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First Isolation of Human Parechovirus Type 4 in Yamagata, Japan

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We have been engaged in an epidemiological study on viral acute respiratory infections primarily based on virus isolation from 6 cell lines using a microplate method, at pediatric clinics working in collaboration with the Yamaga Prefectural health authorities as part of the National Epidemiological Surveillance of Infectious Diseases (NESID) in Japan (1).

Human parechoviruses (HPeVs) are members of a relatively newly created genus designated Parechovirus, of the Picornaviridae family, with at least 16 types identified to date (2). In 3 large-scale studies that involved more than 600 patients with respiratory diseases, the prevalence rate of HPeV infection was 0.4–3%, and these studies detected HPeV1, HPeV3, and HPeV6 but not HPeV4 (2–5). HPeV4 has been detected and isolated infrequently, and only 1–4 cases of HPeV4 detection have been reported alongside reports on detection of other HPeVs, such as HPeV1, HPeV3, and HPeV6 (2,6–8).

On June 9, 2016, an 8-month-old girl, presenting with fever (38.6°C), cough, rhinorrhea, and nasal obstruction, visited the Yamanobe Pediatric Clinic, where she was clinically diagnosed with nasopharyngitis. Her nasopharyngeal specimen was collected on the same day and inoculated onto the LLC-MK2-N cell line. We observed a cytopathic effect (CPE) after one passage and a more pronounced CPE with cell rounding (Fig. 1A), which is similar to that of HPeV3, upon further passaging, followed by cell disruption (Fig. 1B). The microplate method is useful for the simultaneous inoculation of several clinical specimens into multiple cell lines and the verification of the epidemiology of respiratory viruses based on virus isolation (1). However, we generally do not perform passages when we cannot observe a CPE or hemadsorption. To overcome this limitation, we introduced a two-step virus isolation system. We first carried out the molecular screening of the HPeV genome and inoculated HPeV-positive specimens onto the LLC-MK2-N cell line. When this two-step method was used, HPeV1, HPeV3, and HPeV6 could be successfully isolated, and representative iso-
lates could be used for further seroepidemiological studies (13). Furthermore, the HPeV4 strain was successfully isolated for the first time. Virus isolation in cells is important for further studies, such as virological and seroepidemiological analyses, for the control of viral infectious diseases. Based on our experience, molecular screening and virus isolation can be useful for Saffold viruses as they also grow slowly (14).

Herein, we report the first successful isolation of HPeV4 and propose that molecular screening and virus isolation may be useful for epidemiological studies, apart from using the microplate method, for viruses that are known to grow slowly.

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Conflict of interest  None to declare.

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