



NOTE

Virology

Characterization of a novel reassortant H7N3 highly pathogenic avian influenza virus isolated from a poultry meat product taken on a passenger flight to Japan

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ABSTRACT. A new reassortant H7N3 avian influenza virus (AIV) was isolated from a duck meat product that was illegally taken on board a passenger flight from China to Japan in March 2018. Sequencing analysis revealed that the H7N3 isolate, A/duck/Japan/AQ-HE30-1/2018 (Dk/HE30-1) (H7N3), was a reassortant highly pathogenic avian influenza virus (HPAIV) that contained the haemagglutinin (HA) gene of Chinese H7N9 HPAIV. Dk/HE30-1 (H7N3) possessed a novel polybasic sequence motif PEVPKRRRTAR/GLF at the HA cleavage site that has never previously been reported in H7 HPAIVs. The HA antigenicity of Dk/HE30-1 (H7N3) slightly differed from that of H7N9 HPAIVs previously reported. These findings will help further our knowledge of the circulation and genetic evolution of emerging AIVs in endemic areas.

KEY WORDS: highly pathogenic avian influenza virus, H7N3 subtype, reassortant virus

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Zoonotic H7N9 avian influenza viruses (AIVs) have been isolated from humans and poultry in mainland China since their first emergence in 2013 [2]. The spread and circulation of H7N9 AIVs in the field have generated highly pathogenic avian influenza viruses (HPAIVs) [4, 14] and multiple genotypes of H7N9 AIVs [8]. Infections of birds and humans with H7N9 AIVs have only been reported on mainland China, with the exception of some infected humans who have traveled from China [14]. However, H7N9 AIVs have been carried across the national border by international flight passengers carrying contaminated poultry meat products [10].

Continuous monitoring is undertaken at airports and ports in Japan, which has resulted in a new reassortant H7N3 HPAIV being isolated from a duck meat product that was illegally taken on board a passenger flight from China to Japan. This new virus, A/duck/Japan/AQ-HE30-1/2018 (Dk/HE30-1) (H7N3) (accession no. LC416563-70), contained a haemagglutinin (HA) gene segment of Chinese H7N9 HPAIV and had an intravenous pathogenicity index (IVPI) value of 2.99, with ten 6-week-old chickens that were intravenously inoculated with the virus all dying within 48 hr of inoculation. To the best of our knowledge, this is the first report of the isolation of a new reassortant H7N3 HPAIV that would likely be circulating in poultry in China.

The duck meat product that was contaminated with Dk/HE30-1 (H7N3) had been illegally carried in the hand luggage of a flight passenger who had boarded an international flight from Lanzhou Zhongchuan or Shanghai Pudong International Airport, China to Chubu Centrair International Airport, Japan in March 2018 and was detected by a quarantine detector dog. The duck meat product consisted of irregular-shaped pieces of born-in meat that did not appear to contain any organs (Fig. 1). The meat product was

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Fig. 1. Photograph of the duck meat product that was contaminated with the H7N3 highly pathogenic avian influenza virus (HPAIV) isolate.

homogenized in phosphate-buffered saline containing antibiotics (1:10, w/v) and used for virus isolation in 11-day-old embryonated chicken eggs, following the same protocol as previously reported [9, 10]. Three embryonated chicken eggs were then inoculated with 0.2 ml of the inoculum, all of which died within 72 hr. After confirming the presence of the virus in the allantoic fluid by the haemagglutination test, the isolate was identified as H7N3 AIV without noticeable cross-reactivity by haemagglutination inhibition (HI) and neuraminidase (NA) inhibition tests with antisera against the reference AIV strains (H1-16 and N1-9). While the original sample was possibly contaminated with multiple viruses producing genetic reassortment in eggs, the probability of not detecting the contaminated virus (e.g. H7N9 virus) in NA inhibition test could be relatively low. The pathogenicity of the virus was then determined using the IVPI test according to the manual of the World Organisation for Animal Health (OIE) [7]. The species of duck from which the meat originated was identified as Muscovy duck (*Cairina moschata*) by mitochondrial DNA sequencing, as described previously [5].

The complete genome sequence of Dk/HE30-1 (H7N3) was determined to understand its genetic properties and to analyze its genetic relationship with other AIVs that have been isolated in North and South America, Europe, and Asia and listed in GenBank/the European Molecular Biology Laboratory (EMBL)/the DNA Data Bank of Japan (DDBJ) (<https://www.ncbi.nlm.nih.gov/genbank/>) and the Global Initiative on Sharing All Influenza Data (GISAID; <http://platform.gisaid.org/>). Sequence analysis using the Basic Local Alignment Search Tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) showed that the HA and matrix (M) gene segments of Dk/HE30-1 (H7N3) shared 98.6–99.5% nucleotide sequence identity with those of A/chicken/Heinan/ZZ01/2017 (H7N9) isolated in central China, while its NA gene segment shared 99.1% nucleotide sequence identity with that of A/environment/Fujian/S1XA33/2017 (H11N3) isolated in poultry slaughterhouses in southeast China. Its remaining five internal gene segments were closely related to those of other AIVs isolated from poultry originating from China, i.e., the polymerase basic protein 2 (PB2) gene segment of Dk/HE30-1 (H7N3) shared 97.8% nucleotide sequence identity with that of A/duck/Ganzhou/GZ148/2016 (H6N6); the PB1 and nucleoprotein (NP) gene segments of Dk/HE30-1 (H7N3) shared 97.6–98.8% nucleotide sequence identity with those of A/duck/Japan/AQ-HE103/2015 (H1N2), which was isolated from a duck meat product that was illegally brought to Japan on board a passenger flight from China [9]; the polymerase acidic (PA) gene segment of Dk/HE30-1 (H7N3) shared 97.6% nucleotide sequence identity with that of A/duck/Guangxi/135D20/2013 (H3N2); and the nonstructural (NS) gene segment of Dk/HE30-1 (H7N3) shared 98.2% nucleotide sequence identity with that of A/chicken/Guangxi/125C8/2012 (H3N2). Phylogenetic analysis classified Dk/HE30-1 (H7N3) into the same clade as the H7N9 HPAIVs in the Yangtze River Delta Lineage based on the HA gene (Fig. 2A) and into the same clade as HxN3 AIVs that have recently been isolated from poultry or the environment in China based on the NA gene (Fig. 2B), demonstrating that Dk/HE30-1 (H7N3) was a multiple reassortant virus that contained the HA gene of H7N9 HPAIVs.

Dk/HE30-1 (H7N3) was found to possess a novel polybasic cleavage sequence motif PEVPKRRRTAR/GLF in the HA protein that has not been previously reported in H7 HPAIVs. The seventh amino acid in this motif exhibited a mutation from lysine to arginine compared with the motif of H7N9 HPAIVs [6]. Amino acids 226Q and 228G (H3 numbering) in the HA protein of Dk/HE30-1 (H7N3) were consistent with those of the H7N9 HPAIVs, which are associated with avian-type receptor binding [1, 12]. Furthermore, amino acid 292R (N2 numbering) was retained in the NA protein of Dk/HE30-1 (H7N3), indicating the susceptibility of this virus to NA inhibitors such as oseltamivir [2, 11]. Mutations in amino acids E627K and D701N in PB2, and I368V in PB1, which are known to be associated with virulence and transmissibility in ferrets and mice, were not observed in Dk/HE30-1 (H7N3) [3, 13, 15, 16]. The key molecular markers in these gene segments indicate that Dk/HE30-1 (H7N3) may be able to adapt to avian species, as seen in previously reported H7N9 HPAIVs.

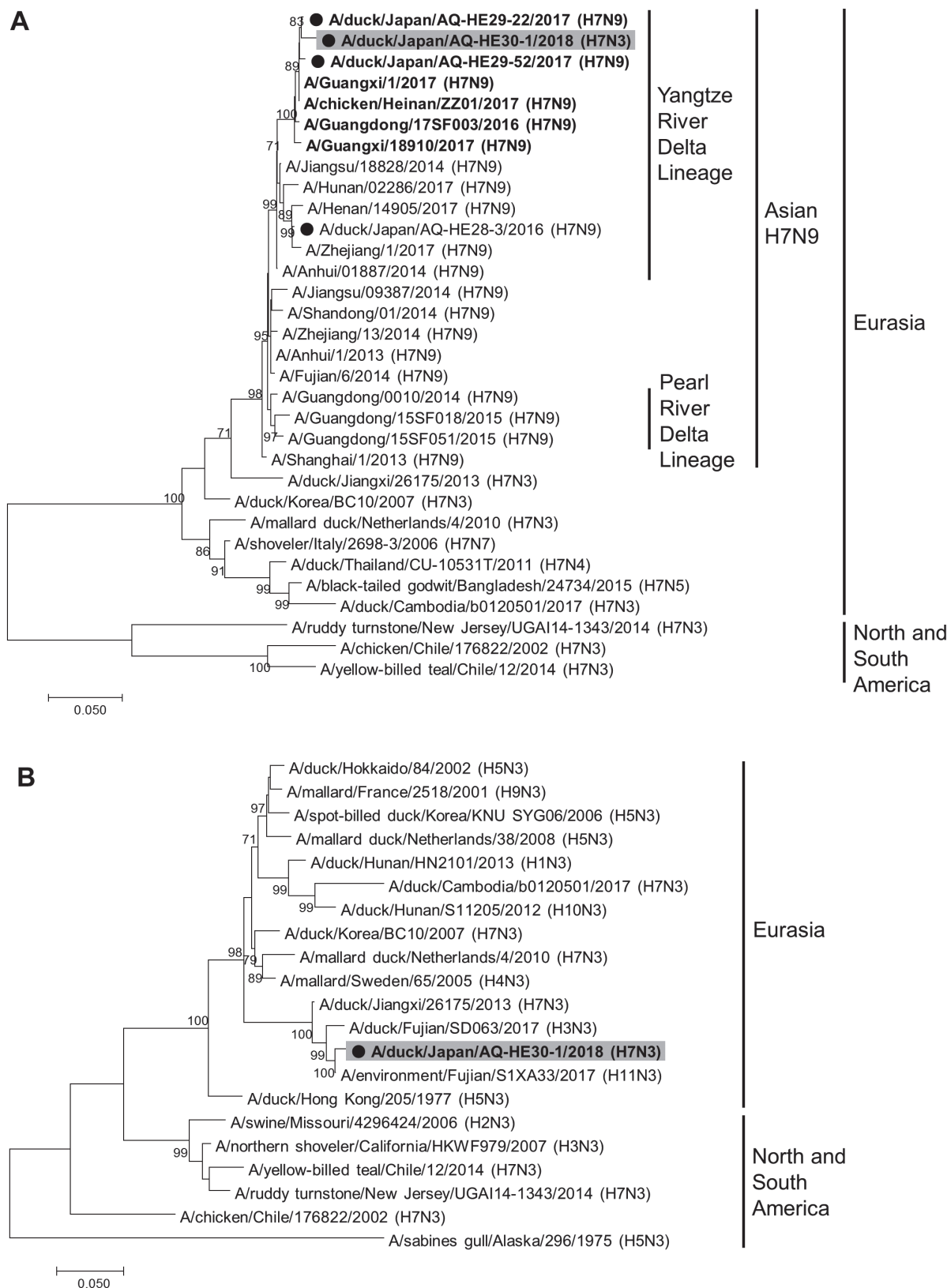


Fig. 2. Phylogenetic analysis of the hemagglutinin (HA) gene of H7 avian influenza virus (A) and the N3 neuraminidase (NA) gene of avian influenza virus (B). The nucleotide sequences of the H7 HA and N3 NA genes were analyzed by the maximum-likelihood method with the corresponding genes of reference strains using MEGA 7.0 software (<http://www.megasoftware.net/>). The horizontal distances in the trees are proportional to the minimum number of nucleotide differences that are required to join nodes and sequences, and the values at the nodes indicate the confidence levels in the bootstrap analysis with 1,000 replications. The virus that was isolated in this study is highlighted in gray, while highly pathogenic avian influenza viruses are indicated in bold. Viruses that have been isolated from illegally brought meat products are marked with black dots.

Table 1. Hemagglutination inhibition assay of A (H7) influenza viruses

Reference antigens	Subtype	Lineage	Reference antisera		
			Ma/NL12	Anhui1	GD17SF003
A/mallard/Netherlands/12/2000	H7N3	-	<u>320</u>	40	320
A/Anhui/1/2013	H7N9	-	160	<u>160</u>	160
A/Guangdong/17SF003/2016	H7N9	YRD HPAI	20	10	<u>160</u>
Test antigens					
A/duck/Japan/AQ-HE28-3/2016	H7N9	YRD	80	160	640
A/duck/Japan/AQ-HE29-22/2017	H7N9	YRD HPAI	20	20	160
A/duck/Japan/AQ-HE29-52/2017	H7N9	YRD HPAI	20	20	160
A/duck/Japan/AQ-HE30-1/2018	H7N3	YRD HPAI	10	<10	40

Homologous titres are underlined. HPAIVs are shown in bold.

Dk/HE30-1 (H7N3) was antigenically analyzed by cross HI test, using a panel of post-infection ferret antisera (Table 1). The ferret antiserum against A/Anhui/1/2013 (H7N9) reacted equally with A/duck/Japan/AQ-HE28-3/2016 (H7N9), a low pathogenic H7N9 strain of Yangtze River Delta (YRD) lineage [10] to the homologous antigen, and that against A/Guangdong/17SF003/2016 (H7N9) did so with A/duck/Japan/AQ-HE29-22/2017 (H7N9) and A/duck/Japan/AQ-HE29-52/2017 (H7N9), highly pathogenic H7N9 strains of YRD HPAI lineage [10]. On the contrary, those against A/Anhui/1/2013 (H7N9) and A/Guangdong/17SF003/2016 (H7N9) showed more than 16-fold and 4-fold lower HI titers, respectively, with Dk/HE30-1 (H7N3), indicating that the HA antigenicity of Dk/HE30-1 (H7N3) slightly differed from that of A/Anhui/1/2013 (H7N9) and A/Guangdong/17SF003/2016 (H7N9). However, in order to understand the antigenic diversity of most recent H7 isolates, more precise analyses are required to reveal the antigenicity of this novel H7N3 HPAIV.

In summary, a multiple reassortant H7N3 HPAIV that contained the HA gene from Chinese H7N9 HPAIV and the NA gene from HxN3 AIV was isolated from a duck meat product originating from China for the first time, providing strong evidence that a new reassortant H7N3 HPAIV has emerged and is being circulated in poultry in China. These findings will help further our knowledge of the circulation and genetic evolution of emerging AIVs in endemic areas.

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