Original Article

Seasonal Patterns of Respiratory Syncytial Virus, Influenza A Virus, Human Metapneumovirus, and Parainfluenza Virus Type 3 Infections on the Basis of Virus Isolation Data between 2004 and 2011 in Yamagata, Japan

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SUMMARY: Most acute respiratory infections (ARIs) are thought to be associated with respiratory viruses that cause similar symptoms. Therefore, assessment of clinical and epidemiologic features of these viruses is important for diagnosing a viral infection. We collected 13,325 nasopharyngeal specimens from patients with ARIs and isolated the virus using a microplate method involving 7 cell lines between 2004 and 2011 in Yamagata, Japan. We isolated a total of 5,483 viruses. Respiratory syncytial virus (RSV), influenza A virus (FluA), human metapneumovirus (hMPV), and human parainfluenza virus type 3 (hPIV3) showed clear yearly seasonal patterns; generally, RSV infections peaked at the end of the year, FluA infections peaked between January and March, hMPV infections peaked between March and April, and hPIV3 showed seasonal outbreaks between May and July. Further, RSV, hMPV, and hPIV3 were commonly isolated in 12.0–13.1% of specimens from children aged less than 4 years, whereas FluA was isolated in 7.3–8.2% of specimens from school-aged children. A generalized view of seasonality and age distribution, particularly on the basis of longitudinal epidemiological data, will be helpful for medical decision-making, including decisions related to the use of rapid test kits, selection of antiviral treatments, restriction of antibiotic therapy, and implementation of infection control strategies.

INTRODUCTION

As many as 90% of the causal agents of acute respiratory infections (ARIs) are thought to be exclusively viral agents, and more than 200 serologically different viruses have been documented as causative agents of ARIs (1–5). Furthermore, many respiratory viruses cause similar symptoms such as fever, rhinorrhea, and cough, and infected patients are often diagnosed with colds, pharyngitis, bronchitis, croup, bronchiolitis, and pneumonia (2,6). Thus, in cases of ARI, it is difficult to make a differential diagnosis with regard to the causative agent in a clinical setting.

Diagnosis of a viral infection begins with assessment of clinical and epidemiologic features (7). The latest information of infectious diseases, such as community outbreaks, also helps in estimating the causative agent. Clinical diagnosis with epidemiological considerations suggests a number of possible etiologic agents, and a cli-

nician must suggest a suspected etiologic agent to the laboratory to allow selection of the appropriate specimens and tests (7).

An efficient viral detection system is of great importance for laboratory diagnosis. Viral cultures, antigen detection, and molecular methods, such as PCR, are currently used as viral detection systems, and each method has its advantages and disadvantages (2,3). In clinical settings, viral antigen detection kits, which are rapid and easy to use, are particularly helpful in making early decisions regarding management and treatment of the patients. Currently, rapid antigen detection test kits are available for influenza viruses, adenoviruses (Ads), and respiratory syncytial virus (RSV), and an immunochromatographic test kit for human metapneumovirus (hMPV) has recently become commercially available in Japan (8,9).

Here, we describe the results of a virus isolation conducted in patients with ARI in Yamagata, Japan between 2004 and 2011, focusing on RSV, influenza A virus (FluA), hMPV, and human parainfluenza virus type 3 (hPIV3), all of which showed clear yearly seasonality trends. The use of rapid antigen detection kits in association with assessment of the epidemiological features of these viruses, such as seasonality and age distribution,

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may help clinicians make an early differential diagnosis and select an appropriate treatment for patients with ARI.

MATERIALS AND METHODS

Patients: Between January 2004 and December 2011, 13,325 nasopharyngeal swab specimens were collected from patients with ARI at two pediatric clinics (Yamanobe and Katsushima Pediatric Clinic) in collaboration with the Yamagata prefectural health authorities for the national surveillance of viral diseases in Japan based on the Infectious Diseases Control Law. Informed consent was obtained from the patients or their guardians before participating in this surveillance program. Among the specimens, 9,692 (72.7%) were from patients aged ≤ 5 years, 2,385 (17.9%) were from patients aged between 6 and 10 years, 897 (6.7%) were from patients aged between 11 and 15 years, 285 (2.1%) were from patients aged > 15 years, and 66 (0.5%) were from patients of unknown age. Nasopharyngeal specimens were placed in tubes containing 3 ml of transport medium, and transported to the Department of Microbiology, Yamagata Prefectural Institute of Public Health for virus isolation (10).

Virus isolation and identification: We have used the HHV6MRG plate, which contains HEF, HEp-2, Vero E6, MDCK, RD-18S, and GMK cell lines, since January 2004. The 96-well tissue culture plates (Greiner bio-one, Frickenhausen, Germany) were used vertically. Two rows of each cell line were prepared as described previously (10,11). HMV-II cell lines were also prepared as separate 96-well microplates and have been used since 2008 primarily for isolating hPIVs (12,13). The composition of the growth medium and maintenance medium used are shown in Table 1. Fetal bovine serum (Gibco, Life Technologies Co., Grand Island, N.Y., USA and Nichirei Biosciences Inc., Tokyo, Japan), calf serum (Thermo Electron Co., Melbourne, Australia), vitamin solution (Sanko Junyaku, Tokyo, Japan), L-glutamine (200 mM; Gibco), Pen Strep (10,000 U/ml and 10,000 μ g/ml, respectively; Gibco), sodium bicarbonate (7.5% solution; Gibco), and crystallized trypsin (T-8003; Sigma, St. Louis, Mo., USA) were added to Eagle's minimum essential medium (MEM; Nissui No3; Nissui Pharmaceutical Co., Tokyo, Japan) or Roswell Park Memorial Institute 1640 medium (RPMI 1640, Nissui No. 2; Nissui). After centrifugation of the specimens at

$1,500 \times g$ for 20 min, 75 μ l of supernatant was inoculated directly onto 2 wells containing each cell line. The remainder of the specimen was stored at -80°C . The inoculated plates were centrifuged for 20 min at $450 \times g$, incubated at 33°C in a 5% CO_2 incubator, and assessed for the cytopathic effect (CPE) for 14 days, except for plates with the Vero E6 cell lines, which were observed for approximately 1 month without changing the medium to isolate hMPVs (11).

When a CPE was observed or the hemagglutination test and/or hemadsorption test was found to yield positive results using guinea pig erythrocytes (0.8%) and/or chicken erythrocytes (0.5%), virus identification was carried out using a neutralization test, hemagglutination inhibition test, reverse transcription-PCR, or sequence analysis as described previously (10–14).

RESULTS

Whole virus isolation: Using the microplate method, we isolated a total of 5,483 viruses, which were categorized as follows: 543 FluA viruses, including FluA/H1, FluA/H3, and FluA/H1pdm, 173 FluB, and 95 FluC viruses; 280 hPIV1, 155 hPIV2, 512 hPIV3, 329 RSV, 414 hMPV, and 158 mumps viruses; 706 Ads, including Ad types 1–6 and 11; 471 coxsackievirus A (CVA) viruses, including CVA types 2, 3, 4, 6, 9, 10, 14, and 16; 477 CVB viruses, including CVB types 1–5; 313 echoviruses, including echovirus types 3, 6, 7, 9, 11, 16, 18, 25, and 30; 15 enterovirus 68 and 54 enterovirus 71 viruses; 173 rhinoviruses; 14 parechovirus type 1 and 34 parechovirus type 3 viruses; 435 cytomegaloviruses, 131 herpes simplex viruses (not typed), and 1 varicella-zoster virus.

Monthly numbers and monthly cumulative numbers for RSV, FluA, hMPV, and hPIV3: Yearly seasonal patterns were clearly observed for RSV, FluA, hMPV, and hPIV3 among viruses isolated during the study period; monthly isolation numbers for these four viruses are shown in Fig. 1. Enteroviruses were excluded since they were commonly isolated in the summer season, but showed different serotypes on a yearly basis. Seasonal variations and fluctuations in the monthly number were observed and recorded on a yearly basis for each virus isolated. Although RSV infections were observed throughout the year, they primarily occurred in the autumn and winter seasons (September–January). RSV infections peaked at the end of each year except in

Table 1. Composition of growth and maintenance medium for the seven cell lines of the HHV6MRG plate and HMV-II plate

Cell line	HEF	HEp-2	Vero E6	MDCK	RD-18S	GMK	HMV-II
Growth medium	MEM ¹⁾ + 10% ²⁾ FBS ³⁾	MEM + 5% CS ⁴⁾ + 1.7% glucose	MEM + 5% FBS + 5% CS	MEM + 5% FBS + 5% CS	MEM + 8% FBS	MEM + 10% CS	RPMI ⁵⁾ + 10% FBS
Maintenance medium	MEM + 2% FBS	MEM + 2% FBS + 1.7% glucose	MEM + 0.2% glucose + 4% vitamin solution + crystallized trypsin (0.8 μ g/ml) ⁶⁾	MEM + 0.2% glucose + 4% vitamin solution + crystallized trypsin (1.4 μ g/ml)	MEM + 2% FBS	MEM + 2% FBS	RPMI + crystallized trypsin (8 μ g/ml)

¹⁾,⁵⁾: Eagle's minimum essential medium (MEM) and Roswell Park Memorial Institute 1640 medium (RPMI) including sodium bicarbonate (final concentration 0.135%) and antibiotics (penicillin 100 U/ml and streptomycin 100 μ g/ml).

²⁾,⁶⁾: Percentage and trypsin density indicate final concentration.

³⁾: Fetal bovine serum.

⁴⁾: Calf serum.

Fig. 1. Monthly virus isolation from patients with acute respiratory infections in Yamagata, Japan. (A) Specimens; (B) RSV; (C) FluA; (D) hMPV; (E) hPIV3.

2011. Outbreaks due to FluA/H1 pandemic virus in 2009 clearly began in the summer (August), peaked in the autumn, and then decreased, whereas in years other than the 2009–2010 season, FluA infections typically began at the end of the year, peaked in between January and March, and then decreased. hMPV infections were commonly observed during the first half of the year and typically peaked in the spring season between March and April, except in 2007, when hMPV infections peaked at the end of the year. Annual virus isolation frequencies of hPIV1–3 increased after introduction of the HMV-II cell line (13), and hPIV3 infections showed clear seasonal outbreaks between May and July every year and rarely appeared during other periods. Generally, the peaks for RSV, FluA, hMPV, and hPIV3 occurred in this order, except that an unusual novel FluA epidemic preceded RSV in 2009 and the peaks of multiple viruses occasionally coincided; for example, RSV and hMPV co-circulated simultaneously in the winter season between 2007 and 2008. Although yearly fluctuations were observed, as shown in Fig. 1, the monthly cumulative numbers for the four viruses shown in Fig. 2 clearly shows that RSV, FluA, hMPV, and hPIV3 infections appeared in this order.

Occurrence ratios of RSV, FluA, hMPV, and hPIV3 by age: Occurrence ratios of RSV, FluA, hMPV, and hPIV3 viruses by age are shown in Fig. 3. These four

viruses accounted for 13.8–15.8%, 10.9–11.2%, and 13.7–14.0% of the specimens tested in the <4 years, 4–10 years, and >10 years age groups, respectively. RSV, hMPV, and hPIV3 viruses, in particular, accounted for 12.0–13.1% of the specimens tested in the <4 years age group.

The occurrence ratios of each virus differed depending on the age group examined. RSV accounted for 3.2–4.1% of specimens in the <4 years age group, while its rate of isolation decreased with increasing age. hPIV3 also accounted for 4.3–6.5% of specimens in the <4 years group, after which the frequency decreased with age before increasing again to 2.5–3.0% in the >10 years group. hMPV accounted for 2.7–2.8% of the specimens in the <2 years group, and its frequency then increased to 4.1–5.2% in infants aged between 2 and 3 years, and consistently accounted for 2.1–3.7% of specimens from children aged more than 3 years. In contrast, the rate of FluA isolation increased with age and accounted for 4.1–8.7% of specimens in children above the age of 3 years and 7.3–8.2% in school-age children between 6 and 15 years old. The rate of FluA isolation in school-age children was significantly higher than that in children less than 6 years old (chi-square test; $P < 0.01$).

Fig. 2. Monthly cumulative numbers of virus isolates from patients with acute respiratory infections in Yamagata, Japan. (A) RSV; (B) FluA; (C) hMPV; (D) hPIV3.

Fig. 3. Proportion of isolated viruses by age among the patients with acute respiratory infections in Yamagata, Japan.

DISCUSSION

Although the exact seasonal appearance of each virus in the community cannot be precisely predicted, generalizations are useful for diagnosis and for planning control strategies. An example of this is in the publication of the numbers of virus isolates from children seen in private pediatric practices in order to establish seasonal variations (2). In designing this study, we identified isolates but also included age factors. In this study, we

found that four respiratory viruses, RSV, FluA, hMPV, and hPIV3, showed clear seasonal patterns and appeared sequentially in this order, although with some variations from year to year.

In general, RSV shows clear seasonality in the temperate zones of the northern hemisphere and yearly epidemics of this virus occur primarily in the late fall, winter, or spring; most outbreaks peak in February or March, whereas the virus is rarely isolated in the summer (3). In Japan, RSV infections occur mainly between October and March each year and peak at the end of each year, although peak values differ slightly between different geographical areas; for example, RSV epidemics occur in the summer in Okinawa, which is located in the southern part of Japan (15). The seasonality of RSV in Yamagata showed the typical pattern for Japan and for temperate regions of the northern hemisphere. Seasonality of FluA in Yamagata was similar to that in Japan and was typical for the temperate region of the northern hemisphere; the standard epidemic pattern peaked during the winter season, except that the 2009/2010 influenza season showed an unusual epidemic pattern associated with a novel influenza virus as shown in Fig. 1 (6,16,17). hMPV can be detected year-round, but infections by this virus typically peak in the late winter to spring, coincident with or slightly later than RSV infections (6,18). As we reported previously,

hMPV infection rates remain high from winter to spring in Yamagata, while the infection rate is low in the fall (11,14). We previously presented detailed data regarding the epidemiology of hPIV1–3 infections and concluded that hPIV3 infections showed clear seasonality with yearly outbreaks in the spring-summer season, and that this seasonality in hPIV3 outbreaks is observed worldwide (13).

Descriptions of the seasonality of the combination of RSV, FluA, hMPV, and hPIV3 are limited. Brandt et al. suggested that RSV may interfere with FluA, FluB, and hPIV under epidemic conditions in Washington D.C. (19). Easton and Eglin showed that RSV infections occurred in the winter, whereas hPIV3 infections occurred yearly in the spring and summer between 1978 and 1987 in the United Kingdom (20). Johnston et al. revealed that FluA and hPIV infections peaked in November 1989, while RSV infections peaked between December and January 1990 in the United Kingdom (21). Nishimura et al. reported that epidemiological interference occurred between RSV and Flu during three winter seasons and that the peak of RSV infections preceded that of influenza in Kobe, Japan between 1999 and 2002 (22). Iwane et al. reported that RSV infections peaked in January 2001, Flu in February, and hPIV in March–May and August–September in 2002 in the United States (23). Nicholson et al. showed that an RSV outbreak preceded the Flu outbreak and that hMPV cocirculated during this outbreak period between 2001 and 2002 in the United Kingdom (24). Wolf et al. showed that RSV infections peaked earlier than or simultaneously with hMPV infections, and it appears likely that FluA infections peaked earlier than or simultaneously with the above two viruses in Israel between 2001 and 2005 (18). Williams et al. reported that RSV infections peaked in December, Flu in January, hMPV in March, and hPIVs in October and April based on their observation between 1982 and 2001 in the United States (25). Fry et al. and Hall showed a clear contrast between yearly RSV infections occurring during the winter and hPIV3 infections occurring during the spring and summer between 1990 and 2003, and 1993 and 1998, respectively, in the United States (26,27). Although longitudinal observations of the seasonality of these four viruses are limited, our data indicate a similar pattern to that suggested by Williams et al., although they did not divide hPIVs by serotype (25).

The relationship between viral infections and age groups is another important epidemiological factor. Previous studies showed that infection with RSV is nearly universal during the first few years of life (3,15). hPIV3 as well as RSV infections are common in infants and young children, and over 80% of children are infected by 4 years of age (3,28). In contrast, FluA infections occur in persons of all ages, and are particularly common among the school-age population (16). It is reported that hMPV is ubiquitous, with virtually all children worldwide infected by 5–10 years of age, which agrees with our previous data (6,29). Our data generally support these epidemiological features related to age and RSV, FluA, hPIV3, and hMPV infections.

The seasonality of respiratory viruses varies considerably depending on the time of year, year-to-year variations, age, geographical location, and population stud-

ied (3). Thus, it is important to conduct epidemiological surveys of ARIs both longitudinally and locally. Such generalizations, particularly based on longitudinal epidemiological data, will be helpful in medical decision-making, including decisions regarding the use of rapid test kits, selection of antiviral treatments, restriction of antibiotic therapy, and infection control strategies.

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Conflict of interest None to declare.

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