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Human Co-Infection with Avian and Seasonal Influenza Viruses, China

To the Editor: In April 2013, a case of co-infection with avian-origin influenza A(H7N9) virus and seasonal influenza A(H3N2) virus was reported in Jiangsu Province, China (1). This case raised concern over the possible occurrence of new reassortants with enhanced transmissibility among humans. Because of the nature of the dynamic reassortment of A(H7N9) virus with A(H9N2) virus in the environment and in poultry (2,3), close surveillance for possible new reassortment in human patients with A(H7N9) infection is needed. We report co-infection in 2 patients in Hangzhou, the capital Zhejiang Province, China, in January 2014. The co-infections involved influenza A(H7N9) virus and a seasonal A(H1N1)pdm09 virus (1 patient) or a seasonal influenza B virus (1 patient).

Of 60 patients with laboratory-confirmed influenza A(H7N9) infections in Hangzhou in April 2013 and in January–February 2014, testing of pharyngeal swab samples indicated that 2 patients were also positive for seasonal influenza virus. The pharyngeal samples were tested by real-time reverse transcription PCR according to protocols provided by the Chinese National Influenza Center. Informed consent for this study was provided by each patient’s spouse.

On January 6, 2014, patient 1 (male, 58 years of age), a resident of Xiaoshan District, had a high fever (39.6°C) and a cough; at a hospital, he received a diagnosis of severe acute interstitial pneumonia. The patient had a history of chronic myelogenous leukemia; his history of exposure to live poultry was not clear. On January 13, infection with influenza A(H7N9) virus was laboratory confirmed; viral RNA from a pharyngeal swab sample collected before oseltamivir treatment was positive for the following: influenza A virus (cycle threshold [C_t] = 26), H7 (C_t = 27), N9 (C_t = 26), influenza A(H1N1)pdm09 virus H1 (C_t = 30), and N1 (C_t = 30). The 2 viruses were named A/Hangzhou/10–1/2014(H7N9) and A/Hangzhou/10–2/2014(H1N1)pdm09. The patient received oseltamivir while in the hospital but died on January 18.

On January 5, patient 2 (male, 54 years of age), also from Xiaoshan District, had fever and a cough; at a hospital, he received a diagnosis of severe acute pneumonia. He had a history of aplastic anemia and had been exposed to live poultry 1 week before symptom onset. On January 18, infection with influenza A(H7N9) virus was laboratory confirmed. Viral RNA from a pharyngeal swab sample collected before oseltamivir treatment was positive for the following: influenza A virus (C_t = 22), H7 (C_t = 23), N9 (C_t = 22), and influenza B virus (C_t = 22). The viruses were named A/Hangzhou/17–1/2014(H7N9) and B/Hangzhou/17–2/2014. This patient received oseltamivir but died on January 22.

The hemagglutinin (HA) and neuraminidase (NA) sequences of viruses from these 2 patients were determined by Sanger sequencing. The specific primers used are listed in online Technical Appendix Table 1 (<http://wwwnc.cdc.gov/EID/article/20/11/14-0897-Techapp1.pdf>). The accession numbers of these sequences and the reference sequences for phylogenetic analyses are

listed in online Technical Appendix Table 2. Phylogenetic analyses (4) revealed that these 2 influenza A(H7N9) viruses were clustered into the clade of A/Shanghai/2/2013(H7N9)-like viruses (Figure). Some amino acid features within the HA and NA of these 2 viruses were the same as those in the A/Shanghai/2/2013(H7N9) strain: L226 and G228 in HA, believed to control host receptor specificity; the cleavage site in HA,

relevant for virulence; a deletion in NA stalk (position 69–73), associated with the adaption to gallinaceous hosts; and R294 in NA, related to virus sensitivity to oseltamivir (5). The HA and NA sequences of A/Hangzhou/10–2/2014(H1N1) pdm09 and B/Hangzhou/17–2/2014 were very close to those of A(H1N1) pdm09 virus and B/Yamagata-lineage viruses that had recently circulated in China (6,7).

Co-infection with A(H7N9) virus and seasonal influenza viruses is probably associated with the overlap of A(H7N9) virus and seasonal virus circulation in both time and space and with increased prevalence of influenza virus infections within the population. From November 2012 through March 2014, outbreaks of A(H7N9) infection (in April 2013 and in January–February 2014) were concurrent with increases in seasonal influenza

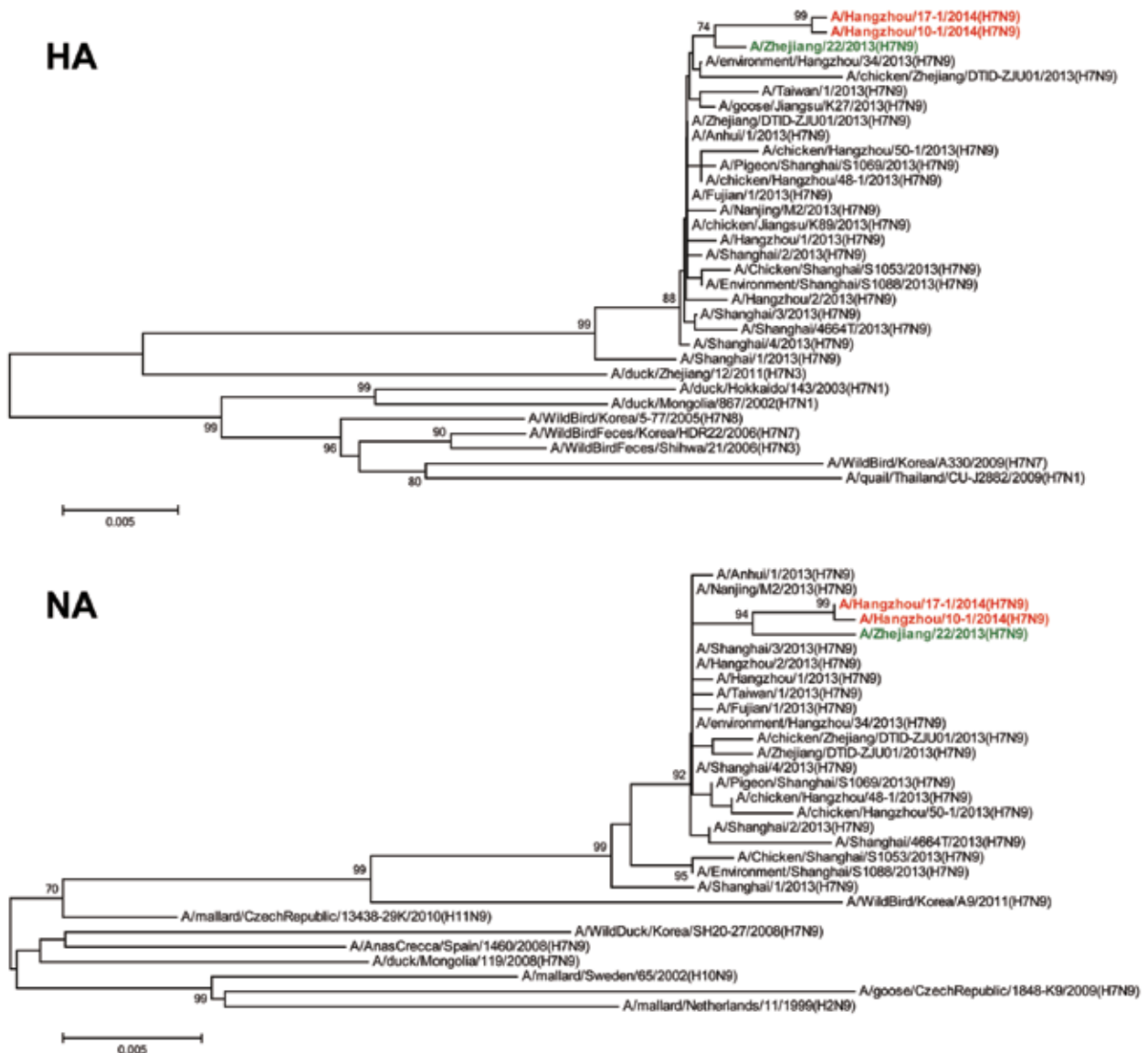


Figure. Phylogenetic analyses of hemagglutinin (A) and neuraminidase (B) of influenza A(H7N9) viruses. The trees were constructed by using the neighbor-joining method with bootstrap analysis ($n = 1,000$) in the MEGA5.0 program (4). Red indicates the 2 viruses isolated from co-infected patients in Hangzhou, China, and green indicates the first strain isolated during the second wave of the influenza A(H7N9) outbreak in China, which started in October 2013. Scale bars indicate nucleotide substitutions per site.

virus infections in Hangzhou (online Technical Appendix Figure). Prompt control of A(H7N9) infection outbreaks and vaccination against seasonal influenza viruses could reduce the potential for co-infections with A(H7N9) virus and seasonal viruses.

Taken together with the previous finding of human co-infection with A(H7N9) virus and A(H3N2) virus (1), our results show that human co-infection with A(H7N9) virus and each of the 3 seasonal influenza viruses currently circulating worldwide can occur. Avian influenza viruses, including A(H7N9), preferentially replicate in the lower respiratory tract of humans (8,9). In contrast, seasonal influenza viruses preferentially infect the upper respiratory tract of humans (10). Coexistence of A(H7N9) virus with either A(H1N1)pdm09 virus or influenza B virus in the pharyngeal swab samples from 2 patients suggests that the upper respiratory tract could provide a location for the A(H7N9) virus to reassort with other influenza viruses. The possibility that seasonal influenza viruses might provide some gene segments that increase the human-to-human transmissibility of possible new reassortants is cause for concern. For detection of such new influenza virus reassortants, extensive surveillance to identify influenza virus co-infections is necessary.

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Misidentification of *Diphyllobothrium* Species Related to Global Fish Trade, Europe

To the Editor: *Diphyllobothriosis*, infection by tapeworms of the genus *Diphyllobothrium* (Cestoda: Diphylllobothriidae) (1), is a well-known disease of humans. In Europe, infections caused by 3 species of *Diphyllobothrium* have recently been reported in humans: *D. latum* is considered to be the principal species infecting persons in Europe (1); 4 cases of *D. dendriticum* infection and 6 cases of *D. nihonkaiense* infection have also been reported (2,3). Except for those caused by *D. latum*, which is autochthonous in northeastern Europe and subalpine lakes, most of the cases in Europe have been imported or caused by consumption of fish imported from areas to which the parasites are endemic (1,3,4).

Diphyllobothriosis is not endemic to Spain, but 7 cases of *D. latum*

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Technical Appendix

Technical Appendix Table 1. Summary of PCR primers used to amplify the HA and NA genes

Gene	Primer	Primer sequence (5'→3')	Source
A(RT)	Uni12	AGCAAAAGCAGG	(1)
H7 & H1	Bm-HA-1	TATTCGTCTCAGGGAGCAAAAGCAGGGG	(1)
	Bm-NS-890R	ATATCGTCTCGTATTAGTAGAAACAAGGGTGT	(1)
H7	H7-H1F17	TGGTATTCGCTCTGATTGC	Designed
	H7-H1R523	TAGTCATCTGCGGAATGC	Designed
	H7-H2F420	TAATGGAGCAACCAGTGCA	Designed
	H7-H2R944	GGACATTTTCCAAGTCCCTG	Designed
	H7-H3F834	TGGAGTACAGGTTGATGCCA	Designed
	H7-H3R1434	TTCAAAGCAACCAGTGCCA	Designed
	H7-H4F1303	TGCTGAACTCTTGGTAGCA	Designed
	H7-H4R1674	AATAGTGCACCGCATGTTTCC	Designed
N9	Bm-N9-1	TATTCGTCTCAGGGAGCAAAAGCAGGGTC	(1)
	Bm-N9-1473R	ATATCGTCTCGTATTAGTAGAAACAAGGGTCTT	(1)
	N9-N1F2	GCGAAAGCAGGGTCAAGAT	Designed
	N9-N1R501	GCTTATCAGGGCGGATACTG	Designed
	N9-N2F416	ATGCTCTCAGCCAAGGAACAA	Designed
	N9-N2R924	TCTATTTGAGCCCTGCCAA	Designed
	N9-N3F857	GCTCATGTTACGGGGAACGA	Designed
	N9-N3R1411	TAGCCCCATCAGGCCAGTT	Designed
H1	H1R2-497	ATGAGGACATGCTGCCGTT	Designed
	H1F2-709	AGTTCAAGCCGGAAATAGC	Designed
	H1R3-1366	AGAACCAACAGTTCGGCAT	Designed
	H1F3-1262	GGGTAAAGAGTTCAACCACCTGG	Designed
	H1R5-1777	CGGAGTAGAAACAAGGGTGTT	Designed
N1	Ba-NA-1	TATTGGTCTCAGGGAGCAAAAGCAGGAGT	(1)
	Ba-NA-1413R	ATATGGTCTCGTATTAGTAGAAACAAGGAGTTTTTT	(1)
	N1R2-489	TTCGATATGGGCTCCTGTC	Designed
	N1F2-421	TGACTCAAGGGGCCCTTGCTA	Designed
	N1R3-1246	TGCTGAACAAAACCTCCCGCT	Designed
	N1F3-917	GAATCGACCGTGGGTGTCTT	Designed

Gene	Primer	Primer sequence (5'→3')	Source
	N1R6-1457	TGCGTCCGAGTAGAAACAAGGAG	Designed
B(RT)	Uni11	AGCAGAAGCRS	(2)
B-HA	BHF1-1	TTTCTAATATCCACAAAATGAAGGCAA	Designed
	BHR1-234	GACAGTCCGGGCATAGTTTC	Designed
	B-HA-F	ATCCACAAAATGAAGGCAATA	(3)
	B-HA-PR	TGCAGGAGGTCTATATTGG	(3)
	BHF3-778	TACCACAAAGCGGCAGAATT	Designed
	BHR3-1451	TGCCAATAGATGCTCGTC	Designed
	BHF4-1285	ACCTTCAAAGACTAAGTGGTGC	Designed
	BHR6-1847	GCGCTCAATAACGTTTCTTTG	Designed
B-NA	BNA-F1	CTGAGGCAAATAGGCCAAAAATG	(2)
	BNA-R1	GGGTTTAGAACAGACTCAACC	(2)
	BNA-280F	AAAGCACTCCTAATTAGCCC	(2)
	BNA-467R	GATGCCTCAGCTTGTCTG	(2)
	BNA-537F	GTCCGCATGCCATGATGGTA	(2)
	BNA-871R	GCTGTGTAAGTGTATCTCTACA	(2)
	BNF-861	AAGAATGCACATGCGGATTTGCTA	Designed
	BNR-1270	AAAGGAGTACCAACCAGGTTCTT	Designed
	BNA-1090F	TGGTACTCTCGAACGATGTC	(2)

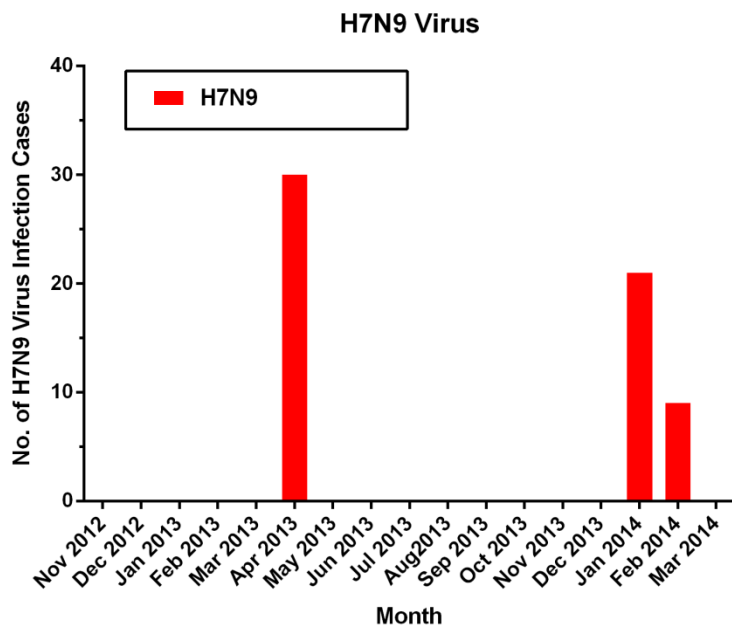
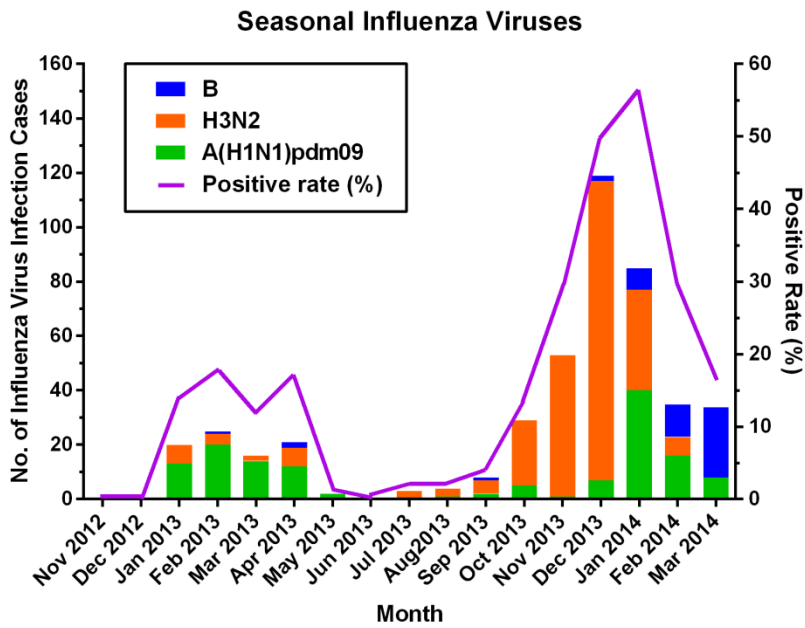
Technical Appendix Table 2. GISAID or GenBank accession numbers of influenza viruses in the construction of the HA and NA phylogenetic trees

Viruses	Segments		Viruses	Segments	
	HA	NA		HA	HA
A/Hangzhou/10-1/2014(H7N9)	EPI509669	EPI509670	A/Chicken/Shanghai/S1053/2013(H7N9)	EPI440685	EPI440684
A/Hangzhou/10-2/2014(H1N1)	EPI509671	EPI511870	A/Environment/Shanghai/S1088/2013(H7N9)	EPI440693	EPI440692
A/Hangzhou/17-1/2014(H7N9)	EPI509672	EPI509673	A/Shanghai/4664T/2013(H7N9)	EPI446962	EPI446965
B/Hangzhou/17-2/2014	EPI509674	EPI509675	A/goose/Jiangsu/K27/2013(H7N9)	EPI442706	
A/Zhejiang/22/2013(H7N9)	EPI477410	EPI477412	A/chicken/Jiangsu/K89/2013(H7N9)	EPI442707	
A/Nanjing/M2/2013(H7N9)	EPI450526	EPI450527	A/duck/Zhejiang/12/2011(H7N3)	JQ906576.1	
A/Zhejiang/DTID-ZJU01/2013(H7N9)	EPI441794	EPI441797	A/duck/Hokkaido/143/2003(H7N1)	AB269694.	
				2	
A/chicken/Zhejiang/DTID-ZJU01/2013(H7N9)	EPI442721	EPI442723	A/duck/Mongolia/867/2002(H7N1)	AB473543.	
				1	
A/Shanghai/1/2013(H7N9)	EPI439486	EPI439487	A/WildBird/Korea/5-77/2005(H7N8)	JX444827.1	
A/Shanghai/2/2013(H7N9)	EPI439502	EPI439500	A/WildBirdFeces/Korea/HDR22/2006(H7N7)	FJ750875.1	
A/Anhui/1/2013(H7N9)	EPI439507	EPI439509	A/WildBirdFeces/Shihwa/21/2006(H7N3)	FJ767723.1	
A/Taiwan/1/2013(H7N9)	EPI445912	EPI445914	A/WildBird/Korea/A330/2009(H7N7)	JN244227.1	
A/Hangzhou/1/2013(H7N9)	EPI440095	EPI440096	A/quail/Thailand/CU-J2882/2009(H7N1)	JX523347.1	
A/Hangzhou/2/2013(H7N9)	EPI442710	EPI442711	A/WildBird/Korea/A9/2011(H7N9)		JN244234.1
A/environment/Hangzhou/34/2	EPI442716	EPI442717	A/mallard/CzechRepublic/13438-29K/2010(H1		JF789602.1

Viruses	Segments		Viruses	Segments	
	HA	NA		HA	HA
013(H7N9)			1N9)		
A/chicken/Hangzhou/48-1/2013(H7N9)	EPI443659	EPI443661	A/goose/CzechRepublic/1848-K9/2009(H7N9)		GU060482. 1
A/chicken/Hangzhou/50-1/2013(H7N9)	EPI454491	EPI454492	A/AnasCrecca/Spain/1460/2008(H7N9)		HQ244409. 1
A/Fujian/1/2013(H7N9)	EPI446746	EPI446747	A/duck/Mongolia/119/2008(H7N9)		AB481213. 1
A/Shanghai/3/2013(H7N9)	EPI443022	EPI443023	A/WildDuck/Korea/SH20-27/2008(H7N9)		JX679164.1
A/Shanghai/4/2013(H7N9)	EPI443025	EPI443026	A/mallard/Sweden/65/2002(H10N9)		CY060360. 1
A/Pigeon/Shanghai/S1069/2013(H7N9)	EPI440701	EPI440700	A/mallard/Netherlands/11/1999(H2N9)		CY122246. 1

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Technical Appendix Figure. Number of seasonal influenza virus infections among patients with influenza-like illness at 2 sentinel hospitals, and positivity rate, by month (upper panel) and number of influenza A(H7N9) virus infections in Hangzhou, China (lower panel). Positivity rate = no. patients with influenza-like illness who were positive for any 1 of the 3 seasonal influenza viruses/no. patients with influenza-like illness tested \times 100.