Table 1. Estimated number of virological type-specific influenza-like illness patients during the 2010–2014 epidemic seasons.

	Estimated number of influenza patients (95% confidence intervals)			
	2010/11	**2011/12**	**2012/13**	**2013/14**
Annual total (10 thousands)				
A(H1)pdm09	648 (633–663)	3 (1–5)	26 (20–32)	674(653–695)
A(H3)	405 (390–421)	1089 (1064–1114)	1073(1053–1093)	254(239–269)
B	284 (271–297)	554(532–575)	229 (215–243)	616 (596–637)
Peak week (10 thousands)				
A(H1)pdm09	140.5 (133.2–147.7)	-	-	110.8 (102.8–118.8)
	Week 4	-	-	Week 5
A(H3)	36.4 (31.9–40.9)	174.4(164.4–184.4)	196.7(188.9–204.5)	36.4(34.4–45.2)
	Week 5	Week 5	Week 4	Week 5
B	35.7 (30.7–40.7)	55.9(49.3–62.4)	26.4(20.8–32.0)	66.5 (59.6–73.3)
	Week 11	Week 10	Week 6	Week 12

The estimated number of cases of influenza A(H1)pdm09 infection were excluded because their weekly numbers of patients were all below 5000.

doi:10.1371/journal.pone.0146520.t001

largest weekly number of influenza type A(H1)pdm09 patients was observed in week 4, year 2011 [1,405,000 (1,332,000–1,477,000)], followed by seasonal influenza [A(H3): 364,000 (319,000–409,000) in week 5, B: 357,000 (307,000–407,000) in week 11] (Table 1). The incidence rates of influenza A(H1)pdm09, A(H3) and B were 0.05, 0.03 and 0.02, respectively. While in the 2011 epidemic season (from September 2011 to May 2012), seasonal type influenza A(H3) was dominant [total estimated number: 10,890,000 (10,640,000–11,140,000)], followed by influenza type B [total estimated number: 5,540,000 (5,320,000–5,750,000)]. This trend was similar in the 2012/13 season; however, in the 2013/14 season, influenza type A(H1) pdm09 was again dominant [total estimated number: 6,740,000 (6,530,000–6,950,000)].

Fig 2 shows the age distribution of patients among the estimated number of virological type-specific influenza cases during the four-year period from 2010 to 2014. During this period, approximately 50% of patients were aged 5–19 or younger, with few patients aged 60 and over, for all of the virus types. Type B influenza incidence showed a particularly strong relationship with younger age groups. For example, in 12 weeks of 2014, the number type B influenza patients of 5–19 years old was 367,000 (95% CI; 318,000–416,000), which was significantly larger than that of 20–59 years old (185,000 (95% CI; 144,000–225,000)).

Discussion

In this study, the number of virological type-specific influenza cases over a four-year period (from week 36, 2010 to week 18, 2014) in Japan was estimated using information from the infectious disease surveillance database. An epidemic of influenza A(H1)pdm09 first occurred in Japan in 2009 [11,12]. Our estimated numbers clearly show the huge impact of influenza A (H1)pdm09 on the Japanese population in the 2010/2011 and 2013/2014 seasons.

The proposed method for the estimation of virological type-specific numbers of influenza was based on a stratified-sampling technique. This technique requires a large number of sentinel medical institutions and a well-designed protocol for selecting the location of these institutions. Infectious disease surveillance in Japan, which includes a large number of sentinel medical institutions equally distributed throughout the nation, is best suited to using a sampling technique to estimate the number of influenza patients. In the United States, the Centers

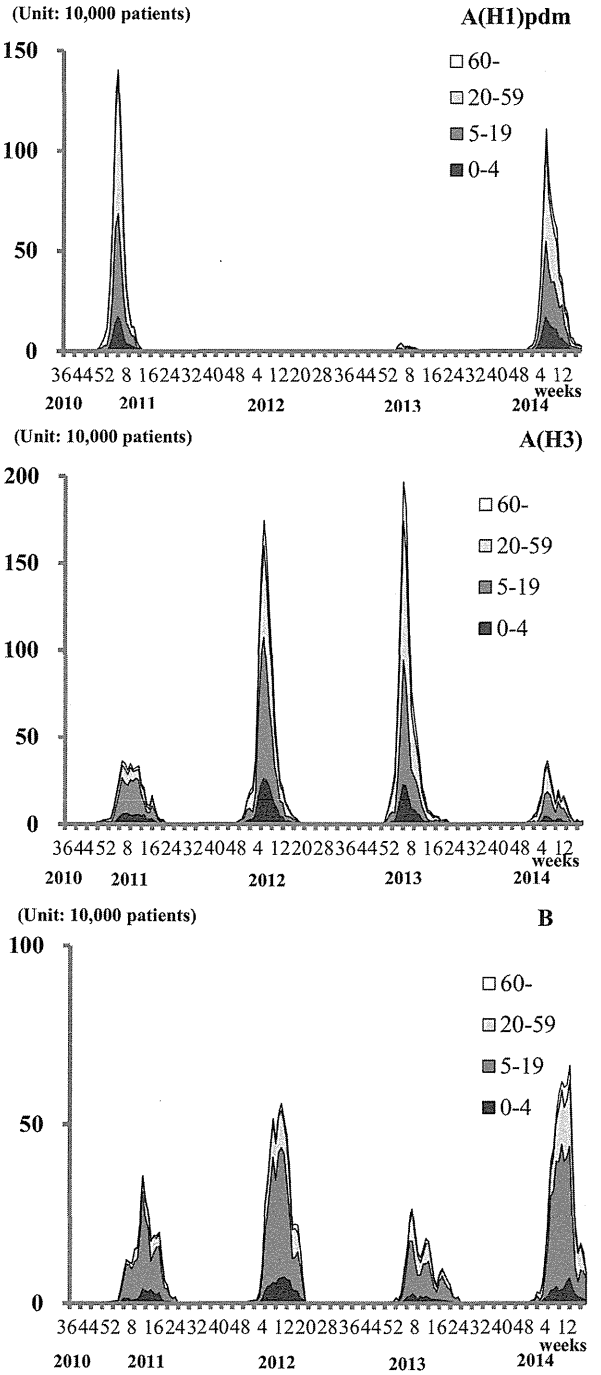


Fig 2. Age distribution of the estimated number of cases of influenza infection according to virological type from week 36, 2010 to week 18, 2014, Japan. Unit of the vertical line: 10,000 patients

doi:10.1371/journal.pone.0146520.g002

for Disease Control and Prevention applied a probabilistic multiplier model for the estimation of the number of influenza A(H1)pdm09 cases nationwide [13]. In this method, a nested structure of six steps was considered for sample collection (including reported cases, test-detected cases, specimen-tested cases, specimen-collected cases, cases seeking medical care for influenza, and total influenza cases) and the multiplier (the inverse of the sampling proportion at each step) was used to estimate the total number of influenza cases [5,13]. This method is straightforward but incorporates many assumptions regarding the "multipliers" in the estimation process. Our estimation method was based on a stratified random sampling technique, the rationale for which was provided by the sampling design. Local public centers selected sentinel medical institutions in Japan based on well-designed criteria to ensure that they were distributed equally across the nation. Although the uncertainty of adherence to these criteria remained in our estimation, a large number of sentinel medical institutions (almost 5000) demonstrated pseudo-stratified random sampling. One study in Japan [14] claimed that our technique might result in an overestimation compared with other methods. This was because the sentinel medical institutions with a high frequency of patient visits were more likely to be included in the infectious disease surveillance system. Improved methods for the unbiased selection of sentinel medical institutions would therefore be desirable [15]. Data on the virological type of influenza cases were derived from laboratory-based infectious agents surveillance. This type of surveillance generated fewer samples compared with sentinel surveillance, leading to instability in the estimation of virological type-specific influenza. Confidence intervals represent the stability of an assessing estimation and the narrow range of confidence intervals shown in Table 1 demonstrate that the annual estimate of the number of virological type-specific influenza cases showed acceptable stability.

Several limitations should mentioned for our study. First, we did not present any regional-specific result that could compare the dominant sub/types of influenza in different regions. In the database of infectious disease agents surveillance, we do not have any regional-based information of influenza sub/types. We suppose that the dominant subtype of influenza in some regions might be different from those of other regions. In case that a regional specific information of influenza virus type is available, we can apply our methods for estimating the regional specific results. The enhancement of infectious disease agents surveillance is desirable to achieve that goal.

Second, we should comment on the inherent bias of our estimation. As we mentioned, the selection of sentinel medical institutions is based on the protocol. In the protocol, the sentinel doctors were encouraged to choose samples randomly, but the decision were totally depend on the doctors. So, we cannot deny the possibility of an inherent bias of specimen selection in the surveillance.

From a public health perspective, the annual estimated number of cases of influenza-like illness throughout the nation provides essential information on which to base future prevention and control strategy decisions to avoid nationwide influenza epidemics. For example, the estimated number of influenza A(H1)pdm09 cases in the 2010/11 season showed a magnitude similar to the first epidemic of this virological type of influenza in 2009 in Japan. This information is vital in preparing for future influenza epidemics [16], with preparations including vaccine storage, increasing medical facilities and provisions, and assigning a budget for ensuing costs. In our opinion, data on the weekly number of patients with virological type-specific influenza could also be made available if this estimation process was built into the infectious surveillance system. This close monitoring of infections would enable early stage intervention to help prevent future influenza epidemics.

Supporting Information

S1 Appendix. The flow diagram of infectious disease surveillance system in Japan. Footnote; This flow diagram cited was Reference 7.

Appendix: The formula for the point estimate and the 95% confidence intervals for the number of virological type-specific influenza-like illness cases.

i is an age category and j is a category of virological type of influence-like illness. A proportion of virological type j in an age-category i sample from the virological surveillance system is p_{ij} and its variance is v_{1ij}. Estimated number of cases of influenza-like illness and its variance in the age category i is α_i and v_{2i}, which is derived from previous studies [8,9]. The estimated number of cases of influenza-like illness from virological type j in the age category i is $\alpha_{ij} = p_{ij}\alpha_i$ and its variance is expressed as $v_{ij} = v_{1ij}v_{2i} + \alpha_i^2 v_{1ij} + p_{ij}^2 v_{2i}$ [17]. Finally, the total estimated number of cases of virological type (j) -specific influenza-like illness and its variance was derived by $\alpha_{\cdot j} = \Sigma_i p_{ij}\alpha_i$ and $v_{\cdot j} = \Sigma_i v_{ij}$. The approximate 95% confidence interval for $\alpha_{\cdot j}$ is given to be $(max\left\{0, \alpha_{\cdot j} - 1.96\sqrt{v_{\cdot j}}\right\}, \alpha_{\cdot j} + 1.96\sqrt{v_{\cdot j}})$.

(PPTX)

Author Contributions

Conceived and designed the experiments: SH YM KT MN. Performed the experiments: SH YM. Analyzed the data: YM. Contributed reagents/materials/analysis tools: TS TM. Wrote the paper: YM SH KT MN MK AO.

References

1. Reed C, Kim IK, Singleton JA, Chaves SS, Flannery B, Finelli L, et al.. Estimated influenza illnesses and hospitalizations averted by vaccination—United States, 2013–14 influenza season. MMWR Morb Mortal Wkly Rep. 2014; 63(49):1151–4. PMID: 25503917
2. Fleming DM, Elliot AJ. Lessons from 40 years' surveillance of influenza in England and Wales. Epidemiol Infect. 2008 Jul; 136(7):866–75. PMID: 18047750
3. Flahault A, Blanchon T, Dorléans Y, Toubiana L, Vibert JF, Valleron AJ. Virtual surveillance of communicable diseases: a 20-year experience in France. Stat Methods Med Res. 2006; 15(5):413–21. PMID: 17089946
4. Lee JS, Shin KC, Na BK, Lee JY, Kang C, Kim JH, et al. Influenza surveillance in Korea: establishment and first results of an epidemiological and virological surveillance scheme. Epidemiol Infect. 2007; 135(7):1117–23. PMID: 17291376
5. Wang X, Yang P, Seale H, Zhang Y, Deng Y, Pang X, et al. Estimates of the true number of cases of pandemic (H1N1) 2009, Beijing, China. Emerg Infect Dis. 2010; 16(11):1786–8. doi: 10.3201/eid1611.100323 PMID: 21029546
6. Ohshiro H, Kawamoto K, Nose T. Surveillance system of infectious diseases in Japan. J Epidemiol. 1996; 6(3 Suppl):S81–5. PMID: 8800278
7. Taniguchi K, Hashimoto S, Kawado M, Murakami Y, Izumida M, Ohta A, et al.. Overview of infectious disease surveillance system in Japan, 1999–2005. J Epidemiol. 2007; 17 Suppl:S3–13. PMID: 18239339
8. Hashimoto S, Murakami Y, Taniguchi K, Shindo N, Osaka K, Fuchigami H, et al. Annual incidence rate of infectious diseases estimated from sentinel surveillance data in Japan. J Epidemiol. 2003; 13(3): 136–41. PMID: 12749600
9. Kawado M, Hashimoto S, Murakami Y, Izumida M, Ohta A, Tada Y, et al. Annual and weekly incidence rates of influenza and pediatric diseases estimated from infectious disease surveillance data in Japan, 2002–2005. J Epidemiol. 2007; 17 Suppl:S32–41. PMID: 18239340
10. Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, the Ministry of Health, Labour and Welfare of Japan. The guideline for National Epidemiological Surveillance of Infectious Diseases (kansensho-hasseidokochosajigyo-jissiyoko). Weekly News of Public Health (Shukan-hoken-eisei) 1999; 998: 14–26. (in Japanese).

11. Ohta A, Hashimoto S, Murakami Y, Kawado M, Taniguchi K, Tada Y, et al. Characteristics of geographical spread and temporal accumulation of the 2009 influenza A (H1N1) epidemic in Japan Based on National Surveillance Data. Jpn J Infect Dis. 2014; 67(5):368–73. PMID: 25241687

12. Shimada T, Gu Y, Kamiya H, Komiya N, Odaira F, Sunagawa T, et al. Epidemiology of influenza A (H1N1)v virus infection in Japan, May-June 2009. Euro Surveill. 2009; 14(24). pii: 19244.

13. Reed C, Angulo FJ, Swerdlow DL, Lipsitch M, Meltzer MI, Jernigan D, et al. Estimates of the prevalence of pandemic (H1N1) 2009, United States, April-July 2009. Emerg Infect Dis. 2009; 15(12):2004–7. doi: 10.3201/eid1512.091413 PMID: 19961687

14. Nakamura Y, Sugawara T, Kawanohara H, Ohkusa Y, Kamei M, Oishi K. Evaluation of estimated number of influenza patients from national sentinel surveillance using the national database of electronic medical claims. Jpn J Infect Dis. 2015; 68(1):27–9. doi: 10.7883/yoken.JJID.2014.092 Epub 2014 Nov 25. PMID: 25420664

15. Hashimoto S, Kawado M, Murakami Y, Ohta A, Shigematsu M, Tada Y, et al. Number of sentinel medical institutions needed for estimating prefectural incidence in influenza surveillance in Japan. J Epidemiol. 2014; 24(3):183–92. PMID: 24584400

16. Fineberg HV. Pandemic preparedness and response—lessons from the H1N1 influenza of 2009. N Engl J Med. 2014; 370(14):1335–42. doi: 10.1056/NEJMra1208802 PMID: 24693893

17. Armitage P, Berry G, Matthews JNS. Statistical methods in medical research fourth edition. Oxon: Blackwell Science, 2002. 161.

Jpn. J. Infect. Dis., 68, 259-261, 2015

Laboratory and Epidemiology Communications

Outbreak of Human Bocavirus 1 Infection in Young Children in Toyama, Japan

Masatsugu Obuchi[1]*, Shin-ichi Yagi[2], Ayako Oguri[3], Takenori Takizawa[1], Hirokazu Kimura[4], and Tetsutaro Sata[1]

[1]Department of Virology, Toyama Institute of Health, Toyama 939-0363; [2]Yagi Pediatric Clinic, Toyama 930-0852; [3]Oguri Pediatric Clinic, Toyama 933-0014; and [4]Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo 208-0011, Japan

Communicated by Makoto Takeda

Human bocavirus 1 (HBoV1), a member of the family *Parvoviridae* and genus *Bocavirus*, was first identified by Allander et al. in 2005 (1). HBoV1 has been detected worldwide in approximately 2% to 20% of patients with an acute respiratory infection (ARI), mainly in children younger than 3 years (y) during the winter and spring seasons (2). The impact of HBoV1 infection of the respiratory tract is often difficult to assess because of its frequent detection in asymptomatic children and co-infection with other respiratory viruses in symptomatic children, at a rate of up to approximately 80% in respiratory specimens (3,4). Although recent studies of serodiagnosis and quantification of the viral load in specimens have provided evidence that HBoV1 is a true respiratory pathogen and not a "bystander" (2), few reports are available regarding outbreaks of HBoV1 infection. Here we report the March 2014 outbreak of HBoV1 infection in young children in Toyama Prefecture, which is approximately 250 km northwest of Tokyo, Japan.

We conducted an investigation to identify respiratory viruses circulating in Toyama Prefecture between October 2013 and June 2014 (the winter and spring seasons in Japan). We studied 104 outpatients with ARIs who were shown to be negative for influenza by a rapid test kit: 67 patients at Yagi Pediatric Clinic located in Toyama City (the prefectural capital) and 37 patients at Oguri Pediatric Clinic located in Takaoka City (the second largest city in Toyama Prefecture). All of the patients were residents of one of these cities and were children under 12 y of age (range, 4 months (mo) to 12 y; median age, 1 y 5 mo). Signed informed consent was provided by the patients' guardian before the sampling. This study was approved by the Ethics Committee of the Toyama Institute of Health.

Nasopharyngeal swabs were collected using FLOQSwabs (Copan Flock Technologies, Brescia, Italy), which were immediately submerged in a conical centrifuge tube containing 2 mL of Nissui Nutrient Broth (Nissui Pharmaceutical, Tokyo, Japan) and stored at −80°C until use. Nucleic acid was extracted from samples using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Duplex real-time reverse transcription (RT)-polymerase chain reaction (PCR) was performed targeting 21 respiratory viruses: human rhinovirus (HRV); respiratory syncytial virus (RSV) A and B; human parainfluenza virus (HPIV) types 1-4; human metapneumovirus (HMPV); influenza A-C viruses; human coronavirus (HCoV) OC43, 229E, NL63, and HKU1; human enterovirus (HEV); human adenovirus (HAdV) B, C, D, and E; and HBoV1. The PCR used previously described primers and probes (5-9) and the QuantiTect Multiplex RT-PCR Kit (Qiagen) according to the manufacturer's instructions. Quantitative real-time PCR was also performed for HBoV1 using the above primer-probe set (6). A 10-fold serial dilution (10^1 to 10^7 copies per reaction) of plasmid DNA containing a fragment of HBoV1 genomic DNA was used to generate a standard curve for the quantification of viral loads in specimens.

In addition, the variable region of the VP1/VP2 genes of HBoV1, corresponding to nucleotide positions 4,172-5,276 of strain ST2 (GenBank accession no. NC_007455), was amplified and sequenced from the HBoV1-positive specimens using the TaKaRa Ex Taq Hot Start Version (Takara Bio, Otsu, Japan) and Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The PCR and sequencing primers were as previously described (10). The sequence data were assembled and analyzed using SEQUENCHER Win V4.10.1 (Hitachi Solutions, Tokyo, Japan) and GENETYX Ver. 10.1.4 (Genetyx, Tokyo, Japan).

Of the 104 tested specimens, one or more respiratory viruses were detected in 88 specimens (85%; 80 single infections, 8 co-infections). In total, 97 viruses were detected, which included the following: HRV (n = 18), RSV (n = 12), HPIV (n = 9), HMPV (n = 12), HCoV (n = 14), HEV (n = 2), HAdV (n = 9), and HBoV1 (n = 21). HBoV1 was thus the most frequently detected virus during the study period. HBoV1 was detected in specimens collected from November 2013 through June 2014: 1 in November, 1 in December, 3 in January, 1 in February, 7 in March, 2 in April, 4 in May, and 2 in June (Fig. 1). HBoV1 was the only virus detected in 14 specimens (67% of the HBoV1-positive specimens), whereas 1 or more respiratory viruses were detected in the remaining 7 HBoV1-positive specimens: co-infection with HCoV in 2 specimens, HMPV in 2, HPIV in 1, HAdV in 1, and HRV and HMPV in 1. Seven of the 14 HBoV1 single infection cases were found in March (Fig. 1), raising the possibility that the virus may have been circulating among children in March in Toyama.

The clinical characteristics of the 7 patients (5 males

Accepted April 17, 2015.
DOI: 10.7883/yoken.JJID.2015.046
*Corresponding author: Mailing address: Department of Virology, Toyama Institute of Health, 17-1 Nakatai-koyama, Imizu-shi, Toyama 939-0363, Japan. Tel: +81-766-56-8143, Fax: +81-766-56-7326, E-mail: masatsugu.obuchi@pref.toyama.lg.jp

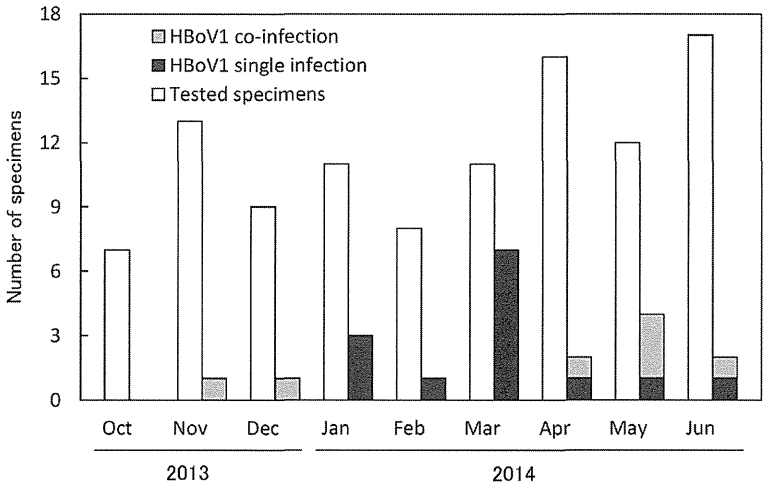


Fig. 1. Monthly distribution of HBoV1-positive cases during the study period. The number of tested specimens (white bars), that of specimens with a single infection of HBoV1 (black bars), and that of specimens with a co-infection of HBoV1 (gray bars) collected in each month are shown.

Table 1. Clinical characteristics and viral roads associated with an HBoV1 single infection in children in March 2014

Patient	Age	Sex	Onset date	Sample date	Clinical diagnosis	Symptom	Body temperature (°C)	Viral load (copies/swab)
1	1 y 3 mo	M	Mar. 4, 2014	Mar. 5, 2014	URTI	Fever, cough, wheezing	38.0	4.2×10^7
2	11 mo	M	Mar. 17, 2014	Mar. 17, 2014	Bronchitis	Fever, cough, wheezing, rhinorrhea, diarrhea	40.0	2.3×10^7
3	10 mo	M	Mar. 14, 2014	Mar. 17, 2014	Bronchitis	Fever, cough	38.5	3.4×10^8
4	1 y 0 mo	F	Mar. 16, 2014	Mar. 17, 2014	Bronchitis	Fever, cough, wheezing, rhinorrhea	39.0	4.3×10^8
5	1 y 1 mo	M	Mar. 15, 2014	Mar. 17, 2014	Bronchitis	Fever, cough, wheezing	39.0	2.6×10^7
6	2 y 4 mo	M	Mar. 31, 2014	Mar. 31, 2014	Bronchitis	Fever, cough, wheezing, rhinorrhea	38.5	$<1.0 \times 10^3$
7	11 mo	F	Mar. 27, 2014	Mar. 28, 2014	Bronchitis	Fever, wheezing	39.3	4.6×10^7

y, year; mo, month; M, male; F, female; URTI, upper respiratory tract inflammation.

and 2 females) with an HBoV1 single infection in March 2014 are shown in Table 1. The patients were aged from 10 mo to 2 y 4 mo (6–11 mo in 3 patients, 12–23 mo in 3, >24 mo in 1). Of the 7 patients, 6 were diagnosed with bronchitis (Patients 2–7), whereas the seventh was diagnosed with an upper respiratory tract inflammation (Patient 1). Common symptoms among these patients were cough (in 6 patients), wheezing (6 patients), and fever (all patients). High fever over 38.5°C occurred in 6 patients. Rhinorrhea was observed in 3 patients, and diarrhea in 1 patient. No patients had underlying diseases.

Many studies have demonstrated a positive correlation between ARI and high copy numbers of HBoV1 DNA in respiratory secretions, and that an HBoV1 single infection is associated with a higher viral load than co-infection with other respiratory viruses (2,11). We thus performed quantitative real-time PCR to measure the viral load in nasopharyngeal specimens obtained from Patients 1–7. In our system, the minimum viral load that would enable reproducible quantification was 10 copies per reaction, corresponding to 10^3 copies per swab. The viral loads ranged from $<1 \times 10^3$ (detection threshold) to 4.3×10^8 copies of HBoV1 genome per swab (Table 1). Only Patient 6 had a low viral load, possibly because of the initial phase of an incipient infection or technical issues such as a loss during sampling. The highest viral load among all 7 patients with co-infections was 1.1×10^5 copies per swab (5 of 7 cases were $< 1 \times 10^3$). These data suggest that HBoV1 was the causative agent of ARI in these patients with a single infection of the virus.

Patients 1 to 6 had visited Yagi Pediatric Clinic. Patients 2, 3, 4, and 6 had been in the same daycare center located near the clinic. In addition, the onsets of the disease in Patients 2, 3, 4, and 5 occurred around the same time (March 14 to 17, 2014). To evaluate the circulating viruses among these children, we performed direct sequencing of PCR amplicons for the VP1/VP2 genes. In the case of Patient 6, the target genes were not successfully amplified, probably because the PCR for the VP1/VP2 genes appeared to be less sensitive than the real-time PCR assay used in this study. Direct sequencing of PCR amplicons in the 6 cases other than Patient 6 showed that all nucleotide sequences were completely identical in the variable region of the VP1/VP2 genes of the viruses (data not shown). These data suggest that an outbreak of HBoV1 infection occurred among young children in March 2014 in Toyama, Japan, and that the virus was transmitted through children in a daycare center and nearby. Several studies demonstrated that a serologic analysis is a more precise approach for the diagnosis of HBoV1 infections

(12,13). However, no serum samples were available in the present study.

Although in the present study we were unable to investigate whether HBoV1 had circulated to other regions in Toyama Prefecture, the present data indicate the possibility that ARIs caused by this virus were prevalent in the spring of 2014 in Toyama Prefecture, Japan. This virus is commonly detected in young children with ARIs and is often associated with severe disease requiring hospitalization (14,15). Therefore, careful monitoring of the prevalence of HBoV1 infection is necessary for the surveillance of ARIs, especially lower respiratory tract infections among young children in the winter and spring seasons.

Acknowledgments We thank Dr. Harutaka Katano, National Institute of Infectious Diseases, Tokyo, Dr. Hidekazu Nishimura, Sendai Medical Center, Miyagi, Dr. Atsushi Kaida, Osaka City Institute of Public Health and Environmental Sciences, Osaka, and Dr. Hiroyuki Tsukagoshi, Gunma Prefectural Institute of Public Health and Environmental Sciences, Gunma, Japan for providing positive control-viral cDNAs or RNAs for the real-time RT-PCR assay.

This work was supported in part by a Grant-in-Aid for Research on Emerging and Re-emerging Infectious Diseases, Labour and Welfare Programs from the Ministry of Health, Labour and Welfare of Japan (H24-Shinko-Ippan-014).

Conflict of interest None to declare.

REFERENCES

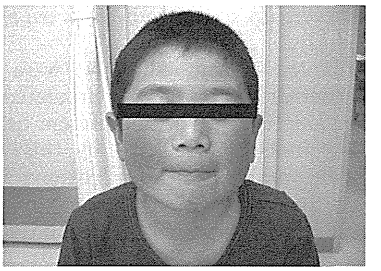
1. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A. 2005;102:12891-6.
2. Dunn JJ, Miller MB. Emerging respiratory viruses other than influenza. Clin Lab Med. 2014;34:409-30.
3. García-García ML, Calvo C, Pozo F, et al. Human bocavirus detection in nasopharyngeal aspirates of children without clinical symptoms of respiratory infection. Pediatric Infect Dis J. 2008; 27:358-60.
4. Schildgen O, Müller A, Allander T, et al. Human bocavirus: passenger or pathogen in acute respiratory tract infections? Clin Microbiol Rev. 2008;21:291-304.
5. Huber I, Campe H, Sebah D, et al. A multiplex one-step real-time RT-PCR assay for influenza surveillance. Euro Surveill. 2011; 16.pii:19798.
6. Katano H, Kano M, Nakamura T, et al. A novel real-time PCR system for simultaneous detection of human viruses in clinical samples from patients with uncertain diagnoses. J Med Virol. 2011;83:322-30.
7. Lu R, Yu X, Wang W, et al. Characterization of human coronavirus etiology in Chinese adults with acute upper respiratory tract infection by real-time RT-PCR assays. PLoS One, 2012;7:e38638.
8. Marcone DN, Videla C, Ricarte C, et al. Rhinovirus detection by real-time RT-PCR in children with acute respiratory infection in Buenos Aires, Argentina. Rev Argent Microbiol. 2012;44:259-65.
9. Templeton KE, Scheltinga SA, Beersma MF, et al. Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza A and influenza B viruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3, and 4. J Clin Microbiol. 2004;42:1564-9.
10. Chieochansin T, Chutinimitkul S, Payungporn S, et al. Complete coding sequences and phylogenetic analysis of human bocavirus (HBoV). Virus Res. 2007;129:54-7.
11. Zhou L, Zheng S, Xiao Q, et al. Single detection of human bocavirus 1 with a high viral load in severe respiratory tract infections in previously healthy children. BMC Infect Dis. 2014;14: 424.
12. Endo R, Ishiguro N, Kikuta H, et al. Seroepidemiology of human bocavirus in Hokkaido Prefecture, Japan. J Clin Microbiol. 2007;45:3218-23.
13. Söderlund-Venermo M, Lahtinen A, Jartti T, et al. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. Emerg Infect Dis. 2009;15:1423-30.
14. Calvo C, Pozo F, García-García ML, et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. Acta Paediatr. 2010;99:883-7.
15. Midulla F, Scagnolari C, Bonci E, et al. Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants. Arch Dis Child. 2010;95:35-41.

＜特集関連情報＞

小児における伝染性紅斑の概要と地域における状況

1．概念・定義

伝染性紅斑（erythema infectiosum）は，ヒトパルボウイルスB19（以下，PVB19）による感染症である。小児では頬がリンゴのように赤くなる（写真）ことから，文字通り“リンゴ病”としてよく知られる。英語でも「頬が赤くなる」との意で“slapped cheek”と称されることもあるが，“fifth disease（第5の発疹症）”の方がより一般的に用いられている模様である。



写真．6歳男児、小学1年生
両頬がやや厚みをもって赤く腫れている

2．疫　学

伝染性紅斑は，5類感染症として小児科定点報告の

（特集つづき）

表．伝染性紅斑 年別，都道府県別累積報告数・累積定点当たり報告数，2010年～2015年第50週

Table. Cumulative number of reported erythema infectiosum cases & cases/sentinel sites, by prefecture and year, 2010 to 2015 (until week 50), Japan

年 year		2010		2011		2012		2013		2014		2015*	
都道府県	Prefecture	累積 cumulative cases	定点当たり cases/ sentinel	累積 cumulative cases	定点当たり cases/ sentinel	累積 cumulative cases	定点当たり cases/ sentinel	累積 cumulative cases	定点当たり cases/ sentinel	累積 cumulative cases	定点当たり cases/ sentinel	累積 cumulative cases	定点当たり cases/ sentinel
総計	Total No.	50,061	16.53	87,010	27.77	20,966	6.67	10,118	3.22	32,352	10.29	92,625	29.43
北海道	Hokkaido	1,726	12.07	6,314	44.46	394	2.77	153	1.08	504	3.52	4,680	32.96
青森県	Aomori	1,012	24.68	602	14.68	151	3.68	80	1.95	957	23.34	956	22.76
岩手県	Iwate	89	2.23	409	10.49	871	21.78	406	10.41	760	19.00	840	21.54
宮城県	Miyagi	1,441	24.84	1,216	20.61	338	5.83	850	14.66	2,871	49.50	1,682	29.00
秋田県	Akita	1,038	29.66	625	18.38	71	2.03	72	2.12	114	3.35	859	25.26
山形県	Yamagata	772	26.62	1,465	50.52	332	11.45	46	1.59	238	8.21	1,310	45.17
福島県	Fukushima	828	17.25	2,178	50.65	672	14.00	85	1.89	718	15.96	2,214	49.20
茨城県	Ibaraki	589	7.96	1,926	25.68	192	2.56	129	1.72	555	7.40	2,549	33.99
栃木県	Tochigi	504	10.72	1,994	42.43	235	5.00	54	1.15	329	6.85	1,662	34.63
群馬県	Gunma	417	6.95	2,088	34.80	247	4.19	78	1.32	291	4.93	1,754	29.73
埼玉県	Saitama	4,317	27.85	6,109	39.16	607	3.92	514	3.32	2,118	13.66	8,368	53.30
千葉県	Chiba	4,306	33.64	3,312	25.88	717	5.52	474	3.62	1,721	13.04	5,154	38.75
東京都	Tokyo	2,929	19.93	6,317	24.30	1,626	6.28	1,608	6.23	5,069	19.65	9,909	38.41
神奈川県	Kanagawa	5,130	25.40	3,949	19.36	1,309	6.42	1,071	5.25	6,338	31.07	6,342	31.09
新潟県	Niigata	1,215	19.92	1,121	18.68	590	9.83	940	15.67	3,060	50.16	1,326	22.47
富山県	Toyama	149	5.32	192	6.62	232	8.00	807	27.83	770	26.55	192	6.62
石川県	Ishikawa	256	8.83	1,169	40.31	115	3.97	40	1.38	470	16.21	889	30.66
福井県	Fukui	782	35.55	594	27.00	91	4.14	19	0.86	76	3.45	326	14.82
山梨県	Yamanashi	486	20.25	702	29.25	70	2.92	30	1.25	36	1.50	691	28.79
長野県	Nagano	554	10.26	1,963	36.35	917	16.98	118	2.23	248	4.68	2,111	39.83
岐阜県	Gifu	160	3.08	993	19.10	812	15.62	91	1.72	89	1.71	1,438	27.65
静岡県	Shizuoka	1,327	15.80	3,231	38.46	223	2.56	121	1.38	1,012	11.50	2,531	28.76
愛知県	Aichi	1,134	6.27	6,083	33.61	1,528	8.44	335	1.84	322	1.77	3,745	20.58
三重県	Mie	1,668	37.07	1,022	23.23	156	3.47	72	1.60	154	3.42	831	18.47
滋賀県	Shiga	515	16.61	835	26.09	48	1.50	42	1.31	57	1.84	1,513	48.81
京都府	Kyoto	671	9.07	1,252	17.39	258	3.53	58	0.79	269	3.68	1,451	19.88
大阪府	Osaka	1,922	9.86	4,295	22.14	927	4.73	347	1.74	691	3.47	5,101	25.63
兵庫県	Hyogo	1,529	11.95	3,383	26.22	805	6.24	313	2.43	665	5.20	2,695	21.05
奈良県	Nara	299	8.54	919	26.26	139	3.97	32	0.94	125	3.68	466	13.71
和歌山県	Wakayama	206	6.65	1,024	33.03	147	4.74	25	0.83	21	0.68	582	19.40
鳥取県	Tottori	245	12.89	454	23.89	513	27.00	28	1.47	17	0.89	205	10.79
島根県	Shimane	82	3.57	777	35.32	459	20.86	26	1.18	22	1.00	101	4.39
岡山県	Okayama	112	2.07	502	9.47	294	5.44	80	1.48	61	1.15	595	11.02
広島県	Hiroshima	330	4.65	1,693	23.85	573	8.07	90	1.27	60	0.85	1,036	14.59
山口県	Yamaguchi	165	3.37	1,225	25.52	687	14.31	89	1.89	101	2.15	715	15.21
徳島県	Tokushima	33	1.43	729	31.70	448	20.36	19	0.86	47	2.04	174	7.57
香川県	Kagawa	68	2.43	437	14.57	278	9.27	63	2.17	44	1.52	543	19.39
愛媛県	Ehime	276	7.46	991	26.78	773	20.89	72	1.95	45	1.25	381	10.30
高知県	Kochi	249	8.30	573	19.10	820	27.33	60	2.00	42	1.40	192	6.40
福岡県	Fukuoka	5,027	41.89	4,469	37.24	185	1.54	129	1.08	530	4.42	5,079	42.33
佐賀県	Saga	423	18.39	963	41.87	41	1.86	37	1.68	127	5.52	820	35.65
長崎県	Nagasaki	1,698	38.59	721	16.39	65	1.48	42	0.95	57	1.30	1,452	33.00
熊本県	Kumamoto	1,274	26.54	816	17.00	167	3.55	77	1.54	77	1.54	1,354	27.08
大分県	Oita	700	19.44	1,065	29.58	371	10.31	59	1.64	130	3.61	2,667	74.08
宮崎県	Miyazaki	420	11.67	2,626	75.03	127	3.63	80	2.22	89	2.47	455	12.64
鹿児島県	Kagoshima	950	17.59	1,294	23.96	120	2.22	86	1.59	140	2.55	2,353	42.78
沖縄県	Okinawa	38	1.12	393	11.56	225	6.82	71	2.15	115	3.38	336	9.88

*2015年は第50週までの暫定値
*provisional number

（感染症発生動向調査：2015年12月16日現在報告数）
(National Epidemiological Surveillance of Infectious Diseases: as of December 16, 2015)

対象疾患である。概ね数年おきに流行がみられる。流行年には春先から夏にかけて患者数が増加して秋になると落ち着くという傾向はあるが，明確にパターン化したものではない（http://www0.nih.go.jp/niid/idsc/idwr/IDWR2015/idwr2015-42.pdf; 2015年12月9日閲覧）。流行はその地域の感受性者の集積状況とも関連すると考えられ，実際に全国と地域とで流行年や季節性に若干の相違が生じている。たとえば，静岡市における大きな流行は2010年と2015年で，2011年は前半に前年の名残り，2014年は初冬から翌年にかけての流行の立ち上がり程度の小流行であった（http://www.city.shizuoka.jp/000_003584.html; 2015年12月9日閲覧）が，全国データとは季節性も含めて異なっていた印象である。

3．症状・所見

リンゴ病自体は，頬が赤くなる（前ページ写真）ことを最大の特徴とする予後良好な感染性疾患である。四肢を中心として特徴的な紅斑を呈することもある。しかしPVB19は，神経（脳炎，脊髄炎，末梢神経痛），循環器（心筋炎，房室ブロック），造血器（溶血性貧血），運動器（関節炎），妊婦の場合には胎児（胎児貧血，胎児水腫）等々，各臓器に対して侵襲性の影響を及ぼすこともあるウイルスである。臨床症状・所見は年齢と関係しており，成人では典型的なリンゴ病の症状よりも，発熱，関節痛，四肢中心の皮疹，頭痛のほか，貧血など造血器系への影響が前面に出ることが少なくない。皮疹は，いったん収まった後でも日光への曝露によって再燃や遷延することがしばしば経験される。また，不顕性感染の頻度が高いとされている。

頬に発疹が出現する1週間～10日ほど前に，前駆症状として感冒様症状がみられることがある。この時期にウイルス血症を起こしており，ウイルスの排泄量が最大になるとされる。特徴的な発疹を呈した時点ではウイルスの排泄はほとんどなく，感染性は失われていると考えられる。通常は飛沫または接触感染である[1)]。

4．診　断

小児科領域では，地域の流行状況を踏まえた上で，主に頬や四肢の特徴的な皮疹と，一方で元気であることの多い全身状態を捉えて，臨床的に「リンゴ病でしょう」と診断することが一般的である。PCR法によるPVB19のDNAの確認や，EIA法によりPVB19に対する血中のIgG/IgM抗体を測定する方法もあるが，小児科の日常診療において頻用されるものではない。

5．治　療

伝染性紅斑に特異的なものはない。皮疹に掻痒感を伴う場合や，年長児で発熱，関節痛，頭痛等を訴えた場合に，対症療法として投薬することはあるが，いずれも頻度は高くない。

6．小児科の日常診療の中で

小児科で診療する伝染性紅斑は，リンゴ病の俗称の通り，“真っ赤なほっぺ”をしているが，発熱もなく元気なことがほとんどである。本人および保護者に対しては，見た目が派手でわかりやすいが，基本的に予後良好な感染症であること，皮疹が出てそれとわかる時点ではすでに周囲への感染性はないため出席停止の対象にはならないこと，ただし，日光に当たることによって皮疹の悪化がみられることがあるので注意すること，などを説明する。

妊娠中に伝染性紅斑に罹患した場合に，胎児に影響が及ぶ場合があることが知られている[2)]。患児の母親が妊婦であった場合には，念のため注意を喚起する。しかし，母親の罹患歴の把握は困難であり，予防法もなく，すでに感染性のある時期は過ぎていること，さらには胎児に影響するリスク自体は高くないことから，無用な不安感を煽るべきではない。抗体検査で感受性の有無を確認すること，自身の症状の推移に注意すること，産科の主治医とよく相談して胎児エコーを励行していただくことなどは，小児科医からでも提案が可能である。

7．まとめ

伝染性紅斑は，小児にとっては時に微笑ましくもある所見を呈する，基本的に予後良好な感染症である。その感染性の時間経過を理解し，目立つ見た目に振り回されるべきではないという認識を，患者や家族，学校や園の関係者と共有することが肝要である。

参考文献

1) http://idsc.nih.go.jp/idwr/kansen/k04/k04_23/k04_23.html
2) 田中敏博，日本臨床 別冊感染症症候群（下）: 694-698, 2013

JA静岡厚生連
静岡厚生病院小児科　田中敏博

＜特集関連情報＞

東京都におけるヒトパルボウイルスB19の検査および疫学状況

ヒトパルボウイルスB19（PVB19）は，感染症法で5類感染症（定点把握対象）として定められている伝染性紅斑の原因ウイルスである。伝染性紅斑は小児に多く発症し，典型的な症例では両頬の平手打ち様紅斑や四肢の網状紅斑など，特異的な皮疹が認められる上に集団発生になる場合が多く，診断しやすいとされる。しかし，成人においては多彩な症状を呈するため，診断が困難なことも少なくない。

東京都健康安全研究センターでは，感染症発生動向調査事業と積極的疫学調査事業においてVP1領域を対象にしたnested-PCR法を用いたPVB19の検査を行っている。2009年1月～2015年10月末までに，伝染性紅斑患者に限らず，不明発疹症を中心に，麻しん，風し

Regional differences in rates of Macrolide-Resistant *Mycoplasma pneumoniae* in Hokkaido, Japan

Nobuhisa Ishiguro, Naoko Koseki, Miki Kaiho, Hideaki Kikuta, Takehiro Togashi, Koji Oba, Keisuke Morita, Naoko Nagano, Masanori Nakanishi, Kyosuke Hazama, Toru Watanabe, Satoshi Sasaki, Atsuko Horino, Tsuyoshi Kenri, Tadashi Ariga, and Hokkaido Pediatric Respiratory Infection Study Group

Received: February 3, 2015. Accepted: June 9, 2015
Published online: July 10, 2015
DOI: 10.7883/yoken.JJID.2015.054

Regional Differences in Rates of Macrolide-Resistant *Mycoplasma pneumoniae* in Hokkaido, Japan

Nobuhisa Ishiguro[1]*, Naoko Koseki[1], Miki Kaiho[1], Hideaki Kikuta[2], Takehiro Togashi[3], Koji Oba[4], Keisuke Morita[5], Naoko Nagano[6], Masanori Nakanishi[7], Kyosuke Hazama[8], Toru Watanabe[9], Satoshi Sasaki[10], Atsuko Horino[11], Tsuyoshi Kenri[11], Tadashi Ariga[1] and Hokkaido Pediatric Respiratory Infection Study Group

[1]Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo 060-8638; [2]Pediatric Clinic, Touei Hospital, Sapporo 007-0841; [3]Sapporo City University School of Nursing, Sapporo 060-0011; [4]Department of Biostatistics, School of Public Health, Graduate School of Medicine, and Interfaculty Initiative in Information Studies, Graduate School of Interdisciplinary Information Studies Library, The University of Tokyo, Tokyo 113-0033, [5]Department of Pediatrics, Japanese Red Cross Asahikawa Hospital, Asahikawa 070-8530; [6]Nagano Pediatric Clinic, Asahikawa 078-8351; [7]Deparment of Pediatrics, Kushiro Red Cross Hospital, Kushiro 085-0032, [8]Hazama Pediatric Clinic, Muroran 050-0073; [9]Watanabe Pediatric Allergy Clinic, Sapporo 006-0831; [10]Department of Pediatrics, Aiiku Hospital, Sapporo 064-0804; and [11]Department of Bacteriology II, National Institute of Infectious Diseases, Tokyo 208-0011, Japan

*Corresponding author: Department of Pediatrics, Hokkaido University Graduate School of Medicine, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan. Tel: +81-11-706-5954, Fax: +81-11-706- 7898, E-mail: nishigur@med.hokudai.ac.jp

Key words: *Mycoplasma pneumoniae*, macrolide resistant, regional difference, Japan

Running head: Macrolide-Resistant *M. pneumonia* in Japan

日本人著者リスト

石黒信久　　北海道大学大学院医学研究科　小児科学分野

小関直子　　北海道大学大学院医学研究科　小児科学分野

海方美紀　　北海道大学大学院医学研究科　小児科学分野

菊田英明　　東栄病院　小児科

富樫武弘　　札幌市立大学　看護学部

大庭幸治　　東京大学大学院　情報学環・学際情報学府

東京大学大学院医学系研究科 公共健康医学専攻　生物統計学分野

森田啓介　　旭川赤十字病院　小児科

長野奈緒子　　ながのこどもクリニック

仲西正憲　　釧路赤十字病院　小児科

間　峡介　　はざま小児科クリニック

渡辺　徹　　わたなべ小児科・アレルギー科クリニック

佐々木聡　　愛育病院小児科

堀野敦子　　国立感染症研究所　細菌第二部

見理　剛　　国立感染症研究所　細菌第二部

有賀　正　　北海道大学大学院医学研究科　小児科学分野

Corresponding author

石黒信久　　北海道大学大学院医学研究科　小児科学分野

〒060-8638　札幌市北区北１５条西７丁目

Tel: 011-706-5954, Fax: 011-706-7898, E-Mail: nishigur@med.hokudai.ac.jp

SUMMARY

Recently, macrolide-resistant (MR) *Mycoplasma pneumoniae* has been emerging and the rate of MR *M. pneumoniae* infection varies by country. However, reports on regional differences in the prevalence of MR *M. pneumonia* within a country are limited. A total of 617 nasopharyngeal swab samples were collected from 617 patients, and DNA of *M. pneumoniae* was identified from 95 of the 617 samples. From 51 of the 95 *M. pneumoniae* -positive samples, the presence of A2063G mutation in the 23S rRNA gene conferring macrolide resistance was detected. The overall macrolide resistance rate was 53.7%, but regional differences in the prevalence of MR *M. pneumoniae* were observed: 0.0% in Muroran, 5.3% in Asahikawa, 55.3% in Sapporo and 100.0% in Kushiro. Statistical differences in the prevalence of MR *M. pneumoniae* were observed between two cities except for the combination of Muroran and Asahikawa. After excluding patients who were prescribed macrolides before collecting nasopharyngeal swab samples in order to avoid the influence of macrolides, statistical differences were still observed: 0.0% in Muroran, 5.6% in Asahikawa, 38.5% in Sapporo and 100.0% in Kushiro.

INTRODUCTION

Mycoplasma pneumoniae is one of the common causative pathogens of community-acquired respiratory tract infections mainly in children and young adults (1). Macrolides are generally considered to be the drugs of choice for treatment of children with *M. pneumoniae* infection (2). Since about 2000, macrolide-resistant (MR) *M. pneumoniae* has been emerging in Asia, Europe, Canada and the USA (3-14). The rates of MR *M. pneumoniae* infection range from 3% to 26% in Europe (9, 15), 63% to 97% in China (16-19) and 25% to 93% in Japan (20-22). The total number of febrile days and the number of febrile days during macrolide administration were longer in patients infected with MR *M. pneumoniae* than in patients infected with macrolide-susceptible (MS) *M. pneumoniae* (23). Therefore, it is important to know the regional rates of MR *M. pneumoniae* infection to predict the duration of fever due to *M. pneumoniae* infection. Differences in the prevalence of MR *M. pneumoniae* in seven surveillance areas throughout Japan have recently been reported (21). However, differences in the prevalence of MR *M. pneumoniae* between cities are not known. The purpose of this study was to investigate the differences in prevalence of MR *M. pneumoniae* between four cities in Hokkaido (83,457 square kilometers in area), the northernmost island of Japan.

MATERIAL AND METHODS

Clinical Specimens

Nasopharyngeal swab samples were collected from pediatric patients who were suspected of having respiratory tract infections associated with *M. pneumoniae* from December 1, 2012 to July 31, 2014 at 8 pediatric clinics and in the department of

pediatrics in 6 hospitals in the cities of Sapporo, Asahikawa, Kushiro and Muroran in Hokkaido, Japan (Figure 1). The nasopharyngeal swab samples were suspended in three ml of BD universal viral transport medium (Becton Dickinson, Sparks, MD, USA) before extraction of DNA.

Real-Time PCR Assay

DNA was extracted with a QIAamp DNA mini kit (Qiagen, Venlo, The Netherlands) from one ml of BD universal viral transport medium and was finally resuspended in 50 μl buffer. DNA of *M. pneumoniae* was identified by real-time PCR using Mp181-F and Mp181-R primer pairs and an Mp181-P probe with one μl of DNA as described elsewhere (24).

Detection of Resistant Point Mutation in Domain V of 23S rRNA

Mutations associated with resistance to macrolides at sites 2063, 2064, and 2617 in the *M. pneumoniae* 23S rRNA domain V gene region were detected by a sequencing method described elsewhere (25). *M. pneumoniae* showing a point mutation in domain V of the 23S rRNA gene was defined as MR *M. pneumoniae*.

Isolation of *M. pneumoniae*

Modified Hayflick medium was used for the isolation of *M. pneumoniae* from patients (26).

Molecular typing of *M. pneumoniae* strains

The *p*1 gene, encoding P1 cytadhesin, an essential factor for pathogenicity of *M. pneumoniae*, was genotyped by a PCR-based method (27).

Statistical Analysis and Ethics

All statistical analyses were performed using JMP software version 11.0.0 (SAS Institute, Cary, NC, USA). The prevalences of macrolide-resistant *M. pneumoniae* were compared by using Fisher's exact test. The multiplicity was adjusted by Bonferroni's

correction method (Adjusted significance level was 0.008 if all combinations of four cities were compared.). All of the necessary ethics approval for this study was obtained from the Institutional Review Board of Hokkaido University Hospital for Clinical Research.

RESULTS

A total of 617 nasopharyngeal swab samples were collected from 617 patients, and DNA of *M. pneumoniae* was identified from 95 of the 617 samples. The average age of the patients was 8.4 years and the gender ratio of males and females was 50:45. From 51 of the 95 *M. pneumoniae*-positive samples, the presence of A2063G mutation in the 23S rRNA gene, a single-base mutation shown to confer macrolide resistance to *M. pneumoniae*, was detected, but other mutations (A2063C, A2063T, A2064G and C2617G) were not detected. From the remaining 44 *M. pneumoniae*-positive samples, these mutations were not detected. The overall macrolide resistance rate was 53.7% (51 of 95), whereas the resistance rate varied in each city: 0.0% (0 of 9) in Muroran, 5.3% (1 of 19) in Asahikawa, 55.3% (21 of 38) in Sapporo and 100.0% (29 of 29) in Kushiro (Table 1). Table 1 shows the *P*-values of Fisher's exact test for comparisons of the prevalences of MR *M. pneumoniae* between two cities. Statistical significance was observed in the prevalence of MR *M. pneumoniae* between two cities except for Asahikawa and Muroran. Macrolides had been administered to 25 (26.3%) of the patients before collecting nasopharyngeal swab samples. Differences in prevalence of MR *M. pneumoniae* were also observed between outpatients and inpatients (Table 2) and between patients visiting hospitals and those visiting clinics (Table 3). Statistical significance was observed in the prevalence of MR *M. pneumoniae* between patients with and those without macrolide pre-administration: 92.0% (23 of 25) in the patients

with macrolide pre-administration and 40.0% (28 of 70) in the patients without macrolide pre-administration (Table 4). After excluding the nasopharyngeal swab samples from 25 patients who had received macrolides, statistical significance in the prevalence of MR *M. pneumoniae* was still observed between different regions (Table 5) and between patients visiting hospitals and those visiting clinics (Table 6), while the statistical difference in prevalence of MR *M. pneumoniae* observed between outpatients and inpatients disappeared (Table 7).

Twenty-three strains of *M. pneumoniae* were isolated from 23 randomly selected samples, and these 23 strains of *M. pneumoniae* were genotyped (Table 8). Four of six strains of MR *M. pneumoniae* isolated from samples collected in Kushiro were genotyped as subtype 1 and two of the six strains were genotyped as variant 2c (Table 8).

DISCUSSION

In the present study, DNA of *M. pneumoniae* was identified from 95 of 617 nasopharyngeal swab samples collected from patients who were suspected of having respiratory tract infections associated with *M. pneumoniae*. Although the overall MR rate was 53.7%, a high degree of regional difference was observed: high MR rate in Kushiro (100.0%) and low MR rates in Asahikawa (5.3%) and Muroran (0.0%). Differences in the prevalence of MR *M. pneumoniae* between prefectures of Japan have been reported (21). In this report, we showed for the first time differences in the prevalence of MR *M. pneumoniae* between cities in one prefecture of Japan. Information on the prevalence of MR *M. pneumoniae* in each city is important for clinicians because the mean prevalence of MR *M. pneumoniae* of 53.7% is not applicable

for clinicians in Kushiro, Asahikawa and Muroran.

The fact that the number of clinics and hospitals that participated in this study was limited raises the following question: Does the difference in macrolide resistance rates arise from the difference in regions or difference in medical institutions? In Sapporo, both MS *M. pneumoniae* and MR *M. pneumoniae* were detected from medical institutions in which *M. pneumoniae* was detected from more than three samples. In Asahikawa, there are 14 pediatric clinics and 5 outpatient pediatric clinics in hospitals. K Hospital and L Clinic, therefore, cover at least 10% of pediatric patients in Asahikawa. K Hospital, located in the western part of Asahikawa, is a second medical care center, and people living anywhere in the city visit the hospital. L Clinic is located in the eastern part of Asahikawa, and people living in the eastern part of the city visit the clinic. M Hospital in Kushiro, which participated in this study, is a secondary medical care center, and people living anywhere in the city visit the hospital. In Muroran, there are 2 pediatric clinics and 2 outpatient pediatric clinics in hospitals. N Clinic covers approximately 30% of pediatric patients living anywhere in Muroran (Table 1). Therefore, it is reasonable to assume that the bias towards MR (Kushiro) or MS (Muroran and Asahikawa) is derived from regional differences, not from institutional differences.

MR *M. pneumoniae* was detected from 80.0% (20 of 25) of inpatients and from only 44.3% (31 of 70) of outpatients (Table 2), and it was detected from 77.4% (48 of 62) of patients visiting hospitals and from only 9.1% (3 of 33) of patients visiting clinics (Table 3). These differences can be at least partially explained by the fact that patients infected with MR *M. pneumoniae* have a fever for a longer duration than do patients infected with MS *M. pneumoniae* (5.1 days vs. 1.7 days, manuscript in preparation).

As shown by our results (Table 4), it has been reported that pre-existing MR *M.*