Influenza preparedness and Response, Global Influenza Programme and Pandemic Influenza Preparedness Framework staff and consultants working across the global, regional, and country levels.

doi: 10.46234/ccdcw2021.226

[#] Corresponding author: Aspen Hammond, hammonda@who.int.

[1] Global Influenza Programme, World Health Organization, Geneva, Switzerland.

Submitted: September 30, 2021; Accepted: October 19, 2021

REFERENCES

1. Zhang WQ, Wood J. The global influenza surveillance and response system-65 years of building trust and sharing and a role model for global health security. Influenza Other Respir Viruses 2018;12(5):566. http://dx.doi.org/10.1111/irv.12548.
2. World Health Organization. Global influenza surveillance and response system (GISRS). https://www.who.int/initiatives/global-influenza-surveillance-and-response-system. [2021-9-20].
3. World Health Organization. FluNet. https://www.who.int/tools/flunet. [2021-9-20].
4. World Health Orgnization. FluID. https://www.who.int/teams/global-influenza-programme/surveillance-and-monitoring/fluid. [2021-9-26].
5. World Health Organization. RSV surveillance. https://www.who.int/teams/global-influenza-programme/global-respiratory-syncytial-virus-surveillance. [2021-9-26].
6. World Health Organization. Global influenza programme. https://www.who.int/teams/global-influenza-programme. [2021-9-20].
7. Ziegler T, Mamahit A, Cox NJ. 65 years of influenza surveillance by a World Health Organization-coordinated global network. Influenza Other Respir Viruses 2018;12(5):558 – 65. http://dx.doi.org/10.1111/irv.12570.
8. World Organisation for Animal Health (OIE). Responding to emergencies through preparedness and resilience. https://www.oie.int/en/home/. [2021-9-20].
9. World Health Organization. Pandemic influenza preparedness (PIP) framework. https://www.who.int/initiatives/pandemic-influenza-preparedness-framework. [2021-9-20].
10. Hay AJ, McCauley JW. The WHO global influenza surveillance and response system (GISRS)-a future perspective. Influenza Other Respir Viruses 2018;12(5):551 – 7. http://dx.doi.org/10.1111/irv.12565.
11. World Health Organization. Implementation of the international health regulations (2005): report of the review committee on the functioning of the international health regulations (2005) in relation to pandemic (H1N1) 2009. 2011. http://apps.who.int/gb/ebwha/pdf_files/WHA64/A64_10-en.pdf?ua=1. [2021-9-20].
12. World Health Organization. GISRS sentinel surveillance for COVID-19: frequently asked questions (FAQ). 2020. https://apps.who.int/iris/bitstream/handle/10665/333616/WHO-2019-nCoV-influenza-FAQ-2020.1-eng.pdf?sequence=1&isAllowed=y. [2021-9-20].
13. World Health Organization. Global influenza programme: external quality assessment project. https://www.who.int/teams/global-influenza-programme/laboratory-network/eqa-project. [2021-9-26].
14. World Health Organization. Influenza surveillance outputs. https://www.who.int/teams/global-influenza-programme/surveillance-and-monitoring/influenza-surveillance-outputs. [2021-9-26].
15. World Health Organization. WHO coronavirus (COVID-19) dashboard. https://covid19.who.int. [2021-9-26].
16. World Health Organization. Maintaining surveillance of influenza and monitoring SARS-CoV-2 adapting global influenza surveillance and response system (GISRS) and sentinel systems during the COVID-19 pandemic, interim guidance. 2020. https://apps.who.int/iris/rest/bitstreams/1316069/retrieve. [2021-9-20].
17. World Health Organization. Operational considerations to expedite genomic sequencing component of GISRS surveillance of SARS-CoV-2. 2021. https://apps.who.int/iris/rest/bitstreams/1332762/retrieve. [2021-9-20].
18. World Health Organization. Global influenza programme: risk assessment summaries of influenza at the human-animal interface. https://www.who.int/teams/global-influenza-programme/avian-influenza. [2021-9-26].

Indexed by PubMed Central (PMC) and Emerging Sources Citation Index (ESCI).

The inauguration of *China CDC Weekly* is in part supported by Project for Enhancing International Impact of China STM Journals Category D (PIIJ2-D-04-(2018)) of China Association for Science and Technology (CAST).

CCDC weekly

Vol. 3 No. 44 Oct. 29, 2021

Responsible Authority
National Health Commission of the People's Republic of China

Sponsor
Chinese Center for Disease Control and Prevention

Editing and Publishing
China CDC Weekly Editorial Office
No.155 Changbai Road, Changping District, Beijing, China
Tel: 86-10-63150501, 63150701
Email: weekly@chinacdc.cn

CSSN
ISSN 2096-7071
CN 10-1629/R1