Short Communication

Epidemiological and Molecular Studies of Measles at Different Clusters in Hokkaido District, Japan, 2007

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SUMMARY: In 2007 eight epidemic clusters (more than 15 cases in each) and other sporadic cases of measles occurred in Hokkaido district, Japan. A total of 850 cases were identified. Approximately half of them were ≥15 years of age, resulting in a huge public health problem in the community associated with school closings, and 31% of the cases reported that they already had a history of vaccination. Of 28 isolates of the measles virus detected, all were identified as genotype D5, identical to the type isolated in other areas of Japan, suggesting that a highly homologous measles virus circulated in Japan. The occurrence pattern of measles patients and molecular epidemiology indicated that the measles virus that spread in Hokkaido district might not be indigenous.

Measles virus (MV) is the monotypic virus in the genus *Morbillivirus* within the family *Paramyxoviridae*, and is the etiological agent of acute and highly contagious infection in humans (1). Infection with MV has clinical signs and symptoms such as cough, coryza, high fever, maculopapular rash and conjunctivitis, can cause death in some cases, and is often complicated with pneumonia and/or encephalitis. In 2001, a national-scale epidemic of measles in Japan occurred, with an estimated 286,000 cases, mainly among infants and young children (2). Consequently measles control was intensified all over the country (through a nationwide public awareness campaign including the establishment of a measles surveillance system by each prefecture and the promotion of timely vaccination with a first dose of live MV-containing vaccine), resulting in a dramatic decrease in cases. In recent years, measles cases have been reported in small numbers (seven cases in 2005 and three cases in 2006) in Hokkaido district. However, in the spring of 2006, a local, small epidemic of measles occurred in certain regions of Kanto district and further spread to Tokyo by the end of 2006. Subsequently, it spread throughout the whole country, including the Hokkaido district during the “Golden Week” holidays in May 2007 (3).

From 1996 - 2007, measles surveillance in Japan consisted of aggregating cases reported from two sentinel (pediatric and adult) surveillance systems. In Hokkaido district, there were 143 pediatric sentinel facilities and 23 hospitals in the adult sentinel surveillance system in 2007. Additionally, the Department of Health and Welfare, Hokkaido Government, recommended that all health practitioners report any clinical or laboratory-confirmed cases to local health officials. Therefore, the number of reported cases appeared to be close to the actual number of measles cases. The vaccination status of the cases was mainly confirmed with vaccination cards or through recall of the cases or of their parents. A database of 2007 measles cases was constructed by the Department of Health and Welfare with the cooperation of local health officials. We selected data regarding the residency, sex, age, date of onset of illness, clinical signs and symptoms and vaccination history.

The first cluster of measles occurred in Muroran city, located approximately 150 km southwest of Sapporo, the capital of Hokkaido. The initial patient officially reported was a 53-year-old woman; the date of onset of clinical illness was 23 April 2007. Subsequently, 97 cases <15 years of age and 145 cases ≥15 years of age were documented by the end of July 2007, comprising a notable peak (Fig. 1A). The second cluster occurred in Kushiro city, located approximately 300 km east of Sapporo, and lasting for approximately 2 months from July to August with 47 cases <15 years of age and 93 cases ≥15 years of age (Fig. 1B). Including other small clusters and sporadic cases, a total of 850 measles cases were ultimately reported in Hokkaido district during 2007: 449 measles cases (52.8%) <15 years of age and 401 cases (47.2%) ≥15 years of age (Fig. 1C). Fortunately, no deaths were associated with this measles epidemic. As noted, approximately half of the measles cases were ≥15 years of age, with the most reported cases between 10 and 14 years of age, leading to short-term school closures (complete or partial) including one elementary school, one junior high school, six high schools and one university.

Of the 850 documented cases, 264 (31.1%) were shown to have already been vaccinated, and 428 (50.4%), including persons ineligible for routine vaccination (i.e., <1 year of age), had no measles vaccination. The vaccination history of the remaining 158 (18.6%) cases was unclear due to the absence or uncertainty of their records.

The World Health Organization recommended that vaccination coverage of children should exceed 95% (4). Since 2002 the Department of Health and Welfare has investigated the rate of vaccination coverage for children when routine physical examinations are carried out at 18 months and 3 years of age. The rate of vaccination coverage at 3 years of age increased to 97.7% in 2006, compared to 93.6% in 2002. Eventually, the number of patients with measles between 1 and 4 years of age was small, just 86 cases (10.1%), in 2007.

The age distribution and vaccination history of the patients
shown in Fig. 1C revealed that in this epidemic the measles infection mainly targeted both unvaccinated and once-vaccinated adolescents in addition to unvaccinated young children. This suggested that one-dose vaccination was indeed insufficient to entirely prevent the infection and emphasized receiving the second dose of the vaccination to give children without sufficient immunity an additional opportunity to be protected against the infection. In contrast, it was reported recently that antibody responses after live viral infection had long half-lives of 50 years or more (5). However, the authors also found that it was unknown whether vaccine-induced immunity is stable for as long as that induced by the natural infection. Thus, for the sake of future vaccination design it is important to clarify the duration of antibody responses and to determine the timing of the additional vaccination.

To investigate the molecular epidemiology of the measles epidemic, throat swabs were obtained from 40 cases in eight different clusters including the cities of Muroran and Kushiro. Viral RNA was extracted directly by using the QIAamp viral RNA Mini Kit (Qiagen, Germantown, Md., USA) according to the manufacturer’s manual. A one-step RT-PCR kit (Qiagen) was used to amplify 574 nucleotides coding for the COOH-terminus of the nucleoprotein (N) and then a nested second PCR was conducted to amplify 533 nucleotides of the N gene as described by Morita et al. (6). Water instead of RNA or first RT-PCR product was added to the reaction mixture as a negative control in each PCR reaction. The nucleotide sequence was determined by the Applied Biosystems 3130xl genetic analyzer (Applied Biosystems, Foster City, Calif., USA) using a BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems). Sequence data of the N gene of MV corresponding to 150 amino acids of the COOH-terminus of the N protein were aligned using the CLUSTAL X program (7), and the genotype was assigned on the basis of a phylogenetic tree constructed with MEGA 3 software (8). The reliability of the tree was estimated using 1,000 bootstrap replications.

Measles viral RNA was detected in 28 (70.0%) cases by the RT-PCR. The PCR product was identified as MV by the sequence data and was indicated the genotype D5 completely identical each other. Based on 450 nucleotides coding for the COOH-terminus of MV N, phylogenetic analysis was performed on five isolates collected in May (Hokkaido.JPN/20.07/21 and Hokkaido.JPN/20.07/27; two from Muroran city), June (Hokkaido.JPN/23.07/57 from Tomakomai city), August (Hokkaido.JPN/33.07/74 from Kushiro city) and December (Hokkaido.JPN52.07/11 from Monbetsu city), and the representative genotype D5 strains as shown in Fig. 2. The phylogenetic tree showed that D5 strains were divided into two major groups: Bangkok.THA 93 and Palau.BLA 93. Japanese isolates including those of Hokkaido in 2007 were clustered in the Bangkok lineage, which differs from those spread in Japan prior to 2002 (Fig. 2). These data suggested that genotype D5 of the MV circulating in Japan was highly homologous in its nucleotide sequence, leading to the genetic stability of MV during this period. The molecular epidemiological data for the Hokkaido isolates of MV in 2007 differed from those for the isolates from Sapporo in 2001 but were homologous to those of the Gunma isolates in 2007 and the measles epidemic that occurred in Hokkaido after the epidemic in Kanto district, indicating that the recent MV isolated in Hokkaido district might not be indigenous.

As mentioned above, the Hokkaido isolates obtained between April and December were highly homologous. This coincided with a report that field isolates collected at different times often show very low levels of nucleotide divergence (9,10). Additionally, while genetic variation of MV is common in countries with endemic transmission, no variation has been observed in countries at or near the measles elimination stage (11-14). Therefore, the findings of our study demonstrated that, mainly due to the increase in successful vaccination, a movement from endemic to epidemic transmission of MV appeared to occur in Japan.

The resurgence of measles in Japan in 2007 led to the change of the sentinel surveillance system to nationwide mandatory case-reporting in January 2008, and all health practitioners were required to report any clinical or laboratory-confirmed case to local health officials (2). Moreover, in addition to the usual two-dose vaccination program for children aged 1 and 6 years old (before entrance to elementary school), a 5-year vaccination catch-up campaign was initiated in April 2008, targeting cohorts aged 13 and 18 years. We conclude that, in Japan, the implementation of high vaccination coverage of children and adolescents seems to be
necessary to prevent future outbreaks.

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REFERENCES