

**Laboratory and Epidemiology Communications**

**Isolation of Influenza A/H3 and B Viruses from an Influenza Patient:  
Confirmation of Co-Infection by Two Influenza Viruses**

Shoichi Toda\*, Reiko Okamoto, Tomoko Nishida, Toshiki Nakao,  
Masatoshi Yoshikawa, Eitaro Suzuki<sup>1</sup> and Shigenori Miyamura

*Division of Biology, Yamaguchi Prefectural Research Institute of Public Health, Yamaguchi 753-0821, and  
<sup>1</sup>Suzuki Pediatric Clinic, Yamaguchi 755-0151, Japan*

Communicated by Ichiro Kurane

(Accepted February 13, 2006)

Influenza A/H3 and B viruses co-circulated in the 2004-2005 epidemic season in Japan, and we confirmed a case of co-infection by both viruses. The two influenza viruses were isolated from a single patient by the plaque purification technique, and co-infection was confirmed.

The patient was a 4-year-old girl whose throat swab specimens were positive for both influenza A and B viruses by a quick diagnostic agent. The virus was isolated from the throat swab by inoculation onto Madin-Darby canine kidney (MDCK) cells. The first culture sample (MDCK1) responded to B/Johannesburg/5/99 antiserum in a hemagglutination inhibition (HI) test using antisera provided by the National Institute of Infectious Diseases (NIID), Tokyo (Table 1). HA genes of both influenza A/H3 and B viruses were detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in the first culture sample. These results suggest that the A/H3 and B influenza viruses co-existed in sample MDCK1. Virus cloning was performed by plaque purification after neutralization with each antiserum (Fig. 1). The influenza A/H3 and B viruses were cloned. The influenza A/H3 virus responded only to A/Wyoming/03/2003 antiserum, and the B virus responded to B/Johannesburg/5/99 antiserum. Thus, we conclude that this patient was co-infected with A/Wyoming/03/2003-like and B/Johannesburg/5/99-like influenza viruses.

In another possible case of co-infection, a 9-year-old girl

was diagnosed with influenza at a sentinel surveillance clinic by a rapid antigen detection kit which gave a positive result for influenza A virus but did not test for influenza B virus. The patient's throat swab specimen was inoculated onto MDCK cells and the first culture sample was tested by HI assay. The virus did not respond to any of the tested antisera (data not shown), however, HA genes of both influenza A/H3 and B viruses were detected by RT-PCR in the virus sample. Unfortunately, plaque cloning of the influenza viruses was not performed in this case.

It is generally believed that co-infection by two influenza viruses is a rare event. In the present study, we confirmed a case of co-infection in a patient who visited her local clinic during the peak period of epidemic caused by influenza A/H3 and B viruses. Although there was also a second case of suspected co-infection with influenza A and B viruses, the first culture sample did not respond to any of the tested antisera in an HI assay. Paired serum samples were not available from these two cases and it was not possible to serologically confirm co-infection. The present study demonstrates the possibility of co-infection by different types of influenza viruses when an epidemic is caused by multiple types of influenza viruses.

This article appeared in the Infectious Agents Surveillance Report (IASR), vol. 26, p. 297-298, 2005 in Japanese.

Table 1. Antigenic analysis of the isolated viruses<sup>1)</sup>

Virus antigens	HI titers with Japanese standard antisera for influenza severance in 2004/05 <sup>2)</sup>				
	A/Moscow/13/98	A/New Caledonia/20/99	A/Wyoming/03/2003	B/Brisbane/32/2002	B/Johannesburg/5/99
Reference antigen					
A/Moscow/13/98 (H3N2)	<b>640</b>	40	<10	<10	<10
A/New Caledonia/20/99 (H1N1)	80	<b>320</b>	<10	<10	<10
A/Wyoming/03/2003 (H3N2)	<10	<10	<b>640</b>	<10	<10
B/Brisbane/32/2002 (Victoria)	<10	<10	<10	<b>320</b>	40
B/Johannesburg/5/99 (Yamagata)	<10	<10	<10	40	<b>1,280</b>
Isolated viruses					
first cultured virus (MDCK1)	<10	<10	<10	10	1,280
cloned A/H3 virus (MDCK5)	<10	<10	640	<10	<10
cloned B virus (MDCK5)	<10	<10	<10	<10	2,560

<sup>1)</sup>: HI titers were tested by using 0.75% guinea pig red blood cell.

<sup>2)</sup>: Reference antigen and antisera were provided by NIID for influenza pathogen surveillance.

\*Corresponding author: Mailing address: Division of Biology, Yamaguchi Prefectural Research Institute of Public Health, Aoi 2-5-67, Yamaguchi 753-0821, Japan. Tel: +81-83-922-7630, Fax: +81-83-922-7632, E-mail: toda.shouichi@pref.yamaguchi.lg.jp

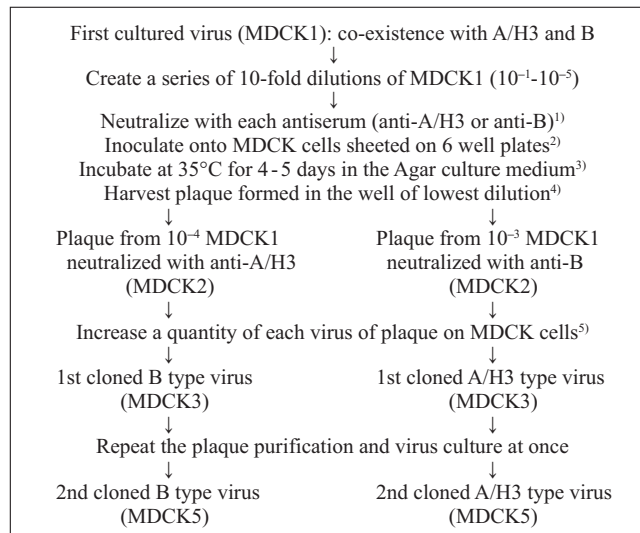


Fig. 1. Plaque purification was carried out twice and the success of cloning was confirmed by RT-PCR.

- <sup>1)</sup>: Antiserum (anti-A/Wyoming/03/2003 or anti-B/Johannesburg/5/99) were used at  $10^{-1}$  dilution, and were neutralized with same quantity of diluted viruses at  $4^{\circ}\text{C}$ , 3 h.
- <sup>2)</sup>: In each plate, 5 wells were used for  $10^{-1}$  -  $10^{-5}$  virus dilutions and 1 well for cell control.
- <sup>3)</sup>: Two milliliters of 0.8% Agar medium with 5  $\mu\text{g}/\text{ml}$  Trypsin and 0.01% DEAE-Dextran was added to each well. After agar became solid (15 min at room temperature), plates were set into  $\text{CO}_2$  incubator.
- <sup>4)</sup>: Plaques were observed as white spots without staining, and harvested by sucking with micropipette tip.
- <sup>5)</sup>: Harvested plaques were suspended in the maintenance medium and inoculated according to the usual method.