

Laboratory and Epidemiology Communications

Isolation of Influenza Virus Type AH3 from a Traveler Returning from Vietnam in July 2005 in Osaka, Japan

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Communicated by Ichiro Kurane

(Accepted October 25, 2005)

On 28 June 2005, the World Health Organization announced that an additional case of human infection with AH5N1 avian influenza was confirmed in Vietnam and that the new case brought the total, in Vietnam, since mid-December 2004, to 60 cases, of which 18 had been fatal. A man who visited Vietnam from 4 July 2005 returned to Osaka, Japan, on 8 July. As he felt a fever beginning on 7 July, he saw a doctor soon after his flight to Japan. He was diagnosed with influenza type A infection using a rapid influenza test. Although he declared that he was traveling around a coast area and that he had not been near chickens in Vietnam, the doctor reported this case to the Izumi Health Center of Osaka Prefecture,

because they could not completely deny the possibility that his influenza was caused by highly pathogenic avian influenza (HPAI). On the afternoon of 8 July, the gargle from the patient was transported to the Osaka Prefectural Institute of Public Health. The specimen was inoculated onto Madin-Darby canine kidney (MDCK) cells. Simultaneously, viral RNA was extracted with QIAamp Viral RNA Mini Kit (QIAGEN K.K., Tokyo, Japan) for analysis by RT-PCR. To obtain an amplicon on a part of the HA1 region of the HA gene in influenza A virus, we used primer pairs as follows: 5'-CAGATGCAGACACAATATGT-3' and 5'-AAACCGGC AATGGCTCCAAA-3' for H1, 5'-CAGATTGAAGTACTA ATGC-3' and 5'-GTTTCTCTGGTACATTCCGC-3' for H3 and 5'-CATACCCAACAATAAAGAGG-3' and 5'-GTGTTC ATTTTGTTAATGAT-3' for H5 (1,2). We carried out RT-PCR with Ready-to-Go RT-PCR Beads (Amersham, Biosciences UK Ltd., Buckinghamshire, UK) and by using a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, Conn., USA)

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Table 1. The HI test results of isolated virus against reference antisera

	Antisera					
	A/Moscow/ 13/98	A/New Caledonia/ 20/99	A/Wyoming/ 03/2003	A/Panama/ 2007/99	B/Brisbane/ 32/2002	B/Johannesburg/ 5/99
Antigen						
A/Moscow/13/98(H1N1)	1,280	nt	nt	nt	nt	nt
A/New Caledonia/20/99(H1N1)	nt	320	nt	nt	nt	nt
A/Wyoming/03/2003(H3N2)	nt	nt	2,560	nt	nt	nt
A/Panama /2007/99(H3N2)	nt	nt	nt	1,280	nt	nt
B/Brisbane/32/2002	nt	nt	nt	nt	10,240	nt
B/Johannesburg/5/99	nt	nt	nt	nt	nt	>20,480
Isolated virus						
A/Osaka/27/2005	<10	<10	1,280	<10	<10	<10

nt: not tested.

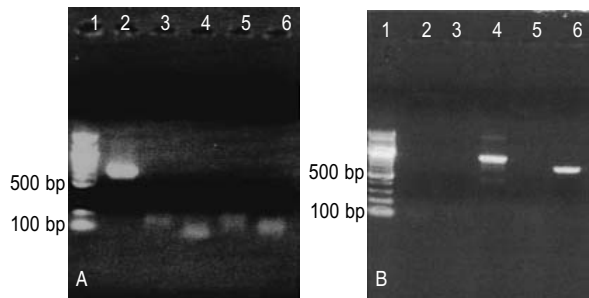


Fig. 1. RT-PCR for a part of the HA1 region of the HA gene in influenza A virus. (A) The RT-PCR products from the extract of the gargle were electrophoresed in 1% agarose gel. Lane 1, marker; lane 2, positive control and primer pairs for H5; lane 3, primer pairs for H1; lane 4, primer pairs for H3; lane 5, primer pairs for H5; lane 6, negative control and primer pairs for H5. (B) The RT-PCR products from the extract of the culture fluid showing the CPE were electrophoresed in 1% agarose gel. Lane 1, marker; lane 2, negative control and primer pairs for H5; lane 3, primer pairs for H1; lane 4, primer pairs for H3; lane 5, primer pairs for H5; lane 6, positive control and primer pairs for H5.

consisting of 1 cycle at 42°C for 30 min, 94°C for 5 min, followed by 40 cycles of 94°C for 30 sec, 45°C for 30 sec, and 72°C for 1 min. However, no PCR products were detected from the extract of the gargle (Figure 1A).

On 11 July, as we observed the cytopathic effects (CPE) in the MDCK cells inoculated with the specimen, we performed a hemagglutination (HA) test with the supernatant of viral culture using erythrocytes of chickens and humans (blood group O). The fluid agglutinated human erythrocytes but not chicken ones. We performed a hemagglutination inhibition (HI) test with human erythrocytes against the reference antisera provided by the National Institute of Infectious Diseases (NIID). As shown in Table 1, the isolate was reacted with anti-A/Wyoming/03/2003 (H3N2) serum only. We also carried out RT-PCR with the culture fluid showing CPE by the method described above. The RT-PCR products were specific for H3 (Figure 1B). The RT-PCR product was purified in an agarose gel and sequenced directly with BigDye Terminator Cycle Sequencing Ready Reaction Kits with AmpliTaq DNA Polymerase, FS (PE Applied Biosystems, Foster City, Calif., USA) by using an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems). A BLAST search showed that the sequence of the isolate possessed 98% homology with A/New York/10/2004 (H3N2). These results indicated that the isolated virus was a human influenza virus type AH3 [named

A/Osaka/27/2005(H3)].

According to the Infectious Disease Surveillance Center, NIID, influenza epidemic seasons in Vietnam are from June to August and from December to March in usual years. In July 2005, an influenza epidemic occurred in Vietnam. As there was no influenza virus activity in Osaka in this month, it is concluded that the patient was infected with influenza virus type AH3 in Vietnam. This is one of the cases in Japan. Some papers have already reported that influenza virus type AH3 was isolated from travelers when they came back to Japan from abroad (3-5). Since HPAI is spreading in Vietnam and its neighbor countries now, it is important that we examine whether influenza of returnees from these countries is conventional human influenza or HPAI, even if patients have no history of contact with humans or chickens infected with HPAI. Furthermore, the examination should include detection of viral antigens, amplification of viral RNA and viral isolation for definite diagnosis.

This article appeared in the Infectious Agents Surveillance Report, vol. 26, p. 222, 2005 in Japanese.

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