Genetic and antigenic characterization of H5 and H7 avian influenza viruses isolated from migratory waterfowl in Mongolia from 2017 to 2019

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Abstract
The circulation of highly pathogenic avian influenza viruses (HPAIVs) of various subtypes (e.g., H5N1, H5N6, H5N8, and H7N9) in poultry remains a global concern for animal and public health. Migratory waterfowls play important roles in the transmission of these viruses across countries. To monitor virus spread by wild birds, active surveillance for avian influenza in migratory waterfowl was conducted in Mongolia from 2015 to 2019. In total, 5000 fecal samples were collected from lakesides in central Mongolia, and 167 influenza A viruses were isolated. Two H5N3, four H7N3, and two H7N7 viruses were characterized in this study. The amino acid sequence at hemagglutinin (HA) cleavage site of those isolates suggested low pathogenicity in chickens. Phylogenetic analysis revealed that all H5 and H7 viruses were closely related to recent H5 and H7 low pathogenic avian influenza viruses (LPAIVs) isolated from wild birds in Asia and Europe. Antigenicity of H7Nx was similar to those of typical non-pathogenic avian influenza viruses (AIVs). While HPAIVs or A/Anhui/1/2013 (H7N9)-related LPAIVs were not detected in migratory waterfowl in Mongolia, sporadic introductions of AIVs including H5 and H7 viruses into Mongolia through the wild bird migration were identified. Thus, continued monitoring of H5 and H7 AIVs in both domestic and wild birds is needed for the early detection of HPAIVs spread into the country.

Keywords Avian influenza · Characterization · Migratory waterfowl · Mongolia · Surveillance

Introduction
Surveillance of avian influenza in wild birds has increased substantially worldwide in recent years because of the spread of H5 highly pathogenic avian influenza viruses (HPAIVs) among domestic poultry and wild birds in Asia, Europe, and Africa [1, 2]. Since the emergence of H5N1 HPAIVs in Asia...
[3], numerous global efforts have focused on elucidating the relative roles of wild bird and poultry movement in virus dissemination. To better understand the ecology of avian influenza viruses (AIVs) in wild birds, the data from wild bird surveillance studies are used to identify factors correlated with AIV detection in wild birds, such as reservoir species, bird health status, age, season, and location [4].

Each of the known subtype (H1–H16 and N1–N9) of influenza A virus (IAV) has been isolated from waterfowl, especially migratory wild ducks, that are infected with the viruses via waterborne transmission at their nesting lakes close to the Arctic Circle in Siberia, Alaska, and Canada during their breeding season in summer. These viruses replicate in columnar epithelial cells, forming crypts in the colon, and they are excreted in feces [5]. The infections of these AIVs do not cause illness in birds; however, current H5 NPAIVs that have been isolated in Asia, Europe, and Africa have caused death in several wild bird species [6].

Mongolia is located on three flyways of wild birds, namely the East Asian–Australasian, Central Asian, and East African–West Asian flyways, through which wild birds migrate from their northern territory in Siberia to the southern regions. In this context, intensive surveillance of AIVs in Mongolia has been conducted since autumn 1996 [7, 8]. Accordingly, the surveillance for migratory waterfowl in central Mongolia is essential for monitoring AIVs that were maintained in nesting lakes in Siberia and that spread southward with their migration, especially if recent H5 NPAIVs in Asia and H7N9 AIVs in China were brought to the north.

In the present study, to monitor AIVs among wild bird populations in Mongolia, fresh duck fecal samples were collected during autumn surveillance from 2015 to 2019. In total, 167 AIVs were isolated from 5,000 fecal samples, and two H5N3, four H7N3, and two H7N7 viruses isolated in 2017 and 2019 were characterized. This study aimed to analyze H5 and H7 AIVs in Mongolia, genetically and antigenically to clarify relations with recent H5 and H7 AIVs, especially HPAIVs and A/Anhui/1/2013 (H7N9)-related low pathogenic avian influenza viruses (LPAIVs).

### Materials and methods

#### Isolation and identification of viruses

In each year, 1000 duck fecal samples were collected in the central region of Mongolia, including the Arkhangai Province (Ugii nuur, 47° 76’ N, 102° 74’ E; Doitiin tsagaan nuur, 47° 37’ N, 102° 31’ E; Durru tsagaan nuur, 49° 00’ N, 101° 12’ E; Tsagaan nuur, 48° 23’ N, 102° 35’ E; Alagzegstei nuur, 47° 37’ N, 102° 32’ E) and Bulgan Province (Khunt nuur 48° 25’ N, 102° 34’ E; Khunt rashaan nuur, 48° 27’ N, 102° 32’ E; Sharga nuur, 48° 55’ N, 101° 56’ E), annually from 2015 to 2019. Fecal samples were stored at a temperature of less than 10 °C and mixed with transport medium [minimal essential medium (Nissui Pharmaceutical, Tokyo, Japan) containing 10,000 U/ml penicillin G, 10 mg/ml streptomycin, 0.3 mg/ml gentamicin, and 0.5% bovine serum albumin] before virus isolation using embryonated chicken eggs. At first, all samples were inoculated into the allantoic cavity of 10-day-old chicken embryos and incubated for 48 h at 35 °C. After incubation, the infectious allantoic fluid was harvested, and the hemagglutination titer was determined using 0.5% chicken red blood cells. For further characterization, the subtypes of influenza viruses were identified via hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests using the reference antisera of AIVs [7].

#### Sequencing and phylogenetic analysis

For the genetic analysis, viral RNA was extracted from the allantoic fluid of infected chicken embryos using TRIzol LS Reagent (Life Technologies, Carlsbad, CA, USA). Viral RNA was reverse-transcribed using the Uni12 primer [9] and M-MLV reverse transcriptase (Life Technologies), then the full-length hemagglutinin (HA) and other internal gene segments were amplified via polymerase chain reaction using gene-specific primer sets [9]. Direct sequencing of gene segments was performed using a BigDye Terminator version 3.1 Cycle Sequencing Kit (Life Technologies) and a 3500 Genetic Analyzer (Life Technologies). For sequencing some isolates, next-generation sequencing was applied as follows. MiSeq libraries were prepared using KAPA RNA Hyper Prep Kit (Illumina, Inc., San Diego, CA, USA) and KAPA Dual-Indexed Adapter Kit (Roche, Basel, Switzerland). The prepared MiSeq libraries were sequenced on a MiSeq by using MiSeq Reagent kit v3 (Illumina, Inc.) with 2 × 300 bp paired-end read length. The sequencing data were analyzed using GENTYX Network version 12 (Genetyx Co., Tokyo, Japan), GeneStudio (https://genestudio.com/), or CLC Genomics Workbench 12 (Qiagen, Hilden, Germany). The gene sequences obtained in the present study have been registered at GenBank (Supplemental Table 1).

Phylogenetic analysis of H5 or H7 HA gene was performed by the maximum likelihood (ML) method using the Tamura–Nei model and bootstrap analysis (n = 1000) using MEGA7.0 software with default parameters [10]. The sequence data from two H5 HA and six H7 HA genes were compared with the reference sequences obtained from public databases, respectively. For the reference sequences, the nucleotide sequences of classical and recent H5 and H7 viruses were downloaded from GenBank/EMBL/DDBJ and Global Initiative on Sharing All Influenza Data (GISAID).
Antigenic analysis

The antigenic properties of representative H7Nx isolates were determined via the cross-HI test using chicken polyclonal antisera. Chicken polyclonal antisera against representative influenza virus strains were prepared, and HI tests were performed as previously described [11]. To visualize the antigenic character of the H7 viruses, the antigenic map was built using the web-based software (https://acmacs-web.antigenic-cartography.org/), and the test result containing cross-HI titers were uploaded to obtain x/y coordinates of each antiserum and antigen.

Results

Isolation of IAVs from the fecal samples of migratory waterfowl

The surveillance was targeted migratory waterfowl in four main lakes located in the Arkhangai and Bulgan Province of Mongolia. In total, 167 influenza viruses were isolated from 5000 fecal samples of migratory waterfowl (Table 1 and Supplemental Table 1); respectively, 40, 10, 21, 73, and 23 AIVs were isolated each autumn from 2015 to 2019. The isolation rate of AIVs for each autumn over 5 years was 1.0–7.3%, and the highest number of isolated viruses was observed in the Arkhangai Province. Among each sampling site, Doitiin Tsagaan nuur in the Arkhangai Province and Khunt nuur in the Bulgan Province were found to be the highest percentage of isolated virus during the overall surveillance time. The majority of HA subtypes of all isolates was H3 and H4.

In the present study, further characterization was conducted using two H5N3 and six H7Nx isolates. The two H5N3 isolates were A/duck/Mongolia/419/2019 and A/duck/Mongolia/926/2019 from the Arkhangai and Bulgan Provinces. Four H7N3 isolates were A/duck/Mongolia/652/2017, A/duck/Mongolia/751/2017, A/duck/Mongolia/782/2017, and A/duck/Mongolia/786/2017 collected in Bulgan Province. Two H7N7 isolates, namely A/duck/Mongolia/1/2019 and A/duck/Mongolia/6/2019, were collected in Arkhangai Province.

Genetic and phylogenetic analysis of H5 and H7 subtype viruses

The full-length HA gene sequences of the H5 and H7 isolates were characterized. The deduced amino acid sequences of the HA cleavage site of the two H5N3 isolates were PQRETR/GLF and PQREIR/GLF, indicating that they were LPAIVs. The HA receptor-binding site of the H5N3 isolates was analyzed, and residues at positions 226 and 228 were identified as Q and G, respectively, suggesting avian-type receptor specificity. None of the H5N3 subtype viruses contained amino acid substitutions E627K in PB2, which is the molecular marker for mammalian adaptation of AIVs and indicates increasing viral pathogenicity to mammals. Furthermore, the HA cleavage sites of the four H7N3 and two H7N7 isolates were analyzed, and the determined sequence...
was PELPKGR/GLF and PEIPKGR/GLF, respectively, suggesting that the viruses were LPAIVs. The HA receptor-binding site of H7Nx isolates was also analyzed, and HA proteins of isolates had residues of 226Q and 228G, indicating avian-type receptor specificity. Similarly, H7Nx LPAIVs did not involve a mutation for potential mammalian adaptation in PB2 protein. Taken together, these data indicate that all H5 and H7 isolates were LPAIVs without mammalian adaptation markers.

The HA gene of these isolates was phylogenetically analyzed by the ML method along with reference strains of HPAIVs and LPAIVs (Figs. 1 and 2). In this phylogenetic analysis, H5 HA genes were divided into two lineages: Eurasian and North American. The Eurasian lineage was clustered into two sub-lineages: Gs/Gd-like and non-Gs/Gd [12]. All H5N3 isolates from migratory waterfowl in Mongolia in 2019 belonged to the non-Gs/Gd sub-lineage that clustered with H5N3 subtype viruses recently reported in Asia and were distinct from Gs/Gd-like viruses. For other gene segments, each segment was classified together with multivariable subtype viruses circulating among wild birds (Supplemental Figs. S1–S5, S7, S8). Moreover, the H7 HA genes were phylogenetically divided into several lineages: Eurasian, Australian, Historical Europe, and North American. Eurasian lineage was further clustered into three sub-lineages: European–Asian, Far Eastern, and Chinese H7N9 [7]. All H7Nx isolates isolated in Mongolia in 2017 and 2019 belonged to the European–Asian
sub-lineage. Reference strains of Chinese H7N9 viruses were classified together with the Far Eastern sub-lineage; therefore, H7N3 and H7N7 isolates of migratory waterfowls had a different ancestor than that of Chinese H7N9 viruses. Other gene segments of H7N3 and H7N7 viruses were also classified with typical LPAIVs circulating in wild birds (Supplemental Figs. S1–S8) [13].

In phylogenetic analysis, the N3 and N7 neuraminidase (NA) genes were classified with Eurasian lineage viruses and had a genetic relationship with other variable N3 and N7 subtype viruses including AIVs isolated in Mongolia in 2010 and 2015. All other internal genes of H7N3 and H7N7 were phylogenetically closely related to recent H7 subtype viruses isolated from wild birds in Asia and Europe.

Antigenic analysis of H7Nx subtype viruses

Representative strains of H7 viruses from Mongolia and the panel of H7 viruses belonging to Eurasian and North American lineage were antigenically analyzed by the cross-HI test using their corresponding antisera (Table 2). HI titers of each antiserum against A/duck/Mongolia/652/2017 (H7N3) and A/duck/Mongolia/1/2019 (H7N7) were closely identical to those against previously isolated H7N2 viruses classified in the European–Asian sub-lineage [7]. The antigenic cartography was built from the cross-HI test which revealed that the present H7N3 and H7N7 strains were antigenically closely related to recent H7 viruses isolated from wild birds. These belonged to the major antigenic group and were distinct from H7 HPAIVs (Supplemental Fig. S9).
Discussion

H5 or H7 HPAIVs as well as LPAIVs are a global threat to livestock production, distribution systems, and human health. However, sustained, comprehensive, coordinated global efforts to monitor the continually changing genetic diversity of AIVs circulating in nature remain insufficient to predict the emergence of novel HPAIVs and human infections of AIVs. Wild aquatic birds have played a role in the evolution of H5 HPAIVs by reassortment with LPAIVs. These factors have contributed to the spread of these viruses to parts of Asia, Europe, Africa, and North America after an outbreak in wild birds at Qinghai Lake in China in 2005 [14]. Circulations of H7 LPAIVs in wild birds and poultry have been reported throughout the world [7, 13, 15–20]. In addition, H7N9 HPAIVs and LPAIVs are at risk to spread by wild bird and human-caused movements [21, 22]. In our surveillance conducted over the past 5 years, targeting migratory waterfowl in four different lakes in the central region of Mongolia, 167 viruses were isolated. No HPAIVs were isolated from the fecal samples of migratory waterfowl in the present study, indicating that the circulation of HPAIVs in the northern nesting lake is still limited. However, these do not fully represent the country state in terms of the infection of IAV in wild birds.

Two AIVs are currently pandemic threats; H5 HPAIVs have spilled over repeatedly to humans since their first identification in 1997, and H7N9 AIVs, initially detected in March 2013, have caused serious human infections across China [23]. In terms of H7N9 virus infection in humans, a total of 1568 laboratory-confirmed human cases have been reported since early 2013 [24]. A novel H7N9 HPAIV variant possessing multiple basic amino acids at the cleavage site of the HA protein was first reported in two cases of human infection in January 2017 [25]. H5N3 LPAIVs were detected from migratory waterfowl in Mongolia in 2019, and HA gene and other gene segments of those H5 isolates were phylogenetically classified in the Eurasian lineage, sharing identical genetic characteristics with recent H5 LPAIVs isolated from wild birds in Asia. Phylogenetic analysis of H7N3 and H7N7 isolates revealed a close relation to H7 LPAIVs belonging to the European–Asian sub-lineage, but no relation to Chinese H7N9 viruses.

Antigenicity monitoring is important for diagnosis and control measures for HPAIVs and LPAIVs. In the present study, representative H7 viruses were analyzed for antigenic characterization. Kim et al. reported the antigenic difference of viruses in Eurasian group I (referred to as the Far Eastern sub-lineage in this study) and group II (Eurasian–Asian sub-lineage) [15]. Our antigenic analyses revealed that the

| Lineage                  | Viruses                                      | Subtype | HI titers of the antiserum |
|--------------------------|----------------------------------------------|---------|-----------------------------|
|                         |                                              | H7N3    | Dk/Hok/W19/13               |
|                         |                                              |         | Ty/Italy/4580/99            |
|                         |                                              |         | Dk/Hok/Vac-2/04             |
|                         |                                              |         | Anhui/1/13                  |
|                         |                                              |         | Ck/NSW/327/97               |
|                         |                                              |         | Dk/TW/Ya103/93              |
|                         |                                              |         | SI/Mass/1/80                |
| Eurasian–Asian           | A/duck/Mongolia/652/2017                     | H7N7    | 10,240                      |
|                         | A/duck/Mongolia/1/2019                       |         | 1280                        |
|                         | A/duck/Hokkaido/W19/2013                     | H7N2    | 2560                        |
|                         | A/turkey/Italy/4580/1999                     | H7N1    | 320                         |
| Far Eastern              | A/duck/Hokkaido/Vac-2/2004                   | H7N7    | 20,480                      |
| Chinese H7N9             | A/Anhui/1/2013                               | H7N9    | 5120                        |
| Australian               | A/chicken/New South Wales/327/1997           | H7N4    | 5120                        |
| Historical Europe        | A/duck/Taiwan/Ya103/1993                     | H7N7    | 320                         |
| North American           | A/seal/Massachusetts/1/1980                   | H7N7    | 10,240                      |

Viruses isolated in this study are shown in bold. Homologous titers are indicated with Italic.

Dk duck, Ty turkey, Ck chicken, Sl seal, Hok Hokkaido, NSW New South Wales, TW Taiwan, Mass Massachusetts
antigenicity of H7 viruses isolated from migratory waterfowl was similar to each other regardless of genetic diversity, and antigenic diversity may emerge after the adaptation of H7 viruses to poultry, same as has been reported for North American H7 viruses [19].

Because of the recent increased numbers of zoonotic infections in poultry and human infections in China, influenza A virus (H7N9) has remained a public health threat. It is possible that wild birds infected with Chinese H7N9 viruses transmit them to surrounding countries, as occurs with H5 HPAIVs. Continued circulation of H7 influenza viruses among poultry and wild birds risks widespread dissemination of these viruses. Accordingly, it is important that H5 and H7 viruses are continually monitored among wild waterfowl.

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Author contributions AU and EB performed genetic analyses and antigenic analyses. AU and EB prepared this manuscript. AU, EB, TH, KB, TA, JT, TU, KhB, KM, TH, TK, MS, YT, ST, MI, KO, TS, NK, YK, JM, MI, and AT conducted sampling of feces from migratory wild birds in Mongolia and performed virus isolation and subtyping. MO, KM, AT, HK, DB, and YS provided laboratory management support and manuscript editing.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Machalaba CC, Elwood SE, Forcella S, Smith KM, Hamilton K, Jebara KB, Swayne DE, Webby RJ, Mumford E, Mazet JA, Gaidet N, Dusazk P, Karesh WB (2015) Global avian influenza surveillance in wild birds: a strategy to capture viral diversity. Emerg Infect Dis 21:1–7. https://doi.org/10.3201/eid2104.141415
2. OIE website (2019) Update on highly pathogenic avian influenza in animals (Types H5 and H7). https://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/
3. Xu X, Subbarao CNJ, Guo Y (1999) Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. Virology 261:15–19. https://doi.org/10.1006/viro.1999.9820
4. Rose K, Newman S, Uhart M, Lubroth J (2006) Wild bird highly pathogenic avian influenza surveillance: Sample collection from healthy, sick and dead birds. FAO Animal Production and Health Manual No.4. https://www.fao.org/3/a-a0960e.pdf. Accessed 20 May 2019
5. Kida H, Yanagawa R, Matsuoka Y (1980) Duck influenza lacking evidence of disease signs and immune response. Infect Immun 30:547–553
6. Lycett SJ, Bodewes R, Pohlmann A, Banks J, Banyai K, Boni MF, Bouwstra R, Breed AC, Brown IH, Chen HL, Dan A, DeLiberto TJ, Diep N, Gilbert M, Hill S, Ip HS, Ke CW, Kida H, Killian ML, Koopmans MP, Kwon JH, Lee DH, Lee YJ, Lu L, Monne I, Passick J, Pybus OG, Rambaut A, Robinson TP, Sakoda Y, Zohari S, Song CS, Swayne DE, Torchetti MK, Tsai HJ, Foucheur RAM, Beer M, Woolhouse M, Kuiken T (2016) Role for migratory wild birds in the global spread of avian influenza H5N8. Science 354:213–217. https://doi.org/10.1126/science.aaf8852
7. Hiono T, Ohkawara A, Ogasawara K, Okamatsu M, Tamura T, Chu DH, Suzuki M, Kuribayashi S, Shichinoho S, Takada A, Ogawa H, Yoshida R, Miyamoto H, Nao N, Furuyama W, Maruyama J, Eguchi N, Uzilizat G, Enkhbold B, Shatar M, Jargalsaihan T, Byambadorj S, Damdjinjav B, Sakoda Y, Kida H (2015) Genetic and antigenic characterization of H5 and H7 influenza viruses isolated from migratory water birds in Hokkaido, Japan and Mongolia from 2010 to 2014. Virus Genes 51:57–68. https://doi.org/10.1007/s11262-015-1214-9
8. Sakoda Y, Sugar S, Batchuluun D, Erdene-Ochir TO, Okamatsu M, Isoda S, Soda K, Takakuwu H, Tsuda Y, Yamamoto N, Kishida N, Matsuno K, Nakayama E, Kajihara M, Yokoyama A, Takada A, Sodnomdalraa J, Kida H (2010) Characterization of H5N1 highly pathogenic avian influenza virus strains isolated from migratory waterfowl in Mongolia on the way back from the southern Asia to their northern territory. Virology 406:88–94. https://doi.org/10.1016/j.virology.2010.07.007
9. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR (2001) Universal primer set for the full-length amplification of all influenza A viruses. Arch Virol 146:2275–2289. https://doi.org/10.1007/s11260-001-002
10. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054
11. Sakabe S, Sakoda Y, Haraguschi Y, Isoda N, Soda K, Takakuwu H, Saijo K, Sawata A, Kume K, Hagiwara J, Tuchiya K, Lin Z, Sakamoto R, Imamura T, Sasaki T, Kokumai N, Kawaoka Y, Kida H (2008) A vaccine prepared from a non-pathogenic H7N7 virus isolated from natural reservoir conferred protective immunity against the challenge with lethal dose of highly pathogenic avian influenza virus in chickens. Vaccine 26:2127–2134. https://doi.org/10.1016/j.vaccine.2008.02.001
12. Nguyen LT, Firestone SM, Stevenson MA, Young ND, Sims LD, Chu DH, Nguyen TN, Nguyen LV, Le TT, Nguyen HV, Nguyen HN, Tien TN, Nguyen TD, Tran BN, Matsuno K, Okamatsu M, Kida H, Sakoda Y (2019) A systematic study towards evolutionary and epidemiological dynamics of currently predominant H5 highly pathogenic avian influenza viruses in Vietnam. Sci Rep 9:1–13. https://doi.org/10.1038/s41598-019-42638-4
13. Le KT, Okamatsu M, Nguyen LT, Matsuno K, Chu DH, Tien TN, Le TT, Kida H, Sakoda Y (2020) Genetic and antigenic characterization of the first H7N7 low pathogenic avian influenza viruses isolated in Vietnam. Infect Genet Evol 78:1–8. https://doi.org/10.1016/j.megid.2019.104117
14. Liu J, Xiao H, Lei F, Zhu Q, Qin K, Zhang XW, Zhang XL, Zhao D, Wang G, Feng Y, Ma J, Liu W, Wang J, Gao GF (2005) Highly pathogenic H5N1 influenza virus infection in migratory birds. Science 309(1206):1–2. https://doi.org/10.1126/science.1115273
15. Kim YI, Kim SW, Si YJ, Kwon HI, Park SJ, Kim EH, Kim SM, Lee IW, Song MS, Choi YK (2016) Genetic diversity and pathogenic potential of low pathogenic H7 avian influenza viruses isolated from wild migratory birds in Korea. Infect Genet Evol 45:268–284. https://doi.org/10.1016/j.meegid.2016.09.005

16. Kim HR, Park CK, Lee YJ, Oem JK, Kang HM, Choi JG, Lee OS, Bae YC (2012) Low pathogenic H7 subtype avian influenza viruses isolated from domestic ducks in South Korea and the close association with isolates of wild birds. J Gen Virol 93:1278–1287. https://doi.org/10.1099/vir.0.041209-0

17. Liu H, Xiong C, Chen J, Chen G, Zhang J, Li Y, Xiong Y, Wang R, Cao Y, Chen Q, Liu D, Wang H, Chen J (2018) Two genetically diverse H7N7 avian influenza viruses isolated from migratory birds in central China. Emerg Microb Infect 7(62):1–12. https://doi.org/10.1038/s41426-018-0064-7

18. Yao Y, Xu CL, Shi JH, Zhu Y, Li YF, Bai T, Li FC, Cai T, Yuan F, Chen T, Yang H, Li WC, Zhang HJ, Zhang H, Shuy L (2015) Phylogenetic and molecular analysis of an H7N7 avian influenza virus isolated in east dongting lake in 2012. Biomed Environ Sci 28:518–526. https://doi.org/10.3967/bes2015.074

19. Xu Y, Bailey E, Spackman E, Li T, Wang H, Long LP, Baroch JA, Cunningham FL, Lin X, Jarman RG, Deliberto TJ, Wan XF (2016) Limited antigenic diversity in contemporary H7 avian-origin influenza A viruses from North America. Sci Rep 6(20688):1–17. https://doi.org/10.1038/srep20688

20. Suttie A, Yann S, Phalla Y, Tum S, Deng YM, Hul V, Horn VS, Barr I, Greenhill A, Horwood PF, Osbjerg K, Karlsson EA, Dussart P (2018) Detection of low pathogenicity influenza A(H7N3) virus during duck mortality event, Cambodia, 2017. Emerg Infect Dis 24:1103–1107. https://doi.org/10.3201/eid2406.170209

21. Shibata A, Okamatsu M, Sumiyoshi R, Matsuno K, Wang ZJ, Kida H, Osaka H, Sakoda Y (2018) Repeated detection of H7N9 avian influenza viruses in raw poultry meat illegally brought to Japan by international flight passengers. Virology 524:10–17. https://doi.org/10.1016/j.virol.2018.08.001

22. Shibata A, Harada R, Okamatsu M, Matsuno K, Arita T, Suzuki Y, Shirakura M, Odagiri T, Takemae N, Uchida Y, Saito T, Sakoda Y, Osaka H (2019) Characterization of a novel reassortant H7N3 highly pathogenic avian influenza virus isolated from a poultry meat product taken on a passenger flight to Japan. J Vet Med Sci 81:444–448. https://doi.org/10.1292/jvms.18-0628

23. Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, Chen J, Jie Z, Qiu H, Xu K, Xu X, Lu H, Zhu W, Gao Z, Xiang N, Shen Y, He Z, Gu Y, Zhang Z, Yang Y, Zhao X, Zhou L, Li X, Zou S, Zhang Y, Li X, Yang L, Guo J, Dong J, Li Q, Dong L, Zhu Y, Bai T, Wang S, Hao P, Yang W, Zhang Y, Han J, Yu H, Li D, Gao GF, Wu G, Wang Y, Yuan Z, Shuy L (2013) Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med 368:1888–1897. https://doi.org/10.1056/NEJMoa1304459

24. World Health Organization (2019) Human infection with avian influenza A(H7N9) virus. https://www.who.int/csr/don/05-september-2018-ah7n9-china/en/. Accessed 23 May 2019

25. Ke C, Mok CKP, Zhu W, Zhou H, He J, Guan W, Wu J, Song W, Wang D, Liu J, Lin Q, Chu DKW, Yang L, Zhong N, Yang Z, Shuy P, Peiris JSM (2017) Human infection with highly pathogenic avian influenza A(H7N9) virus, China. Emerg Infect Dis 23:1332–1340. https://doi.org/10.3201/eid2308.170600

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