Short Communication

Phylogenetic Analysis and Seroprevalence of Influenza C Virus in Mie Prefecture, Japan in 2012

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SUMMARY: Reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR were used to detect 14 (6.6%) influenza C virus (InfC) among 213 clinical samples collected from children with respiratory symptoms in Mie Prefecture, Japan, between January 2012 and December 2012. Virus isolation using Madin-Darby canine kidney cells and/or embryonated chicken eggs was also successful for 3 of the 14 PCR-positive samples. Eleven patients (78.6%) were aged <3 years. Phylogenetic analysis of the hemagglutinin-esterase gene showed that the InfC detected in Mie Prefecture belonged to the C/Sao Paulo/82-related lineage. To determine the seroprevalence of InfC, a total of 575 serum samples from patients aged 1 month to 69 years in Mie Prefecture were screened by hemagglutination inhibition test using the C/Mie/199/2012 (C/Sao Paulo/82-related lineage) strain as the antigen. The samples with an antibody titer of ≥1:16 were designated as antibody-positive. The results showed that 53.7% of the 296 serum samples collected in 2011 and 85.3% of the 279 samples collected in 2012 were positive for antibodies against InfC, suggesting that an outbreak of InfC infection occurred in Mie Prefecture in 2012. Therefore, continuous and proactive monitoring is important to determine the number of InfC-infections and to better understand the epidemiology.

Influenza C virus (InfC) is a causative agent of acute respiratory tract infections in children. It was first isolated from a patient with a slight head cold by Dr. Taylor in the United States in 1947 (1). Reportedly, most children acquire antibodies against InfC by approximately 6–7 years of age and children and adults are repeatedly infected with InfC (2–5). In Japan, InfC is isolated mainly from January to June and, where prevalent, particularly between early spring and early summer (6–8). Furthermore, a biennial increase and decrease in the number of InfC infections detected has been observed, suggesting the possibility of periodic outbreaks (9).

According to the National Epidemiological Surveillance of Infectious Diseases (NESID) system, InfC infection was detected in only 226 cases between the 2002–2003 and 2012–2013 seasons in Japan, whereas there were 79,169 and 15,386 type A and B influenza cases, respectively. Therefore, the number of InfC infections reported to the NESID was extremely low compared with the number of type A or B influenza infections (10). However, it is believed that this is due to the fact that few laboratories perform proactive surveys of InfC infection. Thus, at present, the precise distribution of InfC among cases of respiratory tract infections is not well understood. In this study, we report 14 cases of InfC detected in Mie Prefecture, Japan, in 2012 and provide seroprevalence results of InfC among residents of Mie Prefecture.

Clinical samples (nose discharge and pharynx wipes) were collected from pediatric patients who presented at either of two medical institutions with respiratory symptoms in Mie Prefecture between January 2012 and December 2012. Among the 237 clinical samples that were transported to the Mie Prefecture Health and Environment Research Institute, 24 were positive for influenza A and B viruses using rapid antigen tests. Therefore, 213 samples were analyzed for InfC, exempting the 24 influenza A- and B-positive samples. All 213 patients lived in Kameyama City or Tsu City, which are located in the north-central region of Mie Prefecture.

Each sample was subjected to reverse transcription polymerase chain reaction (RT-PCR) for hemagglutinin-esterase (HE) gene amplification and real-time RT-PCR with a TaqMan probe targeting the NP gene using the methods described by Kimura et al. (11) and...
Matsuzaki et al. (12), respectively. Of the 213 tested samples, 14 (6.6%) were InfC-positive by both RT-PCR and real-time RT-PCR. Of the 14 InfC-positive samples, 12 were obtained from patients residing in Kameyama City and 2 from patients residing in Tsu City. PCR was used to detect the presence of other viruses, as described previously (13–20). In addition to InfC, a total of 155 viruses were detected, which included human rhinovirus (n = 43), human metapneumovirus (n = 36), human parainfluenza virus (n = 30), RS virus (n = 25), human coronavirus (n = 11), human bocavirus (n = 8), and influenza virus (AH3) (n = 2). The remaining 44 samples were negative for all tested viruses.

Next, we isolated viruses from the InfC-positive samples using Madin-Darby canine kidney (MDCK) cells. In brief, the cells were inoculated and then incubated for 7–10 days, and the conditioned media collected from the cultures were then examined for hemagglutination activity (HA) using 0.75% guinea pig erythrocytes and 0.5% chicken erythrocytes. Only samples that were HA-positive in chicken erythrocytes and HA-negative in guinea pig erythrocytes were considered as positive for InfC. Two samples were found to be HA-positive in chicken erythrocytes after propagating twice or thrice in MDCK cells (C/Mie/185/2012 and C/Mie/199/2012). A hemagglutination inhibition (HI) assay was performed using antiserum against C/Ann Arbor/1/50, which was obtained from Yamagata University Faculty of Medicine, to identify the InfC strain. Moreover, 2 InfC strains were isolated from clinical samples using embryonated chicken eggs (C/Mie/199/2012 and C/Mie/231/2012).

The clinical characteristics of the 14 InfC-positive children are presented in Table 1. InfC infections were detected in children aged 4 months to 6 years. Eleven patients (78.6%) were aged <3 years old. The ratio of males to females was 1:1. Body temperatures on initial examination ranged from 36.5°C to 39.8°C. These patients were diagnosed with bronchitis (n = 2), bronchiolitis (n = 7), laryngitis (n = 2), pharyngitis (n = 2), and tonsillitis (n = 1). InfC was detected in 6.8% (9 cases in 133 samples) of patients diagnosed with lower respiratory tract infections, which was similar in frequency among patients diagnosed with upper respiratory tract infections (6.2%; 5 cases in 80 samples). A previous report indicated that most patients with InfC infections were aged <6 years and many patients aged <2 years (approximately 30%) required hospitalization, mainly for pneumonia, bronchitis, or bronchiolitis (6). Therefore, it is essential to consider the potential for increased severity in patients with InfC infection, as InfC was detected in patients with pneumonia from Mie Prefecture in 2011 (21).

The HE gene was sequenced in the 14 InfC-positive samples to determine the lineage of the circulating viruses. Total RNA was extracted from each clinical sample for sequencing analysis; however, RNA extraction from C/Mie/185/2012 and from C/Mie/199/2012 and C/Mie/231/2012 were performed with each virus isolated using MDCK cells and chicken eggs, respectively. Amplification and sequencing of the region from nucleotide 19 to 1083 of the HE gene were performed using the methods described by Kimura et al. (11). The nucleotide sequences were submitted to the DDBJ/GenBank databases and assigned the accession numbers AB751364–AB811846. A phylogenetic tree of the HE gene was constructed using these 14 sequences together with previously published sequences (Fig. 1). Matsuzaki et al. classified the InfC HE gene into five lineages, which are represented by C/Yamagata/26/81 (D28971), C/Kanagawa/1/76 (D34370), C/Aichi/1/81 (D28970), C/Mississippi/80 (M11640), and C/Sao Paulo/378/82.
Phylogenetic Analysis of Influenza C Virus

Fig. 1. Phylogenetic analysis of the HE genes of influenza C viruses detected in Mie Prefecture, Japan, in 2012. The region from nucleotides 64 to 965 was used for analysis. The tree was constructed using neighbor-joining method. Figures at the nodes indicate confidence levels of bootstrap analysis using 1,000 replicates as percentage value. Reference strains: Sao Paulo/378/82 (AB035364), Aichi/1/81 (D28970), Kanagawa/1/76 (D63470), Yamagata/26/81 (D28971), Mississippi/80 (M11640).

Serological analysis of InfC was performed using serum samples collected from 575 persons aged 1 month to 69 years who visited hospitals located in Mie Prefecture for a medical examination (249 persons) or medical checkup (326 persons) between April and September 2012 (279 samples) and from April to September 2011 (296 samples). All serum samples were tested after obtaining informed consent from the patients or their guardians. This seroprevalence study was conducted while protecting the personal information of each participant. Antibody levels were determined by measuring serum HI titers, which were expressed as the reciprocal of the highest serum dilution that inhibited hemagglutination. The C/Mie/199/2012 strain propagated in chicken eggs was used as the antigen for the HI test and HI titration was performed as described previously (2).
The samples with an antibody titer of $\geq 1:16$ were designated as antibody-positive. The results showed that 53.7% of the 296 serum samples collected in 2011 and 85.3% of the 279 samples collected in 2012 were InfC antibody-positive (Figs. 2 and 3). This finding appears to reflect an outbreak of InfC within Mie Prefecture in 2012, although too few serum samples were obtained from infants aged 6–11 months in 2011 to claim that InfC was not in circulation in 2011. Because the serum samples in Fig. 3 were collected from the period immediately after the circulation of InfC in Mie Prefecture in 2012, we believe that this sample size was sufficient to confirm an outbreak of InfC. Although further annual seroprevalence data are needed to define the periodicity of InfC transmission, this is the first seroepidemiological study of InfC performed over two continuous seasons. As shown in Fig. 3, the antibody retention ratio in 2012 was lowest (28.6%) in children aged 6–11 months and increased with age until it reached 96.1% in the 10–19 years age group, suggesting that all the children had been exposed to InfC by the age of 10 years. Although a relatively small number of serum samples were obtained from infants aged 0–5 months, there was a considerable difference in the seroprevalence in this age group from 2011 to 2012. Because the prevalence of antibodies in infants aged 0–5 months is influenced by maternal antibodies, the difference in the prevalence of antibodies in this age group (66.7% in 2012 vs. 0% in 2011) may have been due to the difference in seroprevalence among the mothers in the age group 20–29 and 30–39 years. The high prevalence of antibodies among adults in 2012 indicates that reinfection must have been
common, an observation that supports findings previously reported in Japan (2,27–29).

A local InfC outbreak occurred in the north-central region of Mie Prefecture in 2012, a year in which only 60 cases of InfC infection were reported throughout Japan (30). Patients with InfC infection were reported in Yamagata, Niigata, Hiroshima, and Mie Prefectures and Shizuoka City. In Mie Prefecture, 13 of 14 cases of InfC infection were detected between March and April 2012. There have been no reports of InfC cases after May 2012 in our prefecture, although 19 cases were detected between May and July 2012 in Yamagata Prefecture. Thus, the mechanism of nationwide epidemics of InfC infection is not yet sufficiently understood. The identification of InfC infection based on clinical symptoms is difficult (6). The real-time RT-PCR method (12) used in this study was highly sensitive and rapid; therefore, it is a useful method to diagnose InfC infections. InfC infection is likely to become more prevalent, thus continuous and proactive monitoring studies are highly important to monitor the number of patients with InfC infection and to understand its epidemiology.


Conflict of interest None to declare.

REFERENCES


