TABLE 3. HPV DNA status at entry (TVC)

	HPV (n = 519)	HAV (n = 521)	Total (n = 1040)
HPV-16 and HPV-18 negative	91.5%	88.7%	90.1%
HPV-16 positive	6.2%	6.9%	6.5%
HPV-18 positive	3.3%	4.8%	4.0%
HPV-16 and/or HPV-18 positive	8.5%	11.3%	9.9%

% = n / number of women with available results × 100; positive = positive for HPV DNA by PCR; negative = negative for HPV DNA by PCR.
HPV-16/18 AS04-adjuvanted vaccine group (HPV).

according-to-protocol cohort (ATP cohort) for analysis of immunogenicity and a total vaccinated cohort (TVC) were defined. The TVC was defined as the subject population that had at least 1 dose of the vaccine administered. To be included in the ATP safety cohort, women had to have received at least 1 dose of vaccine according to protocol and did not receive vaccines forbidden by the protocol. The ATP cohort for analysis of immunogenicity was defined as the subject population that (i) met all eligibility criteria, (ii) complied with protocol procedures, (iii) received all 3 doses of vaccine according to protocol, (iv) had no HPV-16 or HPV-18 infection at study entry and at month 6, (v) had received no vaccine forbidden by the protocol, and (vi) had immunogenicity end-point measures available. Women in the ATP immunogenicity cohort had to be negative for the corresponding HPV DNA at both 0 and 6 months. Primary analysis of immunogenicity was done on the ATP cohort, and primary analysis of safety was done on the TVC cohort. In both groups, that is, women who had received the HPV-16/18 AS04-adjuvanted vaccine (HPV-16/18 group) and women who had received the HAV (HAV group), seroconversion rates, seropositivity rates for anti–HPV-16 and anti–HPV-18, and their 95% confidence interval (CI) were calculated at each time point a serological result was available. Geometric mean titers (GMTs) were also calculated with 95% CI. To ensure study blinding, the interim analysis was performed by an independent and external statistician. Therefore, the study blinding is maintained for GlaxoSmithKline personnel, investigators, study collaborators, and subjects.

RESULTS

The study groups are outlined in Figure 1. A total of 1040 eligible women were vaccinated (TVC): 519 women in the HPV-16/18 group and 521 women in the HAV group. The ATP cohort for safety included 1035 women (516 women in the HPV-16/18 group and 519 women in the HAV group), excluding 5 women for noncompliance with the protocol (2 women received a vaccine forbidden in the protocol, and 3 had the study vaccine not administered according to protocol). The ATP cohort for immunogenicity included 806 women (413 women in the HPV-16/18 group and 393 women in the HAV group). A total of 229 women were excluded from the ATP cohort for immunogenicity due to infection with HPV-16 and/or HPV-18 (120 women), lacking serological data (88 women), noncompliance with vaccination or blood sampling schedule (20 women), and protocol violation (1 subject). All women completed visit 4 at month 7 between November 2006 and July 2007. The demographic characteristics of TVC are shown in Table 1; age, height, and body weight were comparable in the 2 study groups; mean age was 22.5 years, and all women were Japanese (Table 1).

Seropositivity status at baseline is shown in Table 2. Of the women in the TVC, 72.9% were negative for antibodies against both HPV-16 and HPV-18, whereas 6.3% of them were positive for both anti–HPV-16 and anti–HPV-18 antibodies. Prevalence of anti–HPV-16 and anti–HPV-18 antibodies were 17.3% and 15.8%, respectively, and 26.8% of all women were positive for anti–HPV-16 and/or anti–HPV-18 antibodies.

Human papillomavirus DNA status at entry is shown in Table 3. The percentage of women negative for both HPV-16 DNA and HPV-18 DNA was 90.1% of the TVC. Human papillomavirus 16 DNA was detected in 6.5% of the women and HPV-18 DNA in 4.0%, and 9.9% of the women were positive for either HPV-16 and/or for HPV-18 DNA.

In the ATP cohort for immunogenicity, the women in the HPV-16/18 group showed 100% seroconversion for anti–HPV-16 and anti–HPV-18 antibodies at month 6 (5 months after the second dose) and month 7 (1 month after the third dose) (Table 4). Anti–HPV-16 GMT was 7441.0 EL.U/mL at month 7, it was 250-fold higher than natural infection (GMT, 29.8 EL.U/mL).[18] Similarly, anti–HPV-18 antibody GMT after dose 3 was 3805.4 EL.U/mL, 168-fold higher than after natural infection (GMT, 22.6 EL.U/mL).[18] The

TABLE 4. Seropositivity rate and GMTs for HPV-16 VLP IgG antibodies and HPV-18 VLP IgG antibodies by prevaccine status (ATP cohort for immunogenicity)

Antibody	Group	Timing	n*	Seropositivity, %	GMT, 95% CI
Anti–HPV-16	HPV group	Enrollment	411	15.6	5.5 (5.1–5.9)
		Month 6	411	100	708.4 (650.1–772.0)
		Month 7	410	100	7441.0 (6854.3–8077.8)
	HAV group	Enrollment	393	13.0	5.3 (4.9–5.7)
		Month 6	393	14.0	5.4 (4.9–5.8)
		Month 7	387	13.4	5.3 (4.9–5.8)
Anti–HPV-18	HPV group	Enrollment	410	14.9	4.5 (4.2–4.8)
		Month 6	410	100	504.6 (464.6–548.1)
		Month 7	409	100	3805.4 (3515.6–4119.1)
	HAV group	Enrollment	390	12.3	4.4 (4.1–4.7)
		Month 6	388	13.7	4.4 (4.1–4.7)
		Month 7	385	14.0	4.5 (4.2–4.8)

*Number of women with prevaccination results available.
HPV-16/18 AS04-adjuvanted vaccine group (HPV), immunoglobulin G (IgG), geometric mean antibody titer calculated on all women (GMT).

TABLE 5. Women reporting solicited AE (TVC)

	HPV (n = 512), %	HAV (n = 510), %
Injection site symptoms		
Pain	99.2	42.0
Redness	88.9	56.3
Swelling	78.3	32.4
General symptoms		
Arthralgia	24.0	11.9
Fatigue	66.6	58.7
Fever	8.0	5.5
Gastrointestinal	33.6	32.7
Headache	48.8	43.4
Myalgia	51.2	25.0
Rash	6.4	4.7
Urticaria	3.1	3.9

HPV-16/18 AS04-adjuvanted vaccine group (HPV).

anti–HPV-16 or anti–HPV-18 antibody titers in the HAV group did not show any increase.

Injection site symptoms (pain, redness, and swelling) were reported more frequently in the HPV-16/18 group than in the HAV group (Table 5). However, most local symptoms were mild and transient, with the mean duration of local symptoms ranging from 3.7 to 4.0 days in the HPV-16/18 group. The occurrence of solicited general symptoms excluding urticaria was higher in the HPV-16/18 group than in the HAV group. Arthralgia, fatigue, headache, and myalgia were reported more frequently in the HPV-16/18 group than in the HAV group. No increase in the occurrence of solicited local and general symptoms was seen with each subsequent dose. Six women in the HPV-16/18 group reported 7 SAEs, and 8 women in the HAV group reported 9 SAEs. All 16 events are shown in Table 6. Study group is not shown because the study is still blinded. With the exception of 2 subjects who experienced traumatic SAEs and who were withdrawn from the study, all SAEs were reported to have resolved. One SAE, a spontaneous abortion, was considered to be possibly related to vaccination by the investigator in charge of the subject because the event occurred approximately 2 weeks after vaccination; the 15 other SAEs were considered not to be related to vaccination. There were no differences in the compliance with vaccination between the 2 study groups; compliance rate for all 3 doses in the HPV-16/18 group was 89.2%, and in the HAV group, it was 90.0 % (Table 7).

TABLE 6. Serious AEs reported (TVC)

Subject	Event	Grade	Outcome	Onset After Vaccination,* d
A	Brain contusion	Moderate	Resolving	40
A	Skull fracture	Moderate	Resolving	40
B	Gastritis	Moderate	Resolved	106
C	Abortion spontaneous	Severe	Resolved	159
D	Acute tonsillitis	Severe	Resolved	78
E	Hepatitis acute	Severe	Resolved	104
F	Chemical abortion	Mild	Resolved	9
G	Automobile accident injury	Moderate	Resolved	15
H	Acute pyelonephritis	Moderate	Resolved	156
I	Avulsion fracture of posterior cruciate ligament attachment of right knee	Severe	Resolving	77
I	Contusion of right lower leg	Severe	Resolving	77
J	Appendicitis	Severe	Resolved	18
K	Pneumothorax spontaneous	Moderate	Resolved	31
L	Spontaneous abortion	Mild	Resolved	15
M	Acute tonsillitis	Moderate	Resolved	99
N	Pneumonia	Severe	Resolved	41

*Day of onset after last dose of vaccine administered.

TABLE 7. Compliance with vaccinations (TVC)

	HPV (n = 519)		HAV (n = 521)		Total (n = 1040)	
	n	%	n	%	n	%
Dose 1	519	100	553	100	1040	100
Dose 2	497	95.8	494	94.8	991	95.3
Dose 3	463	89.2	469	90.0	932	89.6

HPV-16/18 AS04-adjuvanted vaccine group (HPV).

DISCUSSION

An interim analysis of the clinical phase II study for the HPV-16/18 AS04-adjuvanted vaccine in healthy Japanese women aged 20 to 25 years was conducted 1 month after the completion of vaccination. At study entry, 17.3% and 15.8% of the women were seropositive for anti–HPV-16 and anti–HPV-18 antibodies, respectively; 26.8% of the women aged 20 to 25 years in the study had experienced either HPV-16 and/or HPV-18 infection. In the clinical phase III study with approximately 18,000 women aged 15 to 25 years conducted in Asia Pacific, Europe, South America, and North America (Study HPV-008 [580299/008, NCT00122681]),[18] anti–HPV-16 and anti–HPV-18 seropositivity rates at study entry were 17.5% and 11.6%, respectively. The broader age range of women in this clinical study (15–25 years) precludes direct comparison of Japanese data with these published data; however, no meaningful differences were noted between the Japanese and the other populations in terms of seropositivity. At entry of the current study, 6.5% of the women were HPV-16 DNA positive and 4.0% HPV-18 DNA positive; approximately 10% of the women were infected with the vaccine HPV type. A large-scale meta-analysis of Japanese data indicated that in 0.84% of the normal cytologies, HPV-16 is found, and in less than 0.50%, HPV-18 is found.[4] These data were generated in screened women of all ages, whereas the current nationwide study in young women aged 20 to 25 years shows a higher prevalence of these HPV types, particularly HPV-18. These findings strongly suggest that the prevalence of HPV-16 or HPV-18 is increasing in the younger population in Japan. Previous epidemiological analysis showed that the detection rates of HPV-16 and HPV-18 in Japanese women with cervical cancer were 44.8% and 14.0%, respectively,[7] which was considered to be slightly lower than the 54.4% (HPV-16) and 15.9% (HPV-18) found in populations

from other parts of the world[1]; HPV-16 and HPV-18 DNA positivity seems to be increasing in Japanese women aged 20 to 30 years.[8] Therefore it is likely that, in Japan, cervical cancer caused by HPV-16/18 will increase in the future.

In this study, the HPV-16/18 AS04-adjuvanted vaccine showed strong immunogenicity in Japanese women as the anti–HPV-16 and anti–HPV-18 seroconversion rate after the second dose (at month 6) and after the third dose (at month 7) was 100%. In the phase III clinical study (Study HPV-008 [580299/008, NCT00122681]), the seroconversion rate for both HPV types after the second and third vaccinations was more than 99.5%, and at month 7, the anti–HPV-16 GMT was 9341.5 EL.U/mL, and anti–HPV-18 GMT was 4769.6 EL.U/mL.[18] The results of this interim analysis are consistent with those of Study HPV-008 (580299/008, NCT00122681). In the follow-up observation for 6.4 years in Study HPV-007 (580299/007, NCT00120848) (ie, long-term extension study of Study HPV-001 [580299/001, NCT00689741]), none of the women vaccinated with HPV-16/18 AS04-adjuvanted vaccine developed persistent infection with either HPV-16 or HPV-18 and HPV-16/18–associated cervical intraepithelial neoplasia (CIN) 2+ (defined histologically as CIN2, CIN3, adenocarcinoma in situ, and invasive carcinoma).[21] Because immunogenicity of HPV-16/18 AS04-adjuvanted vaccine was not lower in this study than what was observed in other populations, prophylactic efficacy in terms of prevention of persistent infection and precancerous lesions caused by HPV-16 and HPV-18 is also expected to be in line with what was observed in other clinical studies.

Local and general symptoms after vaccination were generally observed more frequently in the HPV-16/18 group, but no increase of the occurrence was seen with the number of doses administered. In addition, symptoms were mild and transient. It needs to be highlighted that, in the current study, the control group received Aimmugen, a generally used HAV that is known to be have excellent tolerability in Japan.[23] Also, the data generated in the present study are similar to those generated in other studies (HPV-001[580299/001, NCT00689741],[16] HPV-008 [580299/008, NCT00122681],[18] and HPV-012 [580299/012, NCT00169494][19]), wherein HPV-16/18 AS04-adjuvanted vaccine was compared with HAV or placebo. Human papillomavirus 16/18 AS04-adjuvanted vaccine has an acceptable safety profile and is well tolerated in Japanese subjects. This is supported by the finding that there was no difference in the compliance with vaccination with all 3 doses between the HPV-16/18 (89.2%) and the HAV (90.0%) groups in the study. The same level of compliance was observed in other studies with HPV-16/18 AS04-adjuvanted vaccine.[16,18] One spontaneous abortion was reported by 1 investigator to be possibly related to vaccination in the study because the event appeared 15 days after vaccination. Meanwhile, the chemical abortion was considered not to be related to vaccination, although it occurred 9 days after vaccination. Consideration of various risk factors by each investigator may indeed lead to differences in clinical judgment. In both subjects, the pregnancy test taken before vaccination was negative. Spontaneous abortions are known to be a common complication of gestation, and its incidence is 10% to 14% in Japan.[24] In a pooled analysis of the safety data of the HPV-16/18 AS04-adjuvanted vaccine in a cohort of almost 30,000 girls and women aged 10 years or older, no differences in pregnancy outcomes were observed between the HPV-16/18 AS04-adjuvanted vaccine group and the control groups.[25]

In many other countries, prophylactic HPV vaccine is administered to adolescent girls as part of routine vaccination programs. The Advisory Committee on Immunization Practices and Center for Disease Control and Prevention in the United States recommend HPV vaccination from the early age of 11 to 12 years.[22] In addition, a health economics study in Japan suggested that routine vaccination of young female adolescents and women aged 12 to 40 years could reduce the onset of cervical cancer by 70% or more and markedly reduce accompanying health care costs (R. Konno, unpublished data). Based on the immunogenicity results of the interim analysis of the present clinical study in Japan, which are very similar to those seen in other clinical trials, clinical effects as observed in foreign populations are also expected in Japan if HPV-16/18 vaccination is introduced, with consequent significant benefits from a public health point of view.

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REFERENCES

1. Castellsagué X, de Sanjose S, Aguado T, et al. HPV and Cervical Cancer in the World: 2007 Report. *Vaccine.* 2007;25S:C1–C26.
2. Ministry of Health, Labour & Walfare. Vital Statistics of Japan. Available at: http://ganjoho.ncc.go.jp/professional/statistics/statistics.html. Accessed May 26, 2008.
3. Marugame T, Matsuda T, Kamo K, et al. Cancer incidence and incidence rates in Japan in 2001 based on the data from 10 population-based cancer registries. *Jpn J Clin Oncol.* 2007;37:884–891.
4. Konno R, Shin HR, Kim YT, et al. Human papillomavirus infection and cervical cancer prevention in Japan and Korea. *Vaccine.* 2008;26512:M30–M42.
5. Wright TC, Bosch FX, Franco EL, et al. Chapter 30: HPV vaccines and screening in the prevention of cervical cancer; conclusions from a 2006 workshop of international experts. *Vaccine.* 2006;24S3: S3251–S3261.
6. Narisawa-Saito M, Kiyono T. Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: roles of E6 and E7 proteins. *Cancer Sci.* 2007;98:1505–1511.
7. Miura S, Matsumoto K, Oki A, et al. Do we need a different strategy for HPV screening and vaccination in East Asia? *Int J Cancer.* 2006; 119:2713–2715.
8. Nakagawa S, Yoshikawa H, Onda T, et al. Type of human papillomavirus is related to clinical features of cervical carcinoma. *Cancer.* 1996;78: 1935–1941.
9. Smith JS. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer.* 2007;121:621–632.
10. Inoue M, Sakaguchi J, Sasagawa T, et al. The evaluation of human papillomavirus DNA testing in primary screening for cervical lesions in a large Japanese population. *Int J Gynecol Cancer.* 2006;16: 1007–1013.
11. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. *J Am Med Assoc.* 2007;297: 813–819.
12. Peto J, Gilham C, Deacon J, et al. Cervical HPV infection and neoplasia in a large population-based prospective study: the Manchester cohort. *Br J Cancer.* 2004;91:942–953.
13. Beby-Defaux A, Bourgoin A, Ragot S, et al. Human papillomavirus infection of the cervix uteri in women attending a health examination center of the French social security. *J Med. Virol.* 2004;73:262–268.
14. Ratnam S, Franco EL, Ferenczy A. Human papillomavirus testing for primary screening of cervical cancer precursors. *Cancer Epidemiol Biomarkers Prev.* 2000;9:945–951.
15. Giannini SL, Hanon E, Moris P, et al. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. *Vaccine.* 2006;24:5937–5949.
16. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet.* 2004;364:1757–1765.

17. Harper DM, Franco EL, Wheeler CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet.* 2006;367:1247–1255.
18. Paavonen J, Jenkins D, Bosch FX, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet.* 2007;369:2161–2170.
19. Pedersen C, Petaja T, Strauss G, et al. Immunization of early adolescent females with human papillomavirus type 16 and 18 L1 virus-like particle vaccine containing AS04 adjuvant. *J Adolesc Health.* 2007; 40:564–571.
20. Keam SJ, Harper DM. Human papillomavirus types 16 and 18 vaccine (recombinant, AS04 adjuvanted, adsorbed) [Cervarix™]. *Drugs.* 2008;68:359–372.
21. Harper DM, Gall S, Naud P, et al. Sustained immunogenicity and high efficacy against HPV-16/18 related cervical neoplasia: long-term follow up through 6.4 years in women vaccinated with Cervarix™ (GSK's HPV 16/18 AS04 candidate vaccine). Presented at: 39th Annual Meeting of the Society of Gynecologic Oncologists; March 10, 2008; Florida. *Gynecol Oncol.* 2008;109:158–159; Abstract 1.
22. Markowitz LE, Dunne EF, Saraiya M, et al. Quadrivalent human papillomavirus vaccine, recommendations of the advisory committee on immunization practices (ACIP). *Morb Mortal Wkly Rep.* 2007;56:1–24.
23. Aimmugen™ [package insert] [in Japanese]. Kumamoto, Japan: Chem-Sero-Therapeutic Research Institute; 2006.
24. Saijo Y, Sato F, Yamada H, et al. Ah receptor, CYP1A2, CYP1A2 and CYP1B1 gene polymorphism are not involved in the risk of recurrent pregnancy loss. *Mol Hum Reprod.* 2004;10:729–733.
25. Descamps D, Hardt K, Spiessens B, et al. Safety of human papillomavirus (HPV)-16/18 AS04 adjuvanted vaccine for cervical cancer prevention: a pooled analysis of 11 clinical trials. *Hum Vaccin.* 2009;5:332–340.

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Short communication

Detection of enteric viruses in rectal swabs from children with acute gastroenteritis attending the pediatric outpatient clinics in Sapporo, Japan

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ABSTRACT

Background: Gastroenteritis is a world-wide disorder. Numerous studies to identify causative viral agents have been reported for hospitalized patients but there are only a few for outpatients with mild symptoms who are usually managed in the outpatient clinics.
Objectives: Our aim was to clarify the epidemiological and clinical characteristics of acute gastroenteritis in children who visited the outpatient clinics with various complaints suggestive of gastroenteritis.
Study design: From December 2003 to December 2005, 877 rectal swabs were collected from patients attending outpatient clinics in Sapporo, Japan. Viral genomes of major five enteric viruses (rotavirus, norovirus, adenovirus, astrovirus and sapovirus) and bocavirus were investigated by RT-PCR or PCR.
Results: At least one viral agent was found in 326 (37.2%) cases of the 877 studied. Rotaviruses were the most prevalent and were detected in 143 (16.3%) followed by norovirus in 116 (13.2%), adenovirus in 42 (4.8%), astrovirus in 40 (4.6%) and sapovirus in 15 (1.7%) cases. Bocavirus was detected in only 4 (0.5%) cases. Frequent diarrhea and frequent vomiting were prominent in rotavirus and norovirus infection, respectively.
Conclusions: The prevalence of each enteric virus in outpatients resembled that previously estimated in hospitalized patients, although the detection rate of rotavirus was slightly low. The contribution of bocavirus appears to be small.

1. Background

Acute gastroenteritis is one of the most common diseases in infants and children, and continues to be a leading cause of morbidity and mortality worldwide.[1] Enteric viruses are the most important etiologic agents of acute gastroenteritis,[2] and norovirus, rotavirus, adenovirus, astrovirus and sapovirus are well known as five representative enteric viruses.

Currently, norovirus is the most common cause of outbreaks of viral gastroenteritis in mainly adults worldwide.[1–3] Rotavirus is also a common enteric pathogen, and usually causes severe diarrhea in children. The remaining 3 enteric viruses are much less often responsible for childhood gastroenteritis. Enteric adenoviruses sometimes cause gastroenteritis. The replication of astrovirus in intestinal tissues has been confirmed,[4] although the pathogenesis has not been established. Sapovirus causes small epidemics of relatively mild gastroenteritis. In addition to these five representative enteric viruses, human bocavirus has often been detected in the stool of children with gastroenteritis,[5–7] although its causative role remains uncertain.

Many epidemiological studies on viral gastroenteritis in children exist; however, they mostly examined soft/liquid stool samples from hospitalized children.[8–10] Few reports have been published of the viral status of children with mild gastroenteritis, sometimes without diarrhea, who are usually managed in outpatient clinics.

2. Objectives

In this study, we collected rectal swab samples irrespective of the presence of diarrhea from children with gastrointestinal symptoms who attended the outpatient clinics. We intended to determine the entire epidemiology of viral gastroenteritis in children. The presence of viral RNA or DNA in the rectal swabs was investigated and clarified the prevalence of the above-mentioned five representative enteric viruses and of human bocavirus in all the children with gastroenteritis.

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3. Study design

Between December 2003 and December 2005, 877 rectal swab samples were obtained from 877 children under 14 years of age who attended outpatient clinics located in different parts of Sapporo, Japan with features suggesting acute gastroenteritis. Written informed consent was obtained from the parents of the children.

We included all cases from mild to severe symptoms, and defined acute gastroenteritis as the occurrence of one of the following: one bout of soft/liquid stool, or vomiting or abdominal pain within the 48 h before visiting the clinic. Cases with possible bacterial gastroenteritis based on the existence of bloody stool were excluded. Experienced staff obtained the rectal swabs, which were immediately vortexed in the collection tube with 1.0 ml of phosphate buffered saline (pH 7.4).

Viral RNA and DNA were extracted from 200 μl of swab extract applying the RNA-Bee (TEL-TEST, Inc., TX) and the DNA-Zol (Molecular Research Center, Inc., O) according to the manufacturer's instructions, respectively, and eluted with 30 μl of water and maintained at −80 °C until used. The presence of norovirus, rotavirus, astrovirus and sapovirus was detected by RT-PCR using the protocols described previously.[9,11–13] Adenovirus and bocavirus were detected by a previously described PCR protocols.[14,15]

Statistical analyses were performed with StatView Software, version 5.0. Analysis was carried out using the nonparametric Kruskal–Wallis test. Simultaneous comparison of several proportions was performed using the chi-square test or Mann–Whitney *U*-test. All tests were two-tailed; *p* values under 0.05 were considered significant.

4. Results

The sex ratio (male:female) of the 877 children was 1.3:1 (502:375). The median age was 46 months (range, 2 months to 14 years). At least one viral agent was found in 326 of the 877 cases (37.2%). A single viral agent was detected in 295 cases, two different viral agents in 28 cases and three in 3 cases (Table 1). Among the 295 cases in which a single viral agent was detected, rotavirus, norovirus, adenovirus, astrovirus, sapovirus and bocavirus were confirmed in 118, 101, 33, 31, 9 and 3 cases, respectively.

Ten different combinations of viruses were seen in 31 samples, rotavirus combined with other viruses was the most frequent pattern in mixed infections. Including cases with single or plural viral infection, rotavirus was detected in 143 of 877 (16.3%) cases, norovirus in 116 (13.2%), adenovirus in 42 (4.8%), astrovirus in 40 (4.6%), sapovirus in 15 (1.7%) and bocavirus in 4 (0.5%).

Table 1
Distribution of mixed and single-virus infections.

Positive	326
One virus detected	295
Norovirus	101
Rotavirus	118
Adenovirus	33
Astrovirus	31
Sapovirus	9
Bocavirus	3
Two viruses detected	28
Norovirus, rotavirus	9
Norovirus, adenovirus	2
Norovirus, astrovirus	3
Rotavirus, adenovirus	5
Rotavirus, astrovirus	4
Rotavirus, sapovirus	4
Adenovirus, bocavirus	1
Three viruses detected	3
Norovirus, rotavirus, sapovirus	1
Rotavirus, adenovirus, astrovirus	1
Rotavirus, astrovirus, sapovirus	1
Negative	551

Rotavirus and norovirus predominated for all ages of children, with rotavirus most common in the under 5 years old group and norovirus predominated in the 6–9 years olds (Fig. 1). Viral gastroenteritis incidence decreased with age; being more frequent in children under 4 years old (44.0%) than in those over 5 years old (22.4%) ($p < 0.001$, chi-square test).

Viral gastroenteritis was most prevalent in winter, but was seen throughout the year (Fig. 2). A peak of norovirus gastroenteritis was seen from November to December and was followed by a peak of rotavirus gastroenteritis occurring from January to April. Adenovirus, astrovirus, sapovirus were detected sporadically throughout the year. Virus detection rate was lower from August to October in 2005 than other periods.

Among the 295 cases from whom a single virus was detected, 229 cases (77.6%) had one or more episodes of diarrhea, and vomiting was reported in 192 patients (65.0%), fever over 37.5 °C was

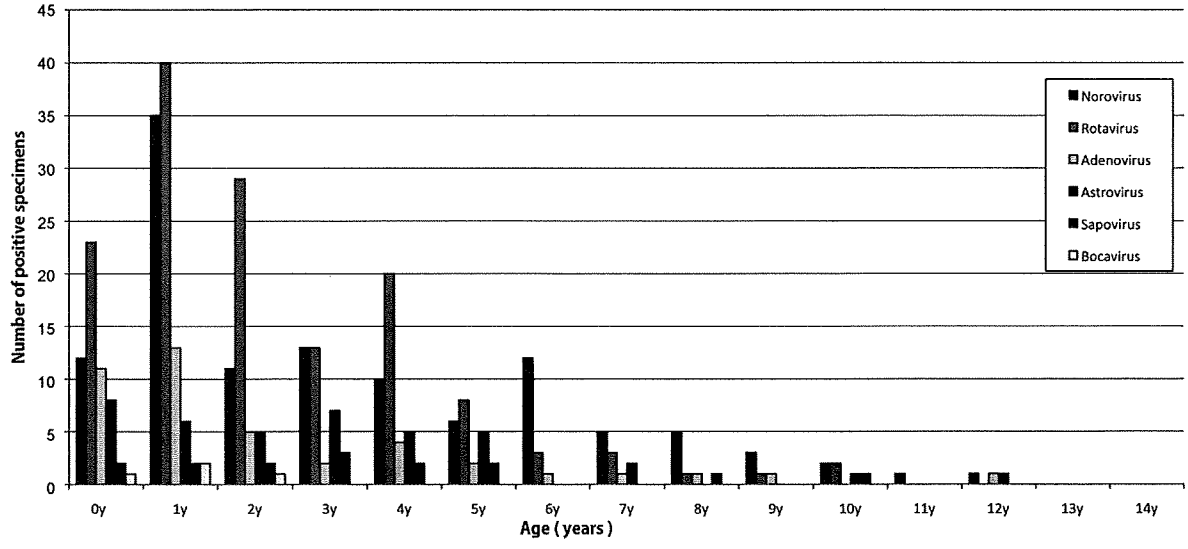

Fig. 1. Age distribution of five enteric viruses and bocavirus.

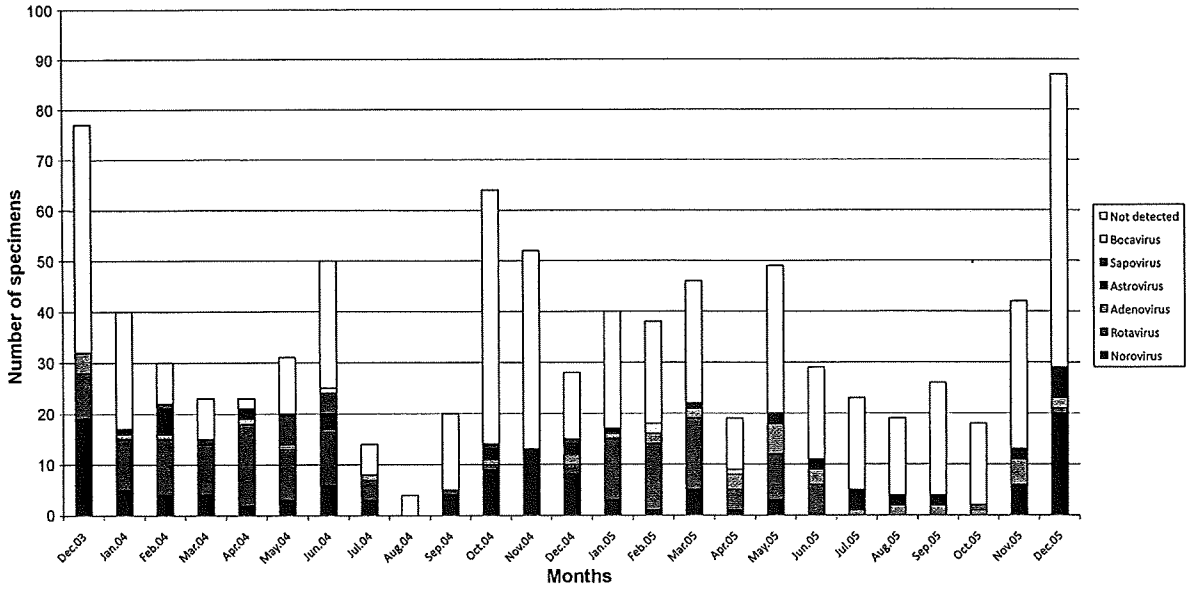

Fig. 2. Monthly distribution of five enteric viruses and bocavirus from November 2003 to December 2005.

Table 2
Correlation between disease severity and causative viral agent in patients in whom a single virus was confirmed.

Disease manifestation	Norovirus ($n=101$)	Rotavirus ($n=118$)	Adenovirus ($n=33$)	Astrovirus ($n=31$)	Sapovirus ($n=9$)	Bocavirus ($n=3$)	p^a	p^b
No. of bouts of diarrhea (stools/24 h)								
0	35	14	3	12	2	0		
1–3	39	47	8	7	5	1		
4–6	13	28	14	8	2	1		
7≦	14	29	8	4	0	1	<0.05	<0.01
No. of bouts of vomiting (episodes/24 h)								
0	18	41	25	15	4	0		
1–2	31	36	3	10	1	1		
3–4	25	19	4	2	1	2		
5≦	27	22	1	4	3	0	<0.01	<0.01
Fever (°C)								
<37.5	78	64	24	14	8	3		
37.5–38.5	18	27	4	12	1	0		
38.5≦	5	27	5	5	0	0	<0.05	<0.01

[a] Kruskal–Wallis test comparing all viruses.
[b] Mann–Whitney *U*-test comparing rotavirus and norovirus.

seen in 104 patients (35.3%) (Table 2). There was significant correlation between the causative viral agents and frequency of diarrhea ($p<0.05$, Kruskal–Wallis test) and vomiting ($p<0.01$), and also with the occurrence and degree of fever ($p<0.05$). Comparing rotavirus with norovirus, diarrhea was more frequent with rotavirus infection ($p<0.01$, Mann–Whitney *U*-test), but vomiting was more frequent with norovirus ($p<0.01$). A higher degree of fever was observed with rotavirus than with norovirus ($p<0.01$).

5. Discussion

We used rectal swabs to examine the prevalence of five enteric viruses and bocavirus in children visiting outpatient clinics with signs of possible gastroenteritis. We included not only severe cases but also mild cases who were complaining only of vomiting or abdominal pain without diarrhea, as our intention was to determine the entire epidemiology of viral gastroenteritis in children. Viruses were identified in 37.2% of the patients, most commonly rotavirus or norovirus. The detection rates of adenovirus, astrovirus and sapovirus were several percent.

The results of the present study on the leading viral causes of gastroenteritis, their seasonal distribution and clinical symptoms were similar to previous studies focused on hospitalized children,[8,16] but the viral detection rate, especially of rotavirus, was a little lower than previous reports.[6,17,18] This may be due either to a greater number of mild cases in our series or to the use of rectal swab samples.

Bocavirus was detected in only 4 samples (0.5%), although this report was the first study to investigate bocavirus in outpatients with gastroenteritis. Previous studies on hospitalized children with gastroenteritis reported detection rates of 0.8–4.6%.[5–7] In the present study, the number of positive cases was too small to discuss the role of bocavirus infections in gastroenteritis.

Examining the rectal swab samples from patients regardless of the presence of diarrhea and comparing norovirus and rotavirus infection, we found that significantly more frequent vomiting, but less diarrhea was observed in norovirus infection; on the other hand, more frequent diarrhea and higher fever was observed in rotavirus infection, as described previously.[19–21]

In conclusion, we showed that the prevalence of each enteric virus in child outpatients including mild cases was similar to that in mainly hospitalized patients. Our findings also showed that rectal swab samples were satisfactory as test material for viral genomes in children with acute gastroenteritis.

Acknowledgments

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References

1. Musher DM, Musher BL. Contagious acute gastrointestinal infections. *N Engl J Med* 2004;**351**:2417–27.
2. Wilhelmi I, Roman E, Sánchez-Fauquier A. Viruses causing gastroenteritis. *Clin Microbiol Infect* 2003;**9**:247–62.
3. Widdowson MA, Monroe SS, Glass RI. Are noroviruses emerging? *Emerg Infect Dis* 2005;**11**:735–7.
4. Matsui SM, Greenberg HB. Astroviruses. In: Fields BN, Knipe DM, Howley PM, editors. *Fields virology*. 4th ed. Philadelphia: Lippincott-Raven; 2001. p. 875–93.
5. Campe H, Hartberger C, Sing A. Role of human bocavirus infections in outbreaks of gastroenteritis. *J Clin Virol* 2008;**43**:340–2.
6. Lee JI, Chung JY, Han TH, Song MO, Hwang ES. Detection of human bocavirus in children hospitalized because of acute gastroenteritis. *J Infect Dis* 2007;**196**:994–7.
7. Susanna K, Lau P, Cyril C, Yip Y, Tak-lun Q, Rodney A, et al. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. *J Clin Virol* 2007;**196**:986–93.
8. Colomba C, De Grazia S, Giammanco GM, Saporito L, Scarlata F, Titone L, et al. Viral gastroenteritis in children hospitalised in Sicily, Italy. *Eur J Clin Microbiol Infect Dis* 2006;**25**:570–5.
9. Malasao R, Maneekarn N, Khamrin P, Pantip C, Tonusin S, Ushijima H, et al. Genetic diversity of norovirus, sapovirus, and astrovirus isolated from children hospitalized with acute gastroenteritis in Chiang Mai, Thailand. *J Med Virol* 2008;**80**:1749–55.
10. Oh DY, Gaedicke G, Schreier E. Viral agents of acute gastroenteritis in German children: prevalence and molecular diversity. *J Med Virol* 2003;**71**:82–93.
11. Nishida T, Kimura H, Saitoh M, Shinohara M, Kato M, Fukuda S, et al. Detection, quantitation, and phylogenetic analysis of noroviruses in Japanese oysters. *Appl Environ Microbiol* 2003;**69**:5782–6.
12. Sakon N, Yamazaki K, Utagawa E, Okuno Y, Oishi I. Genomic characterization of human astrovirus type 6 Katano virus and the establishment of a rapid and effective reverse transcription-polymerase chain reaction to detect all serotypes of human astrovirus. *J Med Virol* 2000;**61**:125–31.
13. Wang YH, Kobayashi N, Zhou DJ, Yang ZQ, Zhou X, Peng JS, et al. Molecular epidemiologic analysis of group A rotaviruses in adults and children with diarrhea in Wuhan city, China, 2000–2006. *Arch Virol* 2007;**152**:669–85.
14. Allard A, Albinsson B, Wadell G. Rapid typing of human adenoviruses by a general PCR combined with restriction endonuclease analysis. *J Clin Microbiol* 2001;**39**:498–505.
15. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005;**102**:12891–6.
16. Onishi N, Hosoya M, Matsumoto A, Imamura T, Katayose M, Kawasaki Y, et al. Molecular epidemiology of norovirus gastroenteritis in Soma, Japan, 2001–2003. *Pediatr Int* 2008;**50**:65–9.
17. Jin M, Xie HP, Duan ZJ, Liu N, Zhang Q, Wu BS, et al. Emergence of the GII4/2006b variant and recombinant noroviruses in China. *J Med Virol* 2008;**80**:1997–2004.
18. Sanchez-Fauquier A, Montero V, Moreno S, Solé M, Colomina J, Iturriza-Gomara M, et al. Human rotavirus G9 and G3 as major cause of diarrhea in hospitalized children, Spain. *Emerg Infect Dis* 2006;**12**:1536–41.
19. Chiristensen ML. Human viral gastroenteritis. *Clin Microbiol Rev* 1989;**2**:51–89.
20. Reither K, Ignatius R, Weitzel T, Seidu-Korkor A, Anyidoho L, Saad E, et al. Acute childhood diarrhoea in northern Ghana: epidemiological, clinical and microbiological characteristics. *BMC Infect Dis* 2007;**7**:104.
21. Chen SY, Chang YC, Lee YS, Chao HC, Tsao KC, Lin TY, et al. Molecular epidemiology and clinical manifestations of viral gastroenteritis in hospitalized pediatric patients in Northern Taiwan. *J Clin Microbiol* 2007;**45**:2054–7.

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北海道における水痘，流行性耳下腺炎，肺炎球菌感染症による入院例についての検討

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要　旨

水痘，ムンプス，肺炎球菌感染症による入院症例について札幌医科大学小児科と，北海道内の関連機関である23施設を対象として，2004～2008年の5年間に亘りアンケート調査を行った．年毎の差はあるものの，年間21～32例の水痘による入院，4～64例のムンプスによる入院，2～6例の化膿性髄膜炎を含む侵襲性肺炎球菌感染症を確認できた．これらの入院施設は道内の小児の全入院施設のほぼ3分の1に相当することから，小児科以外の入院も考慮すると，北海道全体においてはこの数倍の入院例があると推測される．以上より，これら疾患の一般小児における重要性は明らかであり，ワクチンの定期接種化による予防を考慮すべきと考えられた．

キーワード：水痘，流行性耳下腺炎，肺炎球菌感染症，予防接種

はじめに

水痘，ムンプスは予防接種により予防可能であるが任意接種のため接種率は低く，その流行は制御されていない．また，肺炎球菌感染症については現行の23価多糖体ワクチン（PPV23）は，肺炎球菌による髄膜炎を初めとした侵襲性感染症の多くを占める2歳未満児には有効でなく，現在，乳児期においても効果が期待される7価結合型ワクチン（PCV7）の導入が検討されている[1]．これらワクチンの定期接種への導入を検討する際には，これら疾患による重症例・入院例がどの程度発症しているのかの把握が重要である．その目的のためアンケート調査により，北海道の札幌医科大学小児科の関連病院における入院の実数を5年間に亘り調査した．

北海道は日本の国土の22％の面積を占めるが，人口は580万人と約4.5％である．アンケート調査の対象となった札幌医科大学小児科，および道内の23関連施設の入院症例は，北海道全体の入院症例の約1/3に相当すると考えられる．それら入院の実数を把握することで，北海道全体，ひいては本邦全体の入院の概数を推測することも可能である．また，2004～2008年という連続した5年間の入院数を調査することにより，それら感染症の流行の大きさと，その変化の様子を探ることもできる．

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方　法

札幌医科大学付属病院小児科と，その関連病院である小児の入院設備を有する道内23医療機関に，2004～2008年の5年間，毎年アンケート調査を依頼した．内容は1年間の水痘，ムンプス，肺炎球菌感染症による入院の例数とその内容である．更に各施設の小児病棟における1年間の総入院数についても調査した．検索は入院台帳，あるいは退院台帳を用い，個々の例については，必要であれば入院カルテの検索を依頼した．

結　果

依頼した医療機関の全てから回答が寄せられた．年毎の水痘，ムンプス，肺炎球菌感染症による入院数，および総入院数について全ての医療機関からの合計を表1に示す．水痘による入院は年間21～32例であった．ムンプスによる入院は年毎の差が大きく，2006年が64例と多かったが，2007年は13例，2008年は4例に過ぎなかった．肺炎球菌感染症の入院は89例～222例と年毎に2倍以上の差がみられた．年間の総入院数は13,000～17,000例程度であった．総入院数に占める割合は水痘が0.16～0.19％とほぼ一定していたが，ムンプスでは0.03～0.38％と幅が大きかった．肺炎球菌感染症が0.54～1.63％であった．5年間の入院数の合計と総入院数に占める割合は，それぞれ134例：0.18％，143例：0.19％，734例：0.96％であった．

次に，水痘による入院の主な入院理由について年毎に示す（表2）．5年間をまとめると水痘入院患児134例のうち重症のためが50例，熱性けいれんや脱水，急

表1　2004～2008年の北海道内24施設における水痘，ムンプス，肺炎球菌感染症による入院と総入院数

	2004年	2005年	2006年	2007年	2008年	合計
水痘	32（0.19%）	26（0.17%）	30（0.17%）	25（0.17%）	21（0.16%）	134（0.18%）
ムンプス	24（0.14%）	38（0.25%）	64（0.38%）	13（0.09%）	4（0.03%）	143（0.19%）
肺炎球菌感染症	89（0.54%）	140（0.92%）	169（1.01%）	114（0.79%）	222（1.63%）	734（0.96%）
総入院数	16,564	15,040	16,813	14,418	13,403	76,238

（　）は総入院数に占める割合を示す．

表2　水痘による入院の理由

	2004年	2005年	2006年	2007年	2008年	合計
重症水痘	7	6	15	12	10	50
合併症	18	9	10	10	7	54
基礎疾患	2	3	3			8
その他	5	8	2	3	4	22
合計	32	26	30	25	21	134

表3　ムンプスによる入院の理由

	2004年	2005年	2006年	2007年	2008年	合計
無菌性髄膜炎	11	19	35	6	1	72
重症ムンプス	9	7	18	6	2	42
睾丸炎	1	1			1	3
難聴	1					1
他合併症	2	6	5	1		14
その他		5	6			11
合計	24	38	64	13	4	143

性胃腸炎などの合併のためが54例であった．基礎疾患を有しているためが8例あった．その他が22例であるが，この中には乳児であるためや，他疾患で入院中に発症したなどが含まれた．

ムンプスによる入院の理由を年毎に表3に示す．無菌性髄膜炎がどの年も約半数を占めた．5年間をまとめると，143例中無菌性髄膜炎が72例，続いて重症化のためが42例であった．睾丸炎の併発が3例に，難聴が1例にみられた．

最後に，肺炎球菌感染症による入院の理由を表4に示す．5年間の合計では化膿性髄膜炎の起因菌として同定されたのは11例，他の深部感染症・敗血症が6例あった．また，急性中耳炎88例，肺炎308例などが含まれた．その他，321例の報告があったが，この中には起因菌というよりも常在菌である可能性が高いとの記載が多かった．尚，この3つの感染症による死亡例の報告は無かった．

考　察

2004～2008年の5年間にわたるアンケート調査結果をまとめた．いずれの年も回答率100%であった．総入院数は，ここ2年間は減少傾向が見られ，北海道の小児人口の減少，更に感染症流行が少なかったことなどが理由として挙げられたが，その減少は2割程度であることから，この5年間，ほぼ同規模の調査を続けることができたと考えられる．

水痘による入院は，ここ5年間で21～32例と大きな凸凹は無く，ほぼ同じ規模の流行が繰り返されていると推測された．入院理由としては重症水痘によるためより様々な合併症によるためが多く挙げられていたが，これは既存の報告と同様であった[2)3)]．

ムンプスによる入院は2006年に64例と最多であった後，2007年には13例，2008年には4例と激減した．この間のムンプスワクチン接種数の推移については検討していないが，ここ2年間で急に増えたことは考え難く，この入院数の減少は2006年の大きな流行により集団免疫が増強されたことによると考えられた．どの年もムンプスによる入院理由は無菌性髄膜炎が約半数を占め，その重要性が再確認された[4)5)]．睾丸炎[5)]や難聴[6)]の合併例は少なかったが，これらは泌尿器科や耳鼻科にて加療されることが多いため，今回の調査では実態を把握できていない可能性がある．

表4 肺炎球菌感染症による入院の理由

	2004年	2005年	2006年	2007年	2008年	合計
化膿性髄膜炎	3	3	1	3	1	11
深部感染症，敗血症		3	1	1	1	6
急性中耳炎	15	16	16	12	29	88
肺炎	40	68	68	38	94	308
その他	31	50	83	60	97	321
合計	89	140	169	114	222	734

肺炎球菌感染症については総計734例の報告があり，化膿性髄膜炎11例，深部感染症・敗血症6例の侵襲性感染症を確認できたが今までの疫学調査とほぼ同様と考えられた[7]．また，急性中耳炎の起因菌としても5年間で88例の報告があり，その重要性が確認できた．全体としての報告は21施設から3～115例と幅が大きかった．この入院数の多寡は総入院数とは必ずしも相関しておらず，呼吸器感染症について気道材料からの細菌培養がほぼルーチンに行われている施設から多くの入院例が報告された可能性がある．よって肺炎例の報告が5年間で308例と多いものの，その起因菌としての評価には十分に注意を要すると考えられる．

今回の調査対象となった小児の入院施設は北海道内の小児の全入院施設のほぼ3分の1に相当すると考えられ，小児科以外の入院も考慮すると，北海道全体におけるこれら疾患の入院数はこの数倍と概算できる．つまり，水痘による入院は年間100～140例，ムンプスによるものは流行年には300例以上，非流行年には20～60例，肺炎球菌による髄膜炎，敗血症・深部感染症10～30例と予測される．

全国で同様に入院例が生じていると仮定し，人口規模を考えて単純に20倍すると，全国で水痘による入院が年間2,000～2,800例，ムンプスは流行年で6,000例，非流行年で400～1,200例，侵襲性肺炎球菌感染症は200～600例の入院となる．入院に至らず外来にて加療されるケースはこの数倍になるであろうし，これらに，成人の重症水痘[9]や，年長児や成人男性におけるムンプス睾丸炎の併発，耳鼻科で加療されているムンプス難聴の発生も考慮する必要がある．更に，侵襲性肺炎球菌感染症については長期の入院治療，後遺症の発生などによる大きな疾病負担を考慮する必要もある[8]．以上を総合して考えた場合，定期ワクチン導入によりこれら疾患の防衛を図ることは重要と考えられる．

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文 献

1) 神谷 齊，岩田 敏，石和田稔彦，他．小児用7価肺炎球菌結合型ワクチンの医療経済効果．小児科臨床 2008；61：2233—2241.
2) 永井崇雄，浅野喜造，板倉尚子，他．健康小児の自然水痘重症度に関する臨床的検討：I. 1歳以上の年長児例を中心に．小児科臨床 1997；50：273—279.
3) 永井崇雄，浅野喜造，板倉尚子，他．健康小児の自然水痘重症度に関する臨床的検討：II. 乳児例について．小児科臨床 1997；50：453—458.
4) 庵原俊昭．ムンプス（Mumps）．臨床とウイルス 2002；30：28—32.
5) Mason WH. Mumps. In：Kliegman RM, Behrman RE, Jenson HB, et al, eds. Nelson Textbook of Pediatrics. 18th ed. Philadelphia：Saunders Elsevier, 2007：1341—1343.
6) Kawashima Y, Ihara K, Nakamura M, et al. Epidemiological study of mumps deafness in Japan. Auris Nasus Larynx 2005；32：125—128.
7) 砂川慶介，生方公子，千葉菜穂子，他．本邦における小児細菌性髄膜炎の動向．感染症誌 2008；82：187—197.
8) 岩田 敏，石和田稔彦，坂田 宏，他．肺炎球菌による小児髄膜炎・菌血症の疾病負担分析．小児科臨床 2008；61：2206—2220.
9) 高山直秀，味沢 篤，根岸昌功，他．成人水痘入院症例の検討—臨床像，重症度，感染経路および合併症．感染症誌 1997；71：1113—1119.

The number of hospitalized cases with varicella, mumps and *Streptococcus pneumoniae* infection in Hokkaido district

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The number of hospitalized cases with varicella, mumps and *Streptococcus pneumoniae* infection was examined by questionnaire study for Sapporo Medical University Hospital and other 23 city hospital with bed for children yearly during 2004 and 2008. Although there was differences year and year ; 21 to 32 cases with varicella, 4 to 64 cases with mumps, 2 to 6 cases with invasive *Streptococcus pneumoniae* infection including septic meningitis were confirmed yearly. These 24 medical facilities account for one third of total medical facilities with beds for children in Hokkaido district, therefore there might be several times cases might be admitted to hospital with these diseases every year in whole Hokkaido district considering the cases who were treated on facilities not for children. These results showed the importance of these vaccine-preventable diseases in general pediatrics and suggested the necessity of introduction of vaccines for these diseases to regular vaccine programs.