

**Laboratory and Epidemiology Communications**

**An Outbreak of Exanthematous Disease due to Coxsackievirus A9 in a Nursery in Yamagata, Japan, from February to March 2012**

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Between late February and early March of 2012, an outbreak of exanthematous disease was identified in a nursery in Yamagata, Japan. Of the 3 infants and 47 children enrolled at the nursery, 27 (11 boys and 16 girls) children aged between 4 months and 6 years (median, 1 year and 10 months) presented with fever and exanthema. These children were clinically diagnosed with viral exanthema. In addition, one of the children enrolled at the nursery had 2 siblings (aged 4 months and 1

year) who did not attend the nursery, but were diagnosed with the same disease.

To investigate the causative agents of the viral outbreak, we collected throat swabs and serum or whole blood specimens from a total of 11 patients. We isolated the virus and subsequently performed reverse transcription (RT)-PCR analysis (Table 1). For virus isolation, we used the microplate method, which included 6 previously described cell lines: human embryonic lung fibroblast (HEF), human laryngeal carcinoma (HEp-2), African green monkey kidney (Vero E6), Madin Darby Canine Kidney (MDCK), rhabdomyosarcoma (RD-18S), and green monkey kidney (GMK) cell lines (1). After centrifugation of the specimens at 3000 rpm for 20 min, 75  $\mu$ l of the supernatant was inoculated directly onto 2 wells of each cell line. The remainder of each

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Table 1. Clinical information and results of laboratory examination for the patients with exanthematous diseases at a nursery

Patient no.	Age	Clinical sign	Laboratory examination			Status of measles-rubella vaccine
			Specimen	Virus isolation	RT-PCR	
1	3Y	fever, exanthema	throat swab	CVA9	EV(CVA9)	Received
2	2Y	fever, exanthema	throat swab	CVA9	EV(CVA9)	Received
3	1Y	fever, exanthema	throat swab, serum	CVA9 Not done	EV(CVA9), rubella Negative	Not received
4	1Y	fever, exanthema	serum	Not done	Negative	Received
5	1Y	fever, exanthema	throat swab, whole blood	CVA9 Not done	EV(CVA9) Negative	Received
6	6M	exanthema	throat swab	Negative	rubella	Not received
7	7M	fever, exanthema	throat swab	CVA9	EV(CVA9)	Not received
8 <sup>1)</sup>	1Y	fever, exanthema	throat swab	Negative	EV(CVA9)	Not received
9 <sup>1)</sup>	1Y	fever, exanthema	throat swab	CVA9	EV(CVA9)	Not received
10 <sup>2)</sup>	1Y	fever, exanthema, swelling of lymph nodes	serum, throat swab	Not done CVA9	Negative EV(CVA9)	Received
11 <sup>2)</sup>	4M	fever, exanthema	throat swab	CVA9	EV(CVA9)	Not received

<sup>1)</sup>: No. 8 and No. 9 are twins.

<sup>2)</sup>: Although they do not attend the nursery, their older sister goes to the nursery.  
Y, year old; M, month old; CVA9, coxsackievirus A9; EV, enterovirus.

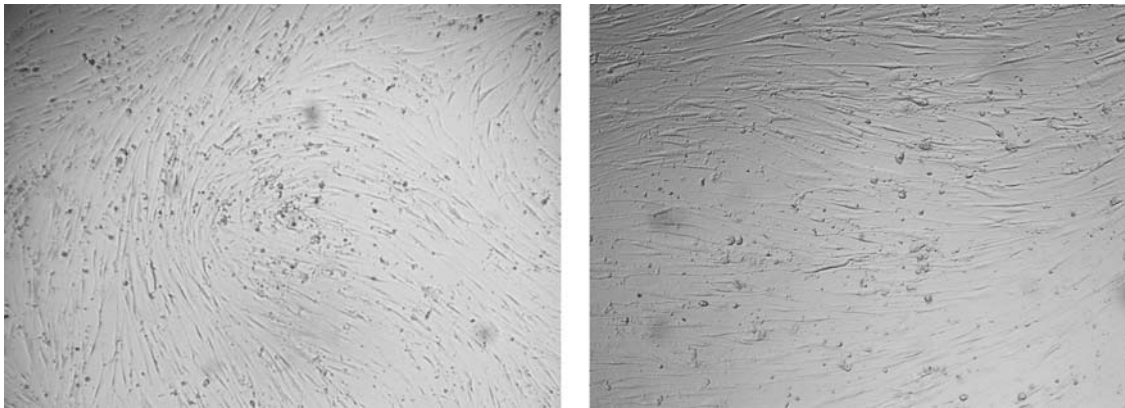


Fig. 1. Cytopathic effect of coxsackievirus A9 virus isolated from Patient no. 1 in HEF cells. Rounding, shrunken, and degenerated cell are observed.

specimen was stored at  $-80^{\circ}\text{C}$ . The inoculated plates were centrifuged for 20 min at 2000 rpm, incubated at  $33^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  incubator, and assessed for cytopathic effect (CPE) over the course of 14 days; the Vero E6 cell lines were observed for approximately 1 month without media changes to isolate human metapneumoviruses (hMPVs) (1,2). When an enterovirus-like CPE was observed (Fig. 1), we used a neutralization test, RT-PCR, and sequence analysis for identification and validation. Moreover, we tried to amplify the genomes of measles virus, rubella virus, enterovirus, and parvovirus using the following primers: MHL1, MHR1, MHL2, and MHR2 for measles (3); Rub-NSL F3 1F (5'-TCCTGCGCCGAAGACT-3'), Rub-NSL B3-6 1R (5'-AGAGGGGGTCCACTTGAG-3'), Rub-NSL F2 nestF (5'-CCACTGAGACCGGCTGCGA-3'), and Rub-NSL B2 nestR (5'-GCCTCGGGGAGGAAGATGAC-3') for rubella; 9895-forward and 9565-reverse for enterovirus (4) and PVB-1 (5'-CACTATGAAAACGGGCAATAAAC-3'), PVB-2 (5'-ATTGATTCTCCGAACTGGTCC-3'), PVB-3 (5'-ATAAACTACAC TTTTGATTTCCCTG-3'), and PBV-4 (5'-TCTCCTGAACTGGTCCCG-3') for parvovirus.

Hence, we successfully isolated coxsackievirus A9

(CVA9) from 8 patients using the HEF and RD-18S cell lines (Table 1). Additionally, we were able to amplify the enterovirus genome, which was confirmed as CVA9 in 9 patients by sequence analysis (Table 1). The presence of the rubella virus genome was detected in 2 patients who had not received the measles-rubella vaccination. However, we could not confirm an increase in antibody titers against the rubella virus using the hemagglutination inhibition test with paired sera for Patient no. 3, in whom we had isolated CVA9 and detected the rubella virus genome from an identical throat swab specimen. Thus, we concluded that this outbreak of exanthematous diseases was due to infection with CVA9. From our experience, we learned that, although difficult at times, differential diagnosis of viral exanthematous disease is extremely crucial.

Now that the measles elimination project has been progressing in Japan, the differential diagnosis of acute exanthematous diseases is critically important. The rashes associated with viral exanthematous diseases such as measles, rubella, enterovirus, parvovirus, herpes simplex, human herpesvirus 6, and varicella are very similar in appearance; they may be clinically indistinguishable in some cases (5,6). We have actively moni-

tored exanthematous diseases and carried out laboratory and differential diagnoses of measles virus infections in Yamagata to eventually eliminate measles entirely. In 2005, we had reported probable cases of measles, and in 2009, we had confirmed imported cases due to genotype D9 (7,8). Furthermore, even though 6 cases of suspected measles surfaced in Yamagata in 2011, we were able to rule out measles infection in all of these cases; we isolated rhinovirus from 1 patient and detected the rubella virus in 2 other patients (9). As a result, for the first time, we could report no cases of measles virus infections in Yamagata Prefecture in 2011. As predicted in 2005 (7), we observed that as the number of measles cases decreases, the role played by public health laboratories becomes increasingly important, as these are the primary agencies that genotype and monitor the circulation and transmission pathways of measles virus and conduct differential diagnosis of viral exanthematous diseases such as rubella and CVA9.

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**Conflict of interest** None to declare.

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