Poultry Infection with Influenza Viruses of Wild Bird Origin, China, 2016

Zhijun Yu,[1] Kaihui Cheng,[1] Yuwei Gao[1]

Author affiliations: Institute of Poultry Science of Shandong Academy of Agricultural Sciences, Jinan, China (Z. Yu); Shandong Academy of Agricultural Sciences, Jinan (Z. Yu, K. Cheng); Dairy Cattle Research Center of Shandong Academy of Agricultural Sciences, Jinan (K. Cheng); Military Veterinary Research Institute of Academy of Military Medical Sciences, Changchun, China (Y. Gao)

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Migratory birds may play a role in transmission of avian influenza virus. We report the infection of black-tailed gulls and chickens in eastern China with avian influenza (H13N2) and (H13N8) viruses. We found that these H13 viruses were transmitted from migratory birds to domestic poultry.

Avian influenza virus with 10 hemagglutinin (HA) subtypes has emerged in poultry (*1*), and the potential role of migratory birds in transmission of avian influenza virus has caused concern (*2*). We report infection with low pathogenicity avian influenza (LPAI) virus of HA subtype 13 (H13) among migratory birds (black-tailed gulls [*Larus crassirostris*]) and domestic poultry (chickens) in Weihai, Shandong Province, eastern China (online Technical Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/24/7/17-1220-Techapp1.pdf).

Weihai is a breeding center for black-tailed gulls that congregate from northern Asia, eastern Asia, Southeast Asia, and North America. These gulls reside along the coastlines of the East China Sea and Japan and have been found as vagrants in Alaska, North America, and the Philippines (*3*). In China, black-tailed gulls perch at the Longxudao wharf (37°23′24.05″N, 122°41′26.16′E), located in the northeastern corner of Weihai. In December 2016, we collected 149 fecal samples from black-tailed gulls at Longxudao wharf and screened them for evidence of influenza virus by reverse transcription PCR, DNA sequencing, and BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) analysis in the GenBank database. After independently inoculating positive fecal samples into the allantoic cavities of specific pathogen-free embryonated chicken eggs, we obtained 6 influenza H13N2 and 60 influenza H13N8 virus isolates.

To assess the epidemiologic characteristics of these H13 isolates, we completely sequenced an H13N2 isolate (A/black-tailed gull/Weihai/115/2016) and an H13N8 isolate (A/black-tailed gull/Weihai/17/2016) (GenBank accession nos. MF461177–92). Phylogenetic analysis indicated that their HA and neuraminidase (NA) segments were derived from the Eurasian lineage, in accordance with their geographic distribution (online Technical Appendix Figures 2, 3). Moreover, the H13N2 and H13N8 isolates possessed high nucleotide sequence identity to the avian influenza virus subtypes previously isolated from Europe, Asia, and North America (online Technical Appendix Table 1). We speculate that avian influenza virus subtypes H13N2 and H13N8 are reassortants between the Eurasian and North American lineages (online Technical Appendix Figures 4, 5).

We next analyzed the timing of the reassortment events that led to the emergence of subtype H13N2 (Figure, panel A). During July 2009, June 2012, July 2009, and June 2015, the following genes, respectively, were transferred from seagulls in Europe: HA, nucleocapsid protein (NP), matrix (M), and nonstructural (NS). During 2004, November 2011, and October 2014, the following genes, respectively, originated from waterfowl in Asia: polymerase basic (PB) 1, polymerase acidic (PA), and NA. In November 2007, the PB2 gene was transferred from avian influenza viruses circulating among wild waterfowl in North America.

We also estimated the timing of the reassortment events that led to the emergence of subtype H13N8 (Figure, panel B). During June 2012, July 2013, July 2013, and June 2015, the following genes, respectively, were transferred from seagulls in Europe: NP, NA, M, and NS. During 2004, November 2011, and September 2012, the following genes, respectively, originated from waterfowl in Asia: PB1, PA, and HA. In November 2007, the PB2 gene was transferred from avian influenza viruses circulating among wild waterfowl in North America.

According to these data, the generation of influenza virus subtypes H13N2 and H13N8 in seagulls seems to have been a complex process and was probably completed in the middle of 2015 (Figure). We also found that subtypes H13N2 and H13N8 possessed some molecular markers associated with increased virulence and transmission among mammals (online Technical Appendix Table 2).

In April 2017, we analyzed serum samples collected from 48 chickens at a chicken farm at Songcun town (37°04′39.96″N, 122°00′38.83″E) in Weihai for serologic evidence of exposure to H13 viruses. We found detectable hemagglutinin inhibition (HI) antibody titers against H13N2 virus in 4 (8.3%) samples and detectable HI antibody titers against H13N8 virus in 14 (29.2%) samples (online Technical Appendix Table 3). When we evaluated reference serum samples known to contain HI antibodies against each of the virus subtypes for potential cross-reactivity, we observed no apparent cross-reactivity of H13

[1]All authors contributed equally to this article.

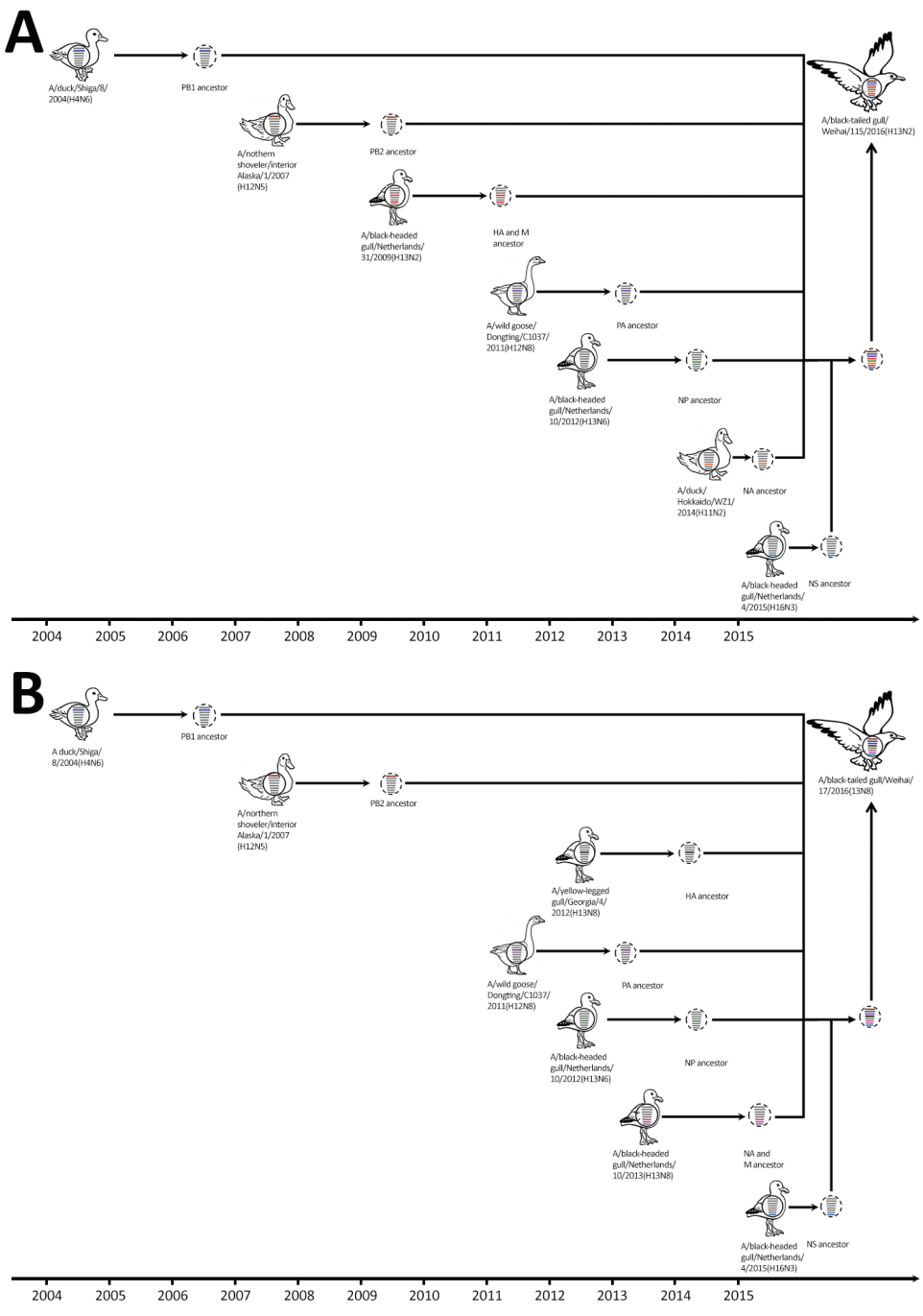

Figure. Hypothetical evolutionary pathway of avian influenza viruses of H13N2 (A) and H13N8 virus (B) subtypes isolated from black-tailed gulls in eastern China, 2016. Dashed virions indicate unidentified viruses. HA, hemagglutinin; M, matrix protein; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, polymerase acidic protein; PB, polymerase basic protein. A color version of this figure showing gene segments origins is available online (https://wwwnc.cdc.gov/EID/article/24/7/17-1220-F1.htm).

antibodies against 7 other HA subtype viruses (online Technical Appendix Table 6). Therefore, although the serum samples' HI antibody titers against H13 viruses were not high, we cannot exclude the possibility that these antibodies were generated in response to independent exposure to H13 viruses.

In March 2013, the novel LPAI H7N9 virus causing serious human infections was detected in eastern China (*4*–*7*); after circulating among domestic poultry, this virus evolved into a highly pathogenic virus (*8*). Therefore, enhanced surveillance is needed to determine whether other LPAI viruses could be introduced into domestic poultry and pose a threat to public health.

In this study, we isolated a large number of LPAI H13 viruses from seagulls at the Longxudao wharf and detected H13-specific seroconversion in chickens at a chicken farm, which is ≈100 km west of this wharf and lies on the migratory route of black-tailed gulls. These findings indicate

that H13 viruses may have been introduced into domestic poultry from migratory birds and that they may have the potential to become a global cross-species threat.

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About the Author

Dr. Yu is a chief of the Monitoring and Early Warning Platform for Cross-species and Virus-induced Diseases in Livestock and Poultry at the Institute of Poultry Science, Shandong Academy of Agricultural Sciences, Jinan, China. His research interests include the epidemiology and evolutionary mechanisms of influenza viruses, the pathogenesis of influenza viruses, and the innate host antiviral immune response.

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Address for correspondence: Zhijun Yu, Shandong Academy of Agricultural Sciences, Institute of Poultry Science, No. 1 Jiaoxiao Rd, Jinan, 250023, China; email: zhijun0215@gmail.com

Rat-Bite Fever in Human with *Streptobacillus notomytis* Infection, Japan

Yoshihiko Ogawa, Kei Kasahara, Sang-Tae Lee, Takamitsu Ito, Hideo Hasegawa, Sachie Hirose, Shigeru Santo, Atsushi Yoshida, Ryuichi Nakano, Hisakazu Yano, Keiichi Mikasa

Author affiliations: Nara Medical University, Nara, Japan (Y. Ogawa, K. Kasahara, S.T. Lee, (R. Nakano, H. Yano) K. Mikasa); Osaka Gyoumeikan Hospital, Osaka, Japan (T. Ito, H. Hasegawa, S. Hirose, S. Santo, A. Yoshida)

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We report a case of rat-bite fever in a 94-year-old woman with *Streptobacillus notomytis* infection. We established an epidemiologic link between exposure to rats and human infection by performing nested PCRs that detected *S. notomytis* in the intraoral swab specimens obtained from rats captured in the patient's house.

Streptobacillus is a genus of gram-negative, filamentous, rod-shaped bacilli belonging to the family *Leptotrichiaceae*. Since 2014, four novel species other than *S. moniliformis* have been reported: *S. hongkongensis* was isolated from 2 human patients, *S. felis* from the lung of a cat, *S. ratti* from black rats, and *S. notomytis* from a spinifex hopping mouse (*1–4*). We report a case of a human infection with *S. notomytis*.

A 94-year-old woman sought treatment at our hospital for general malaise, anorexia, and bilateral knee pain. At admission, her body temperature was 38°C; physical examination revealed swelling in both knees. Her skin was intact, with no rashes or animal bites. Laboratory tests revealed high leukocyte count (1.42×10^9 cells/L) and elevated level of C-reactive protein (19.5 mg/dL).

Bilateral knee arthrocentesis yielded 25 mL of purulent fluid; Gram stain demonstrated the presence of few, thin, gram-negative bacilli with pyrophosphate calcium crystals and neutrophils (Figure). Bacterial culture yielded transparent, small, smooth colonies on 5% sheep blood agar (Kyokuto, Tokyo, Japan) incubated at 37°C under 5% CO_2 for 48 h. However, the automated bacterial identification method (Vitek 2; bioMérieux, Tokyo, Japan) failed to identify the isolate. We evaluated the isolate (NR2245) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using Bruker MALDI BioTyper software version 4.001 library database (Bruker Daltonik GmbH, Bremen, Germany) employing ethanol–formic acid extraction. We identified the isolate as *S. moniliformis*

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Technical Appendix

Detailed Methods

Sample Collection

In the northeast corner of Weihai City is located the Longxudao wharf (GPS position: N37°23′24.05," E122°41′26.16") which a large number of black-tailed gulls perch at. A lot of trawlers moored alongside the longxudao wharf after the fishing, and a small amount of seafood was left at the wharf after the unloading process of the trawlers. The black-tailed gulls ate the seafood, and the feces of these seabirds were left at the longxudao wharf. In December 2016, we picked up one hundred and forty-nine feces specimens of black-tailed gulls at the Longxudao wharf.

RNA Isolation, RT-PCR Amplification, Sequencing, BLAST, and Virus Isolation

All one hundred and forty-nine feces specimens of black-tailed gulls were homogenized in 800μl PBS supplemented with 2000 IU/ml penicillin and 2000 mg/ml streptomycin. The disrupted feces specimens were centrifuged to remove debris and the feces supernatants were used for further study. We screened all feces supernatants by RT-PCR, sequencing, and BLAST for evidence of influenza virus infection. Viral RNA was isolated from the feces supernatants using the RNeasy Mini kit (QIAGEN, Germantown, MD) according to the manufacturer's protocol. Reverse transcription of viral RNA and subsequent PCR were performed using primers specific for H13, H16, N2, N3, N6, and N8 (Technical Appendix Table 4). All positive PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Germantown, MD) according to the manufacturer's protocol and were sequenced by the Tsingke Genomics Institute (Qingdao, China). Sanger sequencing methodology was used to sequence the PCR-amplified viral gene segments. DNA sequences were analyzed using the Lasergene sequence analysis software package (DNASTAR, Madison, WI). There are sixty feces supernatants identified as

H13N8 positive by RT-PCR, subsequent DNA sequencing, and BLAST analysis in the GenBank database (GenBank database URL: https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM = blastn&PAGE_TYPE = BlastSearch&LINK_LOC = blasthome). Moreover, there are other six feces supernatants identified as H13N2 positive by RT-PCR, subsequent DNA sequencing, and BLAST analysis in the GenBank database. Furthermore, all sixty-six AIV-positive feces supernatants were independently inoculated in the allantoic cavities of 10-day-old specific pathogen-free embryonated chicken eggs to isolate viruses. After 72-hour incubation at 37°C, we recovered six H13N2 virus isolates and sixty H13N8 virus isolates. Additionally, serum samples collected from 48 chickens residing in a chicken farm at Songcun town (GPS position: N37°04′39.96″, E122°00′38.83″) in Weihai city were analyzed for serologic evidence of H13N2 and H13N8 AIV exposure.

Genome Sequencing

The viral RNA of A/black-tailed gull/Weihai/115/2016 (H13N2) or A/black-tailed gull/Weihai/17/2016 (H13N8) was independently isolated from the allantoic fluid of inoculated eggs using the RNeasy Mini kit (QIAGEN, Germantown, MD) according to the manufacturer's protocol. Reverse transcription of viral RNA and subsequent PCR were performed using primers specific for each gene segment (Technical Appendix Table 4 and Technical Appendix Table 5). PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Germantown, MD) according to the manufacturer's protocol. Viral gene segments were sequenced by the Tsingke Genomics Institute (Qingdao, China). The GenBank accession numbers of A/black-tailed gull/Weihai/115/2016 (H13N2) and A/black-tailed gull/Weihai/17/2016 (H13N8) are MF461177 to MF461192.

Phylogenetic Analysis

To investigate the molecular and epidemiologic characteristics and to determine the profile of genetic diversity, phylogenetic trees were constructed using molecular evolutionary genetics analysis MEGA 7 (http://www.megasoftware.net/mega.php) with the neighbor-joining (NJ) method to calculate distance.

Hemagglutination Inhibition (HI) Assay

HI tests were performed as previously described (*1*,*2*). Briefly, serum samples were treated with receptor-destroying enzyme (RDE; Sigma-Aldrich, St. Louis, MO, USA; 1 part

serum:4 parts RDE) overnight at 37°C to remove nonspecific inhibitors before analysis. Five parts of 1.5% sodium citrate were added and the samples heat-inactivated at 56°C for 30 min. Serum samples were then serially diluted 2-fold in phosphate buffered saline (PBS). Twenty-five µl of the undiluted and serially diluted serum was mixed with 25µl of test virus containing four hemagglutination units in each well of a microplate and incubated at room temperature for 30 min. Then 50µl of 0.5% chicken erythrocytes was added to each well. Results were read after incubation at room temperature for 30 min. HI antibody titers were defined as the reciprocal of the highest serum dilution that prevented virus-mediated hemagglutination of the chicken erythrocytes.

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Technical Appendix Table 1. Identification of viruses possessing gene segments with the highest nucleotide identity to each segment of A/black-tailed gull/Weihai/115/2016 (H13N2) and A/black-tailed gull/Weihai/17/2016 (H13N8) based on sequences available in GenBank*

Virus, gene	Virus with the highest percentage of nucleotide identity	GenBank accession no.	Identity, %
A/black-tailed gull/Weihai/115/2016 (H13N2)			
PB2	A/northern shoveler/Interior Alaska/1/2007(H12N5)	CY038351.1	94.2
PB1	A/duck/Shiga/8/2004(H4N6)	AB304145.1	96.6
PA	A/wild goose/Dongting/C1037/2011(H12N8)	KC876690.1	97.7
HA	A/black-headed gull/Netherlands/31/2009(H13N2)	KX979380.1	97.1
NP	A/black-headed gull/Netherlands/10/2012(H13N6)	MF148105.1	98.8
NA	A/duck/Hokkaido/WZ1/2014(H11N2)	LC042067.1	98.8
M	A/black-headed gull/Netherlands/31/2009(H13N2)	KX979549.1	98.1
NS	A/black-headed gull/Netherlands/4/2015(H16N3)	KX978185.1	99.2
A/black-tailed gull/Weihai/17/2016 (H13N8)			
PB2	A/northern shoveler/Interior Alaska/1/2007(H12N5)	CY038351.1	94.8
PB1	A/duck/Shiga/8/2004(H4N6)	AB304145.1	96.6
PA	A/wild goose/Dongting/C1037/2011(H12N8)	KC876690.1	97.6
HA	A/yellow-legged gull/Georgia/4/2012(H13N8)	MF147792.1	97.6
NP	A/black-headed gull/Netherlands/10/2012(H13N6))	MF148105.1	98.9
NA	A/black-headed gull/Netherlands/10/2013(H13N8)	KX978567.1	96.9
M	A/black-headed gull/Netherlands/10/2013(H13N8)	KX979140.1	99.3
NS	A/black-headed gull/Netherlands/4/2015(H16N3)	KX978185.1	99.2

*HA, hemagglutinin; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, acidic polymerase PB, basic polymerase.

Technical Appendix Table 2. Analysis of molecular features associated with mammalian virulence, transmissibility, and antiviral resistance*

Protein	Molecular feature or amino acid substitution†	Phenotypic effect	A/black-tailed gull/Weihai/115/2016 (H13N2)	A/black-tailed gull/Weihai/17/2016 (H13N8)
PB2	E158G	Enhanced polymerase activity and increased virulence	E	E
	E627K	Enhanced polymerase activity and increased virulence	E	E
	D701N	Enhanced polymerase activity and increased virulence	D	D
PB1	H99Y	Associated with H5 transmissibility in ferrets	H	H
	I368V	Associated with H5 transmissibility in ferrets	I	I
PA	T97I	Enhanced polymerase activity and increased virulence	T	T
HA	Multibasic cleavage site	Expanded viral tropism; increased virulence in mice	Absent	Absent
	H107Y	Associated with H5 transmissibility in ferrets	H	H
	T160A	Associated with H5 transmissibility in ferrets	K	T
	Q226L	Human-type receptor binding; associated with H5 transmissibility in ferrets	Q	Q
	G228S	Human-type receptor binding; associated with H5 transmissibility in ferrets	S	S
NA	Stalk deletion	Increased virulence in mice	Absent; no deletion	Absent; no deletion
	H274Y	Oseltamivir resistance	H	H
	N294S	Oseltamivir resistance	N	N
M1	N30D	Increased virulence in mice	D	D
	T215A	Increased virulence in mice	A	A
M2	S31N	Amantadine resistance	S	S
NS1	P42S	Increased virulence in mice	S	S

*Single letters refer to the amino acid (aa) found in the noted gene at a specific site. HA, hemagglutinin; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, acidic polymerase PB, basic polymerase. Underlining indicates molecular features that were associated with virulence in mammals or transmissibility of influenza virus and found in H13 isolates in this study.
†Sites are numbered from M, the start codon.

Technical Appendix Table 3. Chicken serum antibodies against subtype H13N2 virus A/black-tailed gull/Weihai/115/2016 (H13N2) or subtype H13N8 virus A/black-tailed gull/Weihai/17/2016 (H13N8)

Sample no.	HI titers against viruses*	
	H13N2	H13N8
3	10	<10
9	10	<10
12	10	<10
30	10	<10
1	<10	10
4	<10	20
7	<10	20
11	<10	20
18	<10	20
21	<10	20
22	<10	20
23	<10	20
24	<10	20
29	<10	20
34	<10	10
36	<10	20
46	<10	10
47	<10	10

*Only display the HI results of HI-positive serum samples against subtype H13N2 or H13N8 virus.

Technical Appendix Table 4. Primer pairs used for the PCR amplification of the HA and NA gene segment

HA or NA subtype	Primers (5′→3′)
H13	H13-F: AGCAAAAGCAGGGGAGAATTC
	H13-R: AGTAGAAACAAGGGTGTTTTTTCTGC
H16	H16-F: AGCAAAAGCAGGGGATATTGTC
	H16-R: AGTAGAAACAAGGGTTTTTTTCCG
N2	N2-F: AGCAAAAGCAGGAGTGAAAAT
	N2-R: AGTAGAAACAAGGAGTTTTTTCTAA
N3	N3-F: AGCAAAAGCAGGTGTGAAAT
	N3-R: AGTAGAAACAAGGTGTTTTTTCTAT
N6	N6-F: AGCAAAAGCAGGGTGAC
	N6-R: AGTAGAAACAAGGGTGTTTTTC
N8	N8-F: AGCAAAAGCAGGAGTTTAAAAT
	N8-R: AGTAGAAACAAGGAGTTTTTTCGT

Technical Appendix Table 5. Primer pairs used for the PCR amplification of the PB2, PB1, PA, NP, M, and NS gene segment of H13N2 and H13N6 viruses

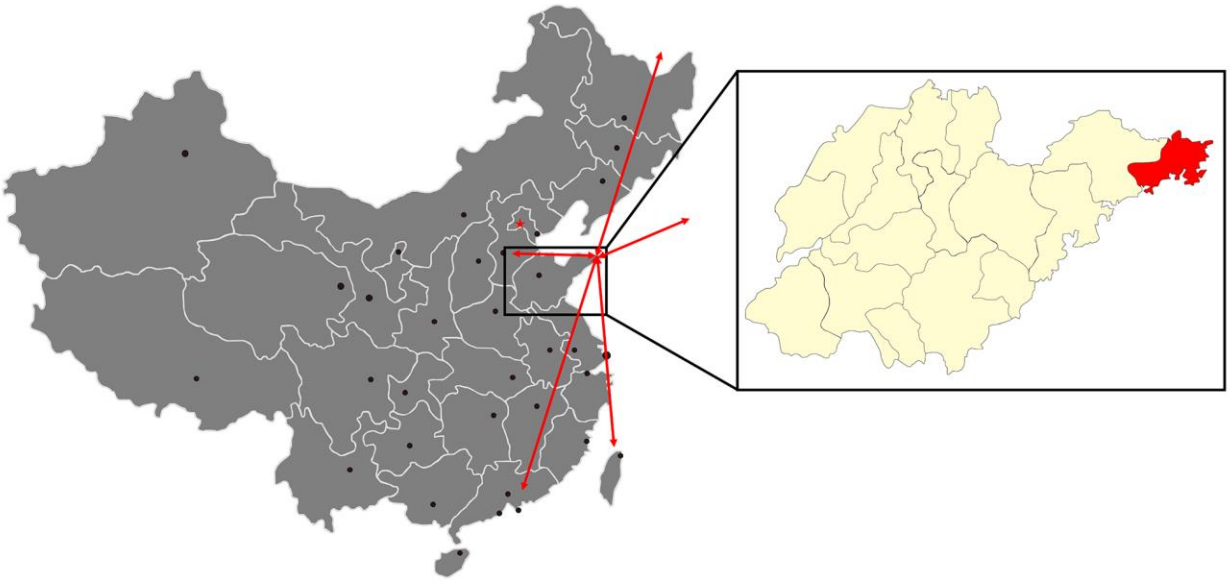
Gene segment	Primers (5′→3′)
PB2	H13-PB2-F: AGCAAAAGCAGGTCAAATATAT
	H13-PB2-R: AGTAGAAACAAGGTCGTTTTTAA
PB1	H13-PB1-F: AGCAAAAGCAGGCAAACCAT
	H13-PB1-R: AGTAGAAACAAGGCATTTTTTCATG
PA	H13-PA-F: AGCAAAAGCAGGTACTGATC
	H13-PA-R: AGTAGAAACAAGGTACTTTTTTGG
NP	H13-NP-F: AGCAAAAGCAGGGTAGATAATC
	H13-NP-R: AGTAGAAACAAGGGTATTTTTCTTC
M	H13-M-F: AGCAAAAGCAGGTAGATA
	H13-M-R: AGTAGAAACAAGGTAGTTTTT
NS	H13-NS-F: AGCAAAAGCAGGGTGACAA
	H13-NS-R: AGTAGAAACAAGGGTGTTTTTTATC

Technical Appendix Table 6. Hemagglutination inhibition antibody titers of reference serum sample against influenza viruses of different subtypes*

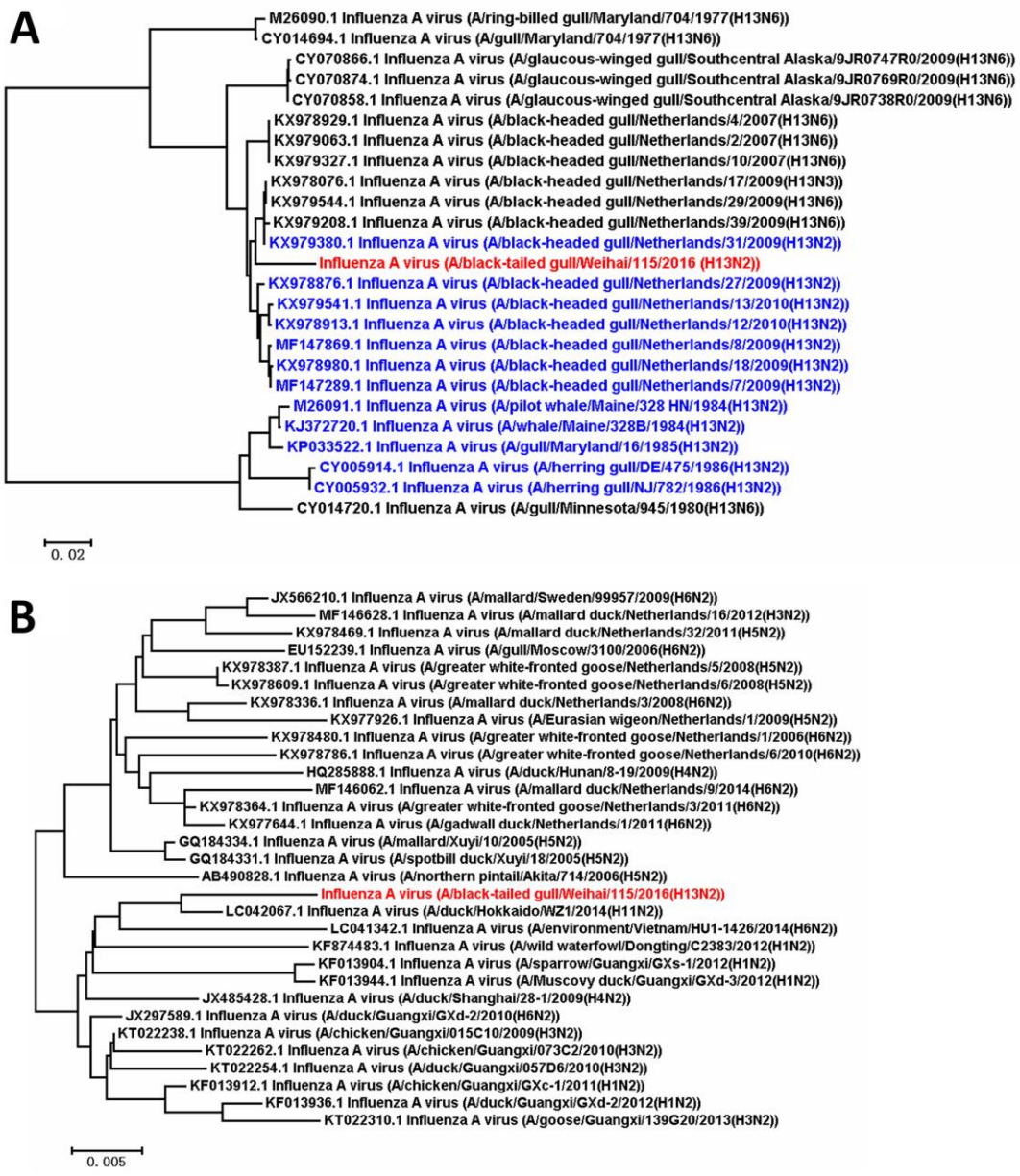
Antigens†	H1	H3	H4	H5	H6	H7	H9	H13N2	H13N8
H1	160	<10	<10	<10	<10	<10	<10	<10	<10
H3	<10	160	<10	<10	<10	<10	<10	<10	<10
H4	<10	<10	160	<10	<10	<10	<10	<10	<10
H5	<10	<10	<10	80	<10	<10	<10	<10	<10
H6	<10	<10	<10	<10	80	<10	<10	<10	<10
H7	<10	<10	<10	<10	<10	80	<10	<10	<10
H9	<10	<10	<10	<10	<10	<10	80	<10	<10
H13N2	<10	<10	<10	<10	<10	<10	<10	80	<10
H13N8	<10	<10	<10	<10	<10	<10	<10	<10	80

*Nine reference serum samples were used in hemagglutination inhibiting serum antibody titers tests. The reference serum samples for H1, H3, H4, H5, H6, H7, and H9 subtype avian influenza viruses were collected from specific pathogen-free chickens and provided by the Military Veterinary Research Institute of Academy of Military Medical Sciences. The reference serum samples for H13N2 and H13N8 subtype avian influenza viruses were collected from specific pathogen-free chickens by our laboratory.

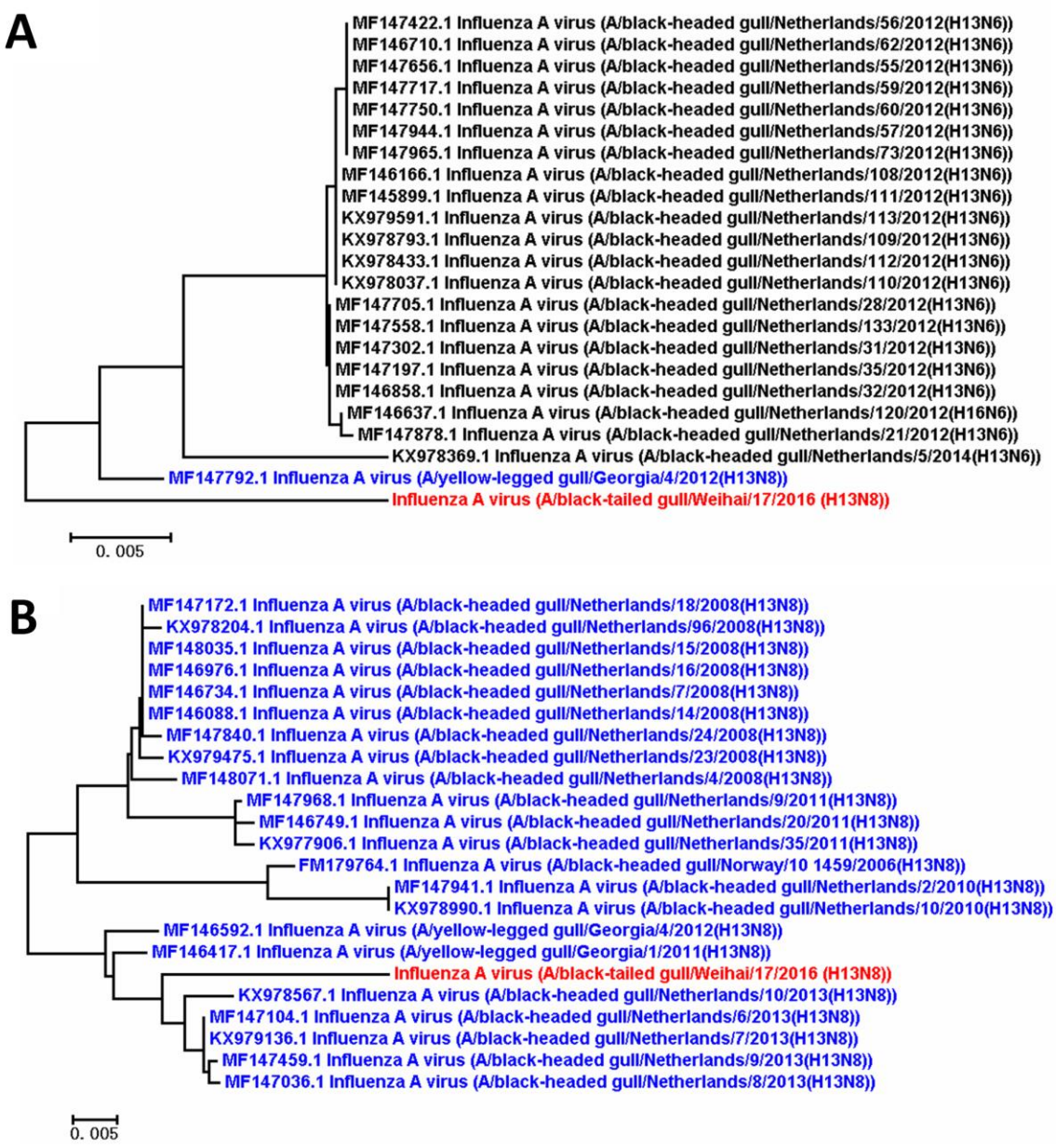
†Nine influenza virus subtypes were used in hemagglutination inhibiting serum antibody titers tests: H1 (A/black-necked crane/Zhaotong/ZT-12/2013 [H1N2]), H3 (A/baikal teal/Shanghai/SH-89/2013 [H3N2]), H4 (A/greylag goose/Changsha/CS-510/2013 [H4N8]), H5 (A/great tit/Panjing/PJ-66/2013 [H5N1]), H6 (A/common teal/Nanji/NJ-227/2013 [H6N1]), H7 (A/Baer's pochard/HuNan/414/2010 [H7N1]), H9 (A/great bustard/InnerMongolia/IM-E2/2012 [H9N2]), H13N2 (A/black-tailed gull/Weihai/115/2016 [H13N2]), and H13N8 (A/black-tailed gull/Weihai/17/2016 [H13N8]).



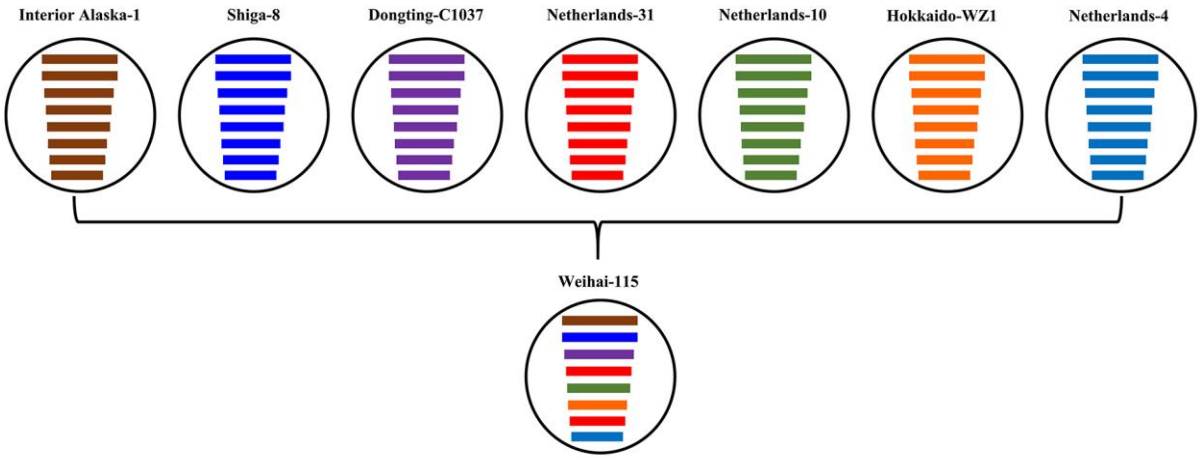
Technical Appendix Figure 1. The migratory routes of the black-tailed gulls (*Larus crassirostris*) to Weihai city, Shandong province, China. Red color area indicates Weihai city. Arrows indicate the migratory routes.



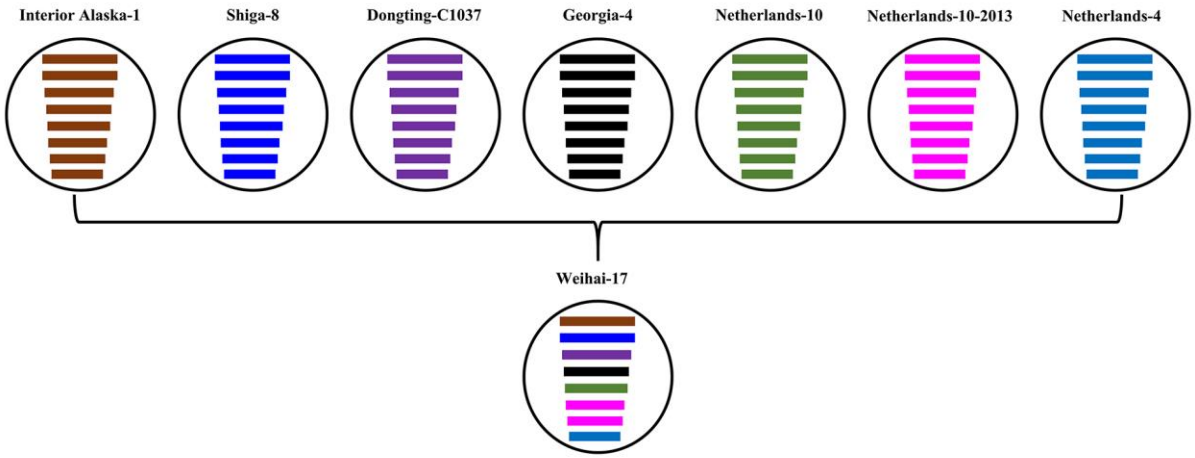
Technical Appendix Figure 2. Phylogenetic trees of HA (A) and NA (B) genes of novel avian influenza (H13N2) virus isolated from black-tailed gull in China, December 2016, with reference sequences. Red, A/black-tailed gull/Weihai/115/2016 (H13N2); blue, other H13N2 avian influenza viruses. Trees were generated by applying the neighbor-joining method in MEGA 7.0 (www.megasoftware.net) on the basis of full-length sequences. Scale bars indicate branch length based on number of nucleotide substitutions per site.



Technical Appendix Figure 3. Phylogenetic trees of HA (A) and NA (B) genes of novel avian influenza (H13N8) virus isolated from black-tailed gull in China, December 2016, with reference sequences. Red, A/black-tailed gull/Weihai/17/2016 (H13N8); blue, other H13N8 avian influenza viruses. Trees were generated by applying the neighbor-joining method in MEGA 7.0 (www.megasoftware.net) on the basis of full-length sequences. Scale bars indicate branch length based on number of nucleotide substitutions per site.



Technical Appendix Figure 4. Putative genomic compositions of the novel avian influenza (H13N2) virus isolated from black-tailed gull in China, December 2016, with their possible donors. The 8 gene segments (from top to bottom) in each virus are polymerase basic protein 2 (PB2), polymerase basic protein 1 (PB1), polymerase acidic protein (PA), hemagglutinin (HA), Nucleoprotein (NP), neuraminidase (NA), matrix protein (M), and nonstructural protein (NS). Each color indicates a separate virus background. Interior Alaska-1, A/northern shoveler/Interior Alaska/1/2007(H12N5); Shiga-8, A/duck/Shiga/8/2004(H4N6); Dongting-C1037, A/wild goose/Dongting/C1037/2011(H12N8); Netherlands-31, A/black-headed gull/Netherlands/31/2009(H13N2); Netherlands-10, A/black-headed gull/Netherlands/10/2012(H13N6); Hokkaido-WZ1, A/duck/Hokkaido/WZ1/2014(H11N2); Netherlands-4, A/black-headed gull/Netherlands/4/2015(H16N3). The simplified schematic illustration is based on nucleotide distance comparison and phylogenetic analysis.



Technical Appendix Figure 5. Putative genomic compositions of the novel avian influenza (H13N8) virus isolated from black-tailed gull in China, December 2016, with their possible donors. The 8 gene segments (from top to bottom) in each virus are polymerase basic protein 2 (PB2), polymerase basic protein 1 (PB1), polymerase acidic protein (PA), hemagglutinin (HA), Nucleoprotein (NP), neuraminidase (NA), matrix protein (M), and nonstructural protein (NS). Each color indicates a separate virus background. Interior Alaska-1, A/northern shoveler/Interior Alaska/1/2007(H12N5); Shiga-8, A/duck/Shiga/8/2004(H4N6); Dongting-C1037, A/wild goose/Dongting/C1037/2011(H12N8); Georgia-4, A/yellow-legged gull/Georgia/4/2012(H13N8); Netherlands-10, A/black-headed gull/Netherlands/10/2012(H13N6); Netherlands-10–2013, A/black-headed gull/Netherlands/10/2013(H13N8); Netherlands-4, A/black-headed gull/Netherlands/4/2015(H16N3). The simplified schematic illustration is based on nucleotide distance comparison and phylogenetic analysis.