# Human Bocavirus: Prevalence and Clinical Spectrum at a Children's Hospital

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*Background.* Molecular methods of pathogen discovery have recently led to the description of several new respiratory viruses. Human bocavirus (HBoV), a proposed member of the family Parvoviridae, is one of the most recently described respiratory viruses. Initial reports indicate that HBoV is a common cause of respiratory tract infection in children.

*Methods.* A total of 1474 nasal scraping specimens collected over a 20-month period were screened by polymerase chain reaction for the presence of HBoV nucleic acid. Positive results were confirmed with a second polymerase chain reaction assay from a different genomic region. The medical records of patients with positive results were reviewed for demographic and clinical data.

*Results.* HBoV DNA was identified in 82 samples (5.6%). The peak rate of HBoV infection occurred during the period of March through May in both 2004 and 2005. Sixty-three percent of infected patients were <12 months of age. The most common symptoms were cough, rhinorrhea, and fever. Other symptoms of interest included diarrhea and a "paroxysmal" cough that was clinically suspected to be caused by *Bordetella pertussis*.

*Conclusions.* HBoV DNA is commonly present in children with upper and lower respiratory tract infections. The presence of a pertussis-like cough and diarrhea in association with HBoV infection merits further investigation.

Upper respiratory tract infections (URTIs) are among the most common infections in children, occurring 3–8 times per year in infants and young children [1]. According to the Centers for Disease Control and Prevention's *National Vital Statistics Report* [2], ~4 million children were born in the United States in 2003, suggesting that 12–32 million episodes of URTI occur each year among children aged 1–2 years. URTI can lead to acute asthma exacerbations, acute otitis media, and lower respiratory tract infection [3–5]. Viral respiratory infections in children are often due to rhinoviruses, respiratory syncytial virus (RSV), and parainfluenza viruses, which are most frequently identified by culture or antigen detection [1]. In clinical practice, a specific agent is often not identified, owing to the lack of sensitive tests or the presence of an as-yet unknown pathogen. Jennings et al. [6] used a variety of techniques to detect 7 different respiratory viruses and were able to detect a pathogen in 87% of children with acute respiratory infections, although some cases lacking a microbiologic diagnosis remained.

Molecular methods for identifying fastidious organisms have expanded our knowledge of infectious agents. Human metapneumovirus and several new coronaviruses were discovered using PCR amplification with nonspecific primers followed by a genetic comparison with known viruses [7] or by amplification with primers that broadly cross-hybridize with the sequence of a known pathogen [8]. By use of the former technique, Allander et al. [9] recently identified a virus designated "human bocavirus" (HBoV) on the basis of its homology to viruses in the family Parvoviridae. Additional screening revealed a prevalence of carriage of 3.1% among children with lower respiratory tract infections. With use of PCR data, we describe the prevalence of HBoV infection among children hospitalized at or seen in the emergency department at a large children's hospital and further describe the clinical illness associated with HBoV infection in children.

## PATIENTS, MATERIALS, AND METHODS

***Study participants and specimen collection and processing.***
This study was a retrospective analysis of nasal scraping specimens placed in saline for diagnostic testing for respiratory viruses by antigen detection. Specimens obtained from patients between birth and the age of 18 years were included. All specimens were included, regardless of the results from screening for RSV, influenza viruses A and B, parainfluenza viruses, and adenovirus. Specimens were submitted from the emergency department, inpatient facilities, and associated clinics at the Children's Hospital of San Diego, California. After receiving institutional review board approval, specimens collected during the period from 1 January 2004 through 30 November 2005 were stored at −70°C. Nucleic acid was extracted from 50–200 μL of sample using QIAamp MinElute Virus Spin Kit (Qiagen) and was stored at −70°C. All pre-PCR processing was undertaken in a separate location from PCR and post-PCR analysis.

***PCR optimization and screening.*** FastStart SYBR Green Master kit (Roche) and the primer set targeting the NP-1 region described by Allander et al. [9] were mixed in accordance with the manufacturer's instructions, with the exception that a primer concentration of 0.5 μmol/L was used for each primer. We optimized our PCR reaction using a plasmid containing a 560-bp region of the NP-1 region of HBoV that was graciously donated by Dr. Tobias Allander. After optimizing primer concentrations and cycling parameters, we were able to consistently detect 17 copies/μL of plasmid DNA.

After optimization, we tested each sample individually, adding 2.5 μL of nucleic acid to 7.5 μL of mastermix. With use of the Lightcycler instrument (Roche), the specimens were subjected to 1 cycle of 95°C for 10 min, followed by 45 cycles of 95°C for 0 s, 68°C for 0 s, 56°C for 10 s (temperature transition rate, 0.5°C/s), and 72°C for 16 s. Fluorescence was measured at the end of each extension step. Final melting analysis was achieved with continuous monitoring of fluorescence from 82°C to 98°C at a temperature transition rate of 0.05°C/s. The result for a specimen was considered to be positive if a single melting peak was measurable between 86.5°C and 89.0°C.

All samples with positive results were subjected to a second round of PCR analysis using primers targeting the NS1 gene described by Sloots et al. [10]. The FastStart SYBR Green Master kit and the primer set were mixed in accordance with the manufacturer's instructions, with the exception that we used a primer concentration of 0.5 μmol/L and subjected it to 1 cycle of 95°C for 10 min, followed by 35 cycles of 95°C for 0 s, 68°C for 0 s, 58°C for 8 s (temperature transition rate 0.5°C/s), and 72°C for 16 s. Melting analysis was achieved with continuous monitoring of fluorescence from 78°C to 98°C at a transition rate of 0.05°C/s. A single melting peak between 83.5°C and 86.0°C was considered to be a positive result.

***Sequencing.*** We sequenced samples with positive results obtained from 10 patients distributed over the 21-month study period. Amplicons from both primer sets were sequenced for each patient selected. Sequences were submitted to GenBank and registered under accession numbers DQ471802-DQ471811 for the NP-1 primers and DQ471812-DQ471821 for the NS1 primer set. DNA bands were located on 3% agarose gel with ethidium bromide. The QIAquick Gel Extraction Kit (Qiagen) was used in accordance with the manufacturer's recommendations. The DNA concentration was quantified by spectrophometer at 260 nm and 280 nm. Sequencing of DNA samples was performed by SeqWright (Houston, TX). Sequences were then aligned, and the homology was assessed using ClustalW (http://www.ebi.ac.uk/clustalw).

***Patient data collection.*** After receiving institutional review board approval, records from patients with positive sample results were reviewed for demographic data, clinical symptoms, laboratory results, radiographic findings, and the use of antimicrobials. Records were not available for patients seen in an outpatient clinic. Underlying illnesses were defined as chronic or persistent pulmonary disease (including asthma and bronchopulmonary dysplasia), anatomic cardiac malformations or congestive heart failure, inherited or acquired immunodeficiency, malignancy, chronic neurological disease, chronic gastrointestinal disease, chronic renal disease, or any other specific genetic defects listed in the chart. Prematurity was defined as a gestational age of <36 weeks at birth. Hypoxia was defined as an oxygen saturation <92% in room air. Patients with cyanotic heart disease were excluded from the analysis of oxygen saturations. An abnormal lung examination was defined as the presence of wheezes or crackles on auscultation or, in children with underlying chronic lung disease, as a change from baseline lung examination findings. Probable bacterial pneumonia was defined as an abnormal finding from a pulmonary examination, fever, and a focal "infiltrate" or "consolidation" on the official radiology report. Radiographic findings consistent with bronchiolitis or viral lower respiratory tract infections included hyperexpansion, peribronchial thickening, or atelectasis, as described in the official radiology report. The clinician's initial diagnosis was taken from either the admission history and physical examination or the emergency department discharge paperwork. A diagnosis of "rule-out pertussis" was given to patients for whom the clinician specifically listed the suspicion of pertussis as a primary diagnosis in the medical record.

Patients with proven or probable bacterial or fungal infections (bacteremia or fungemia; urinary tract infection with documented bacteriuria or funguria; *Bordetella pertussis* infection diagnosed by PCR of a nasopharygeal swab specimen, as diagnosed by the clinical microbiology laboratory; acute otitis media documented during the physical examination; or bacterial pneumonia, as described above) and those with a concomitant positive test result for another respiratory pathogen
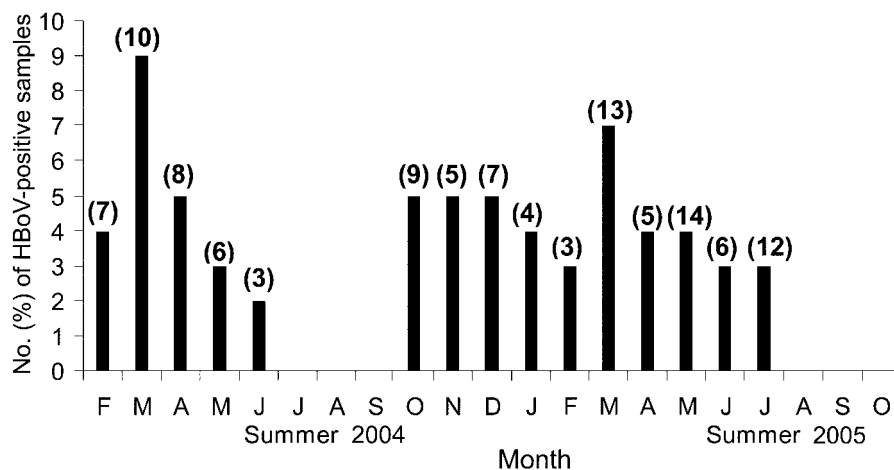
**Figure 1.** Total number of human bocavirus (HBoV)–positive samples, by month. Months are denoted by their first letters.

were removed from the analysis of symptoms, physical examination findings, and laboratory findings.

## RESULTS

***Screening and patient demographic characteristics.*** A total of 1474 specimens were collected, extracted, and screened for the presence of the HBoV NP-1 gene by PCR, of which 82 samples (5.6%) yielded positive results. All 82 samples with positive results also tested positive for the NS1 gene by PCR. One patient had 2 HBoV-positive samples that were submitted 7 days apart and was therefore presumed to have a single illness. RSV was detected in 173 patients (12%), and 36 patients (2%) had *B. pertussis* infection diagnosed by PCR of a nasopharyngeal swab specimen. HBoV was detected in 9 patients (11%) infection with RSV and in 1 patient (1%) with *B. pertussis* infection. There were no coinfections with adenovirus, parainfluenza, or influenza viruses, which were present in 1%, 2%, and 0.1% of all samples respectively.

A seasonal distribution was noted, with an absence of detectable HBoV in August and September of 2004 and 2005. Ten percent of all samples tested in March 2004 and 14% of all samples tested in May 2005 were positive for HBoV. Although a symmetric waxing and waning of HBoV infections occurred during the winter of 2003–2004, the winter of 2004–2005 had more sporadic activity (figure 1).

Fifty-two patients (63%) with HBoV infection were aged ≤12 months (range, 10 days to 16 years) (figure 2). The median age was 12 months. Data on sex were available for 72 patients; 46 (64%) of the patients with HBoV-positive samples were boys.

***Clinical findings associated with the presence of HBoV.*** Complete medical records were available for 68 (83%) of the 82 patients with HBoV-positive samples. An underlying illness was present in 21 (31%) of these patients. Underlying illnesses included asthma or brochopulmonary dysplasia in 11 patients

(52%), inherited or acquired neuromuscular disorders in 7 (33%), and trisomy in 1 (5%); 2 patients (9.5%) had undergone organ transplantation. Thirteen infants (19%) who were infected with HBoV were premature, with a median gestational age of 28 weeks. A total of 47 patients (57%) with HBoV infection had been hospitalized, with a median duration of hospital stay of 3 days. Thirty (63%) of the hospitalized patients required administration of oxygen for a median duration of 3 days. Eight patients (17%) were admitted to the intensive care unit for at least part of their hospitalization, with a median duration of stay in the intensive care unit of 3 days.

Fifty-four HBoV-positive patients (67%) with no viral antigen detected and no obvious bacterial or fungal coinfection were used for the final clinical analysis. The most common symptom was cough, which occurred in 46 patients (85%). Of the patients with cough, 10 (19%) were described as having a "paroxysmal" cough. Other common symptoms included difficulty breathing, nasal congestion or rhinorrhea, fever, diarrhea, and, less commonly, conjunctivitis and rash (table 1). Four of the 5 patients with a rash had blanching maculopapular erythema on the chest, and one-half of those patients had macular erythema of the face. No diagnostic testing for human herpesvirus–6 or parvovirus B19 was pursued for these patients, whose ages ranged from 2 months to 10 years.

Thirty-three patients (62%) had clinical evidence of lower respiratory tract infection. Hypoxia was present in 24 patients (44%) who were infected with HBoV at the time of presentation. The median abnormal oxygen saturation was 87% on room air (range, 70%–90%). Thirty-three patients (62%) had increased work of breathing on the physical examination, 11 (20%) were described as being "in distress," and 28 (52%) had abnormal findings during lung auscultation.

Of the 54 patients included in the analysis, bronchiolitis was the most common diagnosis; it was documented in 14 patients
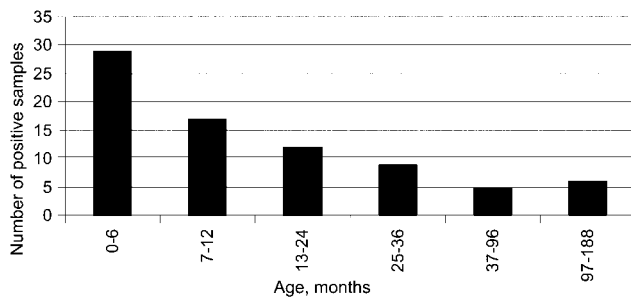
**Figure 2.** Age, in months, of patients with human bocavirus infection.

(26%). Other common diagnoses included pneumonia, rule-out pertussis, asthma, and URTI, which occurred in 13 patients (24%) each. Health care providers commonly listed multiple diagnoses. The most common combination was asthma and pneumonia, which was diagnosed in 6 patients (11%). Although the primary diagnosis in 13 patients was rule-out pertussis, a diagnostic test for *B. pertussis* was performed for 21 (39%) of the patients infected with HBoV, suggesting a high level of suspicion in an additional 8 patients. Nine patients were treated presumptively with a macrolide antibiotic for suspected *B. pertussis* infection. One patient received a diagnosis of acute gastroenteritis, although 9 patients (16%) had diarrhea as part of their clinical illness.

A bacterial coinfection was noted in 13 patients (19%). Seven patients (10%) had acute otitis media, 3 (7%) had bacterial pneumonia, 2 (3%) had a bacterial urinary tract infection with concomitant URTI symptoms, and 1 (<1%) had pertussis. In addition 1 patient (<1%) had URTI symptoms and a permanent central catheter infection with *Candida albicans*.

***Laboratory and radiographic findings associated with the presence of HBoV.*** Excluding patients with proven or probable bacterial infection, the WBC count in remaining patients was 3000–31,000 cells/mm$^3$ (median WBC count, 13,300 cells/mm$^3$). The median neutrophil, band, lymphocyte, and monocyte percentages were 40%, 10%, 39%, and 10% respectively. There were no notable abnormal findings in the routine chemistry panel. The C-reactive protein level, which was determined for 6 patients, ranged from 0.4 to 7.3 mg/dL (median level, 0.7 mg/dL).

Chest radiography was performed for 48 patients (78%) with HBoV infection. Of these radiographs, 16 (34%) were normal, 5 (10%) showed a focal infiltrate, and 27 (56%) had findings consistent with bronchiolitis or other presumed viral lower respiratory tract involvement.

***Sequencing of the NP-1 and NS1 amplicons.*** The regions that were amplified for the NP-1 gene and the NS1 gene were highly conserved over the 21-month period. Homology within the amplified region of the NP-1 gene was 98%–100%, and

within the NS1 gene, it was 95%–100%. There was no noticeable pattern of genetic variation over time (figure 3).

## DISCUSSION

A new age of pathogen discovery has been ushered in with the use of molecular techniques to identify organisms that have resisted conventional isolation methods. With the discovery of each new infectious agent, many questions arise regarding the clinical spectrum, frequency, significance, and diagnostic method of choice. In the original description of HBoV, Allander et al. [9] suggested that this agent caused 3.1% of lower respiratory tract infections among children from Stockholm, Sweden. Soon thereafter, a wintertime prevalence of 5.2% in Queensland, Australia, was reported in children with acute respiratory tract infections [10]. In our study population, which consisted mainly of hospitalized patients, we were able to identify HBoV in 82 children, yielding a prevalence of 5.6% over a continuous 21-month period, with a peak prevalence as high as 14% in the spring and a virtual absence of activity during the summer months. Sequencing of the HBoV-positive samples from 10 patients demonstrated little genetic variation within the amplified regions of the NP-1 and NS1 genes over 21 months.

We show an association between detection of HBoV in the upper respiratory tracts of children and lower respiratory tract infection. We found that HBoV infections of the upper respiratory tract in children were also found in conjunction with known complications of viral respiratory infections, such as asthma, acute otitis media, and pneumonia, although the frequency of these complications in our study may have been exaggerated as a result of the high percentage of hospitalized patients. The most common reason for HBoV-infected children to be hospitalized was respiratory distress and hypoxia associated with a diagnosis of bronchiolitis, supporting the concept that HBoV may directly infect the lower respiratory tract. Diagnostic testing of samples from the lower respiratory tract will

**Table 1. Symptoms of patients with human bocavirus infection, excluding those with another known source of infection.**

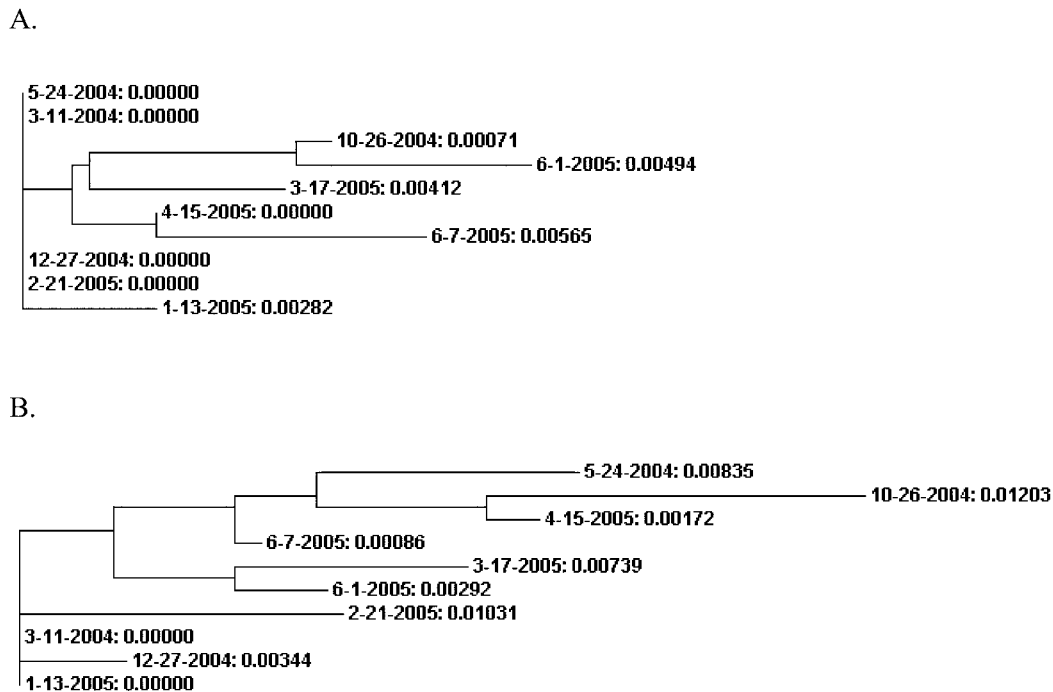| Symptom | No. (%) of patients | Duration of symptom, days Median (range) | Mean |
|---|---|---|---|
| Any cough | 46 (85) | 4 (1–15) | 5.6 |
| Rhinorrhea | 36 (67) | 5 (1–15) | 6.5 |
| Fever | 32 (59) | 2 (1–7) | 2.6 |
| Difficulty breathing | 26 (48) | 1 (1–2) | 1.1 |
| Paroxysmal cough | 10 (19) | 4 (1–15) | 6.8 |
| Diarrhea | 9 (16) | 3 (1–10) | 3.3 |
| Conjunctivitis | 5 (9) | 2 (1–4) | 2.4 |
| Rash | 5 (9) | 2 (1–5) | 2.6 |

A.



B.



**Figure 3.** Phylogenetic tree for the amplified products of the NP-1 gene *(A)* and the NS1 gene *(B)*. Specimens are labeled with the date they were collected, followed by the phylogenetic distance.

be needed to confirm HBoV as a causative agent of lower respiratory tract infection. HBoV was also associated with URTIs, alone or in combination with other presumably unrelated illnesses, such as bacterial urinary tract infections.

We found several aspects of HBoV infection that had not been previously described, including the relatively high frequency of paroxysmal cough and a clinical suspicion of pertussis. Nineteen percent of all identified HBoV-infected patients had a paroxysmal cough, leading to further testing for pertussis and presumptive treatment in one-half of those cases. Although coughing paroxysms have been linked to the presence of pertussis toxin in a rat model [11], the presence of paroxysms in *Bordetella parapertussis*, which does not contain toxin, raises questions about the etiology of such a cough in *B. pertussis* infection [12]. Whether coughing paroxysms in HBoV-infected patients are due to nasopharyngeal irritation and drainage, tracheal inflammation, or lower airway infection is unknown.

Additionally, 16% of patients with HBoV detected in the upper respiratory tract also had diarrhea, either isolated or in association with respiratory symptoms. Although we did not test stool specimens for the presence HBoV, diarrhea in HBoV-patients is an intriguing finding, considering that canine parvovirus causes severe gastrointestinal infections in dogs [13], and some of the earliest members of the family Parvoviridae were identified in stool samples. Some controversy surrounds the clinical relevance of these initial findings, because the elec-

tron micrographic appearance of parvoviruses can be confused with that of other gastrointestinal pathogens, such as astrovirus [14], and the particles were also found in asymptomatic children [1]. Parvovirus B19 is not considered to be an intestinal pathogen, although 2 case reports have described diarrhea in patients with active infection [15, 16].

Four children were identified with maculopapular erythematous rashes, which were most often distributed across the chest and trunk, and one-half of these children also had involvement of the face, which is reminiscent of the rash associated with parvovirus B19 [17]. In addition, 5 children had conjunctival injection, which has only rarely been described in conjunction with parvovirus B19 infection [18].

Although the strength of this study is in the relatively large number of children tested and identified with HBoV, we recognize the problems with this retrospective study. The presence of HBoV DNA in children with respiratory infection does not prove causality, particularly because testing was often done in response to the presence of such symptoms. Likewise, because all of our samples were submitted for testing of ill children, the prevalence we report may not reflect the true prevalence in the community. To determine its true epidemiology, it will be important to determine the prevalence of HBoV infection among healthy children and the prevalence of antibody at different ages. In addition, the majority of our patients were admitted to the hospital, and because testing may have been per-

formed only for children with more-severe illness, the severity of symptoms may have been exaggerated. Finally, although PCR is a powerful tool, there are potential problems associated with such high sensitivity. Small amounts of DNA from prolonged shedding following prior infection cannot be differentiated from DNA from active infection, and even small amounts of contamination within the laboratory can cause false-positive results. We reduced the chance that our results were false positives by using separate locations for processing and running samples and by confirming all initial positive results with a second PCR assay using a different genetic region.

In conclusion, we reinforce the previous finding that HBoV is a relatively common cause of URTIs in children and that it can be associated with symptomatic lower respiratory tract illness. In addition, we suggest that HBoV can cause a disease that may be confused with *B. pertussis* infection by inducing a paroxysmal cough. Finally, we propose that HBoV may occasionally manifest with gastrointestinal and dermatologic findings.

## Acknowledgments

## References

1. Feigin RD, Cherry JD, Demmler GJ, Kaplan SL. Textbook of pediatric infectious diseases, 5th ed, vol. 1. Philidadelphia: Elsevier, **2004**.
2. Hamilton B, Martin JA, Sutton PD. Births: preliminary data from 2003. National vital statistics report. Hyattsville, MD: National Center for Health Statistics, **2004**.
3. Cappelletty D. Microbiology of bacterial respiratory infections. Pediatr Infect Dis J **1998**; 17(8 Suppl): S55–61.
4. Rovers MM, Schilder AG, Zielhuis GA, Rosenfeld RM. Otitis media. Lancet **2004**; 363:465–73.
5. Tan WC. Viruses in asthma exacerbations. Curr Opin Pulm Med **2005**; 11:21–6.
6. Jennings LC, Anderson TP, Werno AM, Beynon KA, Murdoch DR. Viral etiology of acute respiratory tract infections in children presenting to hospital: role of polymerase chain reaction and demonstration of multiple infections. Pediatr Infect Dis J **2004**; 23:1003–7.
7. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med **2001**; 7: 719–24.
8. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. J Infect Dis **2005**; 191:492–8.
9. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA **2005**; 102:12891–6 (erratum: Proc Natl Acad Sci USA 2005; 102:15712).
10. Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. J Clin Virol **2006**; 35:99–102.
11. Parton R, Hall E, Wardlaw AC. Responses to *Bordetella pertussis* mutant strains and to vaccination in the coughing rat model of pertussis. J Med Microbiol **1994**; 40:307–12.
12. Wirsing von Konig CH, Finger H. Role of pertussis toxin in causing symptoms of *Bordetella parapertussis* infection. Eur J Clin Microbiol Infect Dis **1994**; 13:455–8.
13. Squires RA. An update on aspects of viral gastrointestinal diseases of dogs and cats. N Z Vet J **2003**; 51:252–61.
14. Oliver AR, Phillips AD. An electron microscopical investigation of faecal small round viruses. J Med Virol **1988**; 24:211–8.
15. Bateman HE, Kirou KA, Paget SA, Crow MK, Yee AM. Remission of juvenile rheumatoid arthritis after infection with parvovirus B19. J Rheumatol **1999**; 26:2482–4.
16. Matano S, Kinoshita H, Tanigawa K, Terehata S, Sugimoto T. Acute parvovirus B19 infection mimicking chronic fatigue syndrome. Intern Med **2003**; 42:903–5.
17. Young NS, Brown KE. Parvovirus B19. N Engl J Med **2004**; 350:586–97.
18. Scharre JE, Veith J. Conjunctivitis associated with fifth disease in a child: a case report. J Am Optom Assoc **1996**; 67:763–6.