Epidemiological Profil and Clinical Associations of Human Bocavirus and Other Human Parvoviruses

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(See the editorial commentary by McIntosh, on pages 1197–9, and the major article by Kesebir et al., on pages 1276–82.)

Background. Human bocavirus (HBoV) and PARV4 are newly discovered human parvoviruses. HBoV, which was firs detected in respiratory samples, has a potential role in the development of human respiratory disease. The present study compared the frequencies, epidemiological profiles and clinical backgrounds of HBoV and PARV4 infections with those of other respiratory virus infections, by evaluating diagnostic samples referred to the Specialist Virology Laboratory (SVL) at the Royal Infi mary of Edinburgh (Edinburgh, United Kingdom).

Methods. Anonymized samples and study subject information were obtained from the respiratory sample archive of the SVL. Samples were screened for HBoV, PARV4, B19, respiratory syncytial virus (RSV), adenoviruses, influenz viruses, and parainfluenz viruses by use of nested polymerase chain reaction.

Results. HBoV infection was detected in 47 (8.2%) of 574 study subjects, ranking third in prevalence behind RSV infection (15.7%) and adenovirus infection (10.3%). Peak incidences of HBoV were noted among infants and young children (age, 6–24 months) during the midwinter months (December and January) and were specificall associated with lower respiratory tract infections. HBoV infections were frequently accompanied by other respiratory viruses (frequency, 43%), and they were more prevalent among individuals infected with other respiratory viruses (17%), frequently adenovirus or RSV. All respiratory samples were negative for PARV4.

Conclusions. In the present study, HBoV was a frequently detected, potential respiratory pathogen, with a prevalence and an epidemiological profil comparable to those of RSV. Identificatio of HBoV infections may be clinically important in the future.

Adoption of nucleic acid testing (NAT) by diagnostic virology laboratories provides the scope for much more rapid and effective testing of samples obtained from individuals with respiratory and other infections. However, it remains a considerable challenge to devise NAT-based assays, such as the polymerase chain reaction (PCR) assay, that can detect the wide range of viruses potentially associated with respiratory illness. Currently, PCR-based screening for well-established pathogenic viruses, including respiratory syncytial virus (RSV), parainfluenz virus (PIV) types 1–3, influenz A and B viruses, adenovirus, and, more recently, human metapneumovirus (HPMV), detects viral infection in a majority of individuals with often severe respiratory illnesses. With these targets, multiplexing of primers produces a screening assay that represents a reasonable compromise between target range and practicality, testing large numbers of referred clinical specimens. However, a substantial proportion of respiratory infections remain undiagnosed, not least because screening for several other viruses associated with upper and lower respiratory tract infections (URTIs and LRTIs, respectively) is not frequently performed. Screening tests for respiratory viruses often omit influenz C virus, human rhinoviruses, and coronaviruses, as well as parechoviruses and enteroviruses.

Against this background of incomplete screening by both PCR-based screening methods and the virus-isolation methods that preceded them, the extent to which other undiscovered viruses contribute to respiratory

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disease in humans is largely unknown. When several direct methods of virus detection (i.e., PCR, immunofluo escence, and culture) were combined with serological testing for the virus targets mentioned above (but not for PIV-4, parechoviruses, influenz C virus, and group 2 coronaviruses), a viral cause was detected for 88% of cases of bronchiolitis and other diagnosed LRTIs requiring the hospitalization of young children [1]. These finding indicate that, even with relatively exhaustive testing, there still remains a potential window for the diagnosis of other, uncharacterized respiratory pathogens. The proportion of unaccounted infections may indeed vary in different geographical areas with different virus epidemiological profile (reviewed in [1]).

Recently, a new human parvovirus was discovered through the application of a random PCR/cloning technique performed on pooled respiratory samples, followed by bioinformatic analysis of the sequences of the resulting clones [2, 3]. This virus has been named "human bocavirus" (HBoV), reflectin its distant relatedness and resemblance to the genome organization of 2 other parvoviruses, bovine parvovirus 1 and minute virus of canines, that are currently classifie as belonging to the *Bocavirus* (i.e., bovine/canine) genus in the family *Parvoviridae*. At approximately the same time, by use of similar methods for the detection of virus in blood samples obtained from individuals with acute infections of undiagnosed etiology, another human parvovirus was discovered and was named "PARV4" [4]. This virus shows no close resemblance to any other known mammalian parvoviruses and will probably be classifie as the sole member of a new genus within the parvovirus family.

By use of a PCR-based detection method using primers from the NP-1 gene, HBoV was frequently detected among diagnostic respiratory samples in the original study from Stockholm [3] and, more recently, in studies from Australia, Japan, Canada, and France [5–8]. These studies used a variety of study designs and sample-selection criteria, but all showed associations between HBoV detection and LRTIs for respiratory samples found to be negative for other respiratory viruses. Much less is currently known about the distribution of PARV4 and its clinical associations; PARV4 DNA has been detected in pools of plasma samples [9], but whether it is a respiratory pathogen is unknown.

To investigate the involvement of these new parvoviruses in respiratory disease, diagnostic respiratory samples were screened for HBoV and PARV4 DNA by means of highly sensitive nested PCR. Samples were referred from October 2005 to February 2006, covering the period of peak incidence of respiratory tract infections in Scotland. The results of diagnostic testing for other viruses were combined to enable a comparison of the epidemiological profile and clinical associations of the viruses, as well as to assess the potential etiological role of these recently discovered parvoviruses in human respiratory disease.

MATERIAL AND METHODS

Test samples. The study involved a total of 924 samples that had been referred to the Specialist Virology Laboratory (SVL), Royal Infi mary of Edinburgh, for respiratory virus testing. The samples included 767 nasopharyngeal swabs or aspirates, 48 bronchoalveolar lavage flui samples, 20 tracheal swabs or aspirates, 22 sputum samples, 43 samples of "other" types, and 24 samples for which no type was recorded. Routine detection of respiratory viruses comprised nested PCR for RSV, influenz A and B viruses, PIV-1–3, and human adenovirus, on the basis of assays described elsewhere [10, 11], modifie for a nested format.

All samples were firs anonymized and then deposited in the SVL respiratory sample archive before testing. Approval was obtained from the Lothian Regional Ethics Committee to retain information during the anonymization process for epidemiological purposes while strictly protecting patient confidentialit . The stored data comprised each subject's age group and partial postcode, any recorded symptoms or clinical information, the referral source, the month of sample collection, and the results of other virological or bacteriological testing of the sample.

Detection of HBoV and other parvoviruses. Total nucleic acid was extracted from clinical specimens by use of the MinElute Virus Spin kit (Qiagen), according to the manufacturer's instructions. Two nested PCR methods were developed for the detection of HBoV. Samples were initially screened using the following primers from a highly conserved region of the NS-encoding region among complete genome sequences within the *Parvoviridae* (positions in the published, complete genome sequence of HBoV [ST2; GenBank accession number DQ000496] are shown in parentheses): outer sense and antisense primers 5′-TATGGGTGTGTTAATCATTTGAAYA-3′ (nt 1585) and 5′-GTAGATATCGTGRTTRGTKGATAT-3′ (nt 1794) and inner sense and antisense primers 5′-AACAAAGGATTTG-TWTTYAATGAYTG-3′ (nt 1600) and 5′-CCCAAGATACACT-TTGCWKGTTCCACCC-3′ (nt 1703). Individual positive samples were retested by means of a second PCR assay performed with primers from the NP-1 gene. The outer primers used were 5′-CCAGCAAGTCCTCCAAACTCACCTGC-3′ (nt 2325) and 5′-GGAGCTTCAGGATTGGAAGCTCTGTG-3′ (nt 2723); inner primers followed the sequence of the primer sequences 188F and 542R, which are published elsewhere [3], with the sequence error corrected in an erratum to the publication. Assay sensitivity was determined using a dilution series in carrier DNA (0.05 μg/mL) of the recombinant full-length HBoV ST1 clone (provided by Tobias Allander). DNA that was amplifie from primers from confi med samples was sequenced using the Big Dye Terminator kit (Applied Biosystems).

Parvovirus B19 DNA was screened using nested PCR with the use of primers and methods described elsewhere [12]. Assay sensitivity for single copies (expressed as international units)

of the target sequence was determined using a dilution series of the Parvovirus B19 International Standard (data not shown; National Institute for Standards and Biological Controls). Nucleic acid was screened in pools of 10 samples, and pools with positive results were split to identify component samples as described for HBoV.

PARV4 DNA was detected using nested PCR with the use of the following primers from a conserved region of the NS-encoding region (positions in the published sequence of PARV4 [GenBank accession number NC_007018] are shown in parentheses): outer sense and antisense primers 5′-GCTGCTGCCATAGCAAATCTTAGTCC-3′ (nt 1312) and 5′-GGGTGCTAACCACAGGGCCATCAAC-3′ (nt 1588) and inner sense and antisense primers 5′-AACCAAAATTTCCCATTTAATGACTG-3′ (nt 1363) and 5′-GGTAGGTATGTAGTCTTCGCTGCC-3′ (nt 1524). Assay sensitivity was determined using a dilution series of a cloned PARV4-related DNA sequence (provided by Sally Baylis, National Institute for Standards and Biological Controls). Nucleic acid was screened in pools of 10 samples, as described for HBoV.

RESULTS

Study group. A total of 924 respiratory samples (83% of which were nasopharyngeal swabs or aspirates) obtained from 574 different individuals (328 males and 246 females) were available from the archive for screening for parvoviruses. These samples represented >90% of all samples referred to the SVL between October 2005 and February 2006 for respiratory virus screening. These samples were predominantly referred from infants and young children (624 samples were obtained from 357 study subjects <5 years of age). More than 90% of study subjects were treated in accident and emergency departments or inpatient wards in referral hospitals in Edinburgh or neighboring hospitals.

By use of multiplexed nested PCR, all samples were screened for a range of respiratory viruses, including RSV, influenz A and B viruses, PIV-1–3, and adenoviruses. The following viruses were detected in the study samples: RSV ($n = 115$), adenovirus ($n = 72$), influenz A virus ($n = 4$), influenz B virus ($n = 43$), PIV-1 ($n = 6$), PIV-2 ($n = 27$), and PIV-3 ($n = 2$). Respiratory viruses were detected in 245 (27%) of all 924 samples; in 23 of these 245 samples, coinfections with 2 viruses were noted (usually RSV and adenovirus [$n = 18$] but also PIV-2 and adenovirus [$n = 2$], PIV-1 and adenovirus [$n = 1$], influenza B virus and RSV [$n = 1$], and influenz B virus and PIV-2 [$n = 1$]).

Assay sensitivity. The sensitivity of the HBoV PCR assay using the NS and NP-1 primers was determined by limiting dilution analysis of a cloned HBoV sequence of predetermined (spectrophotometric) original concentration. PCR analyses performed with the 2 sets of primers showed equivalent sensitivity, with both primer sets able to detect close to single copies of target sequence in a carrier DNA solution (table 1). The newly developed PARV4 nested PCR was similarly able to detect single copies of the PARV4 sequence (table 1).

Frequency of parvovirus infections. The 924 study samples were screened for HBoV, parvovirus B19, and PARV4 DNA sequences by means of separate nested PCRs performed initially in pools of 10 samples; pools with positive results were split to identify individual positive samples. For HBoV, a total of 54 individual positive samples were identifie with the screening primers from NS; of these, 53 were also found to be positive by PCR performed with primers from the NP-1 gene (sample prevalence, 5.7%). These 53 samples with confi med positive results were obtained from a total of 47 different individuals, yielding an overall prevalence of infection of 8.2% in the study group (8.0% among males and 8.5% among females). The amplicon from the NP-1 primers was directly sequenced in samples obtained from 10 different HBoV-positive individuals. Eight sequences were identical to the prototype ST2 strain, 1 sample showed a single substitution, and 1 sample showed 3 substitutions over the 309 bases sequenced between the inner primers (data not shown). Four samples from 3 different study subjects were positive for parvovirus B19. No samples were positive for PARV4.

HBoV was the third most frequently detected virus in the viral screening of respiratory samples. RSV, which was the most frequently detected virus, was amplifie from 115 samples obtained from 90 different individuals; the next most frequently detected virus was adenovirus (amplifie from 72 samples obtained from 59 individuals). HBoV was more frequently detected in samples in which other respiratory viruses were present (table 2), with HBoV DNA detected in 9% of coinfected samples, compared with 4% of samples in which no virus was detected. HBoV was detected most frequently among samples containing adenovirus (10 [14%] of 72 adenovirus-positive samples were HBoV positive). HBoV was found even more frequently among samples containing 2 other viruses (in 4 [17%]

Table 1. Sensitivity of polymerase chain reaction assay for human bocavirus (HBoV) and PARV4.

	No. of positive replicates detected/total no. of replicates tested, by the no. of input copies per reaction				
Virus, primer	1000	100	10	1	0.1
HBoV					
NS	12/12	12/12	12/12	5/12[a]	1/12
ST-1	9/9	9/9	9/9	5/9[b]	0/9
PARV4, NS	8/8	16/16	16/16	7/16[c]	1/16

[a] 0.54 copies detected per replicate, on the basis of the Poisson distribution ($f = -\ln(f_0)$, where f_0 is the frequency of negative reactions).
[b] 0.81 copies detected per replicate.
[c] 0.56 copies detected per replicate.

Table 2. Frequency of human bocavirus (HBoV) codetection.

Virus codetection status	HBoV-positive samples, no. (%)	HBoV-negative samples, no. (%)
No virus detected	30 (4)	643 (96)
Coinfection detected		
With 1 virus		
RSV	6 (6)	90 (94)
Influenza A virus	1 (25)	3 (75)
Influenza B virus	4 (10)	37 (90)
PIV-1	0	5
PIV-2	1 (4)	23 (96)
PIV-3	0	2
Adenovirus	7 (14)	44 (86)
Total	19 (9)	204 (91)
With 2 viruses		
RSV and adenovirus	3 (17)	15 (83)
Other viruses	1 (20)	4 (80)
Total	4 (17)	19 (83)
All	23 (9)	223 (91)

NOTE. PIV, parainfluenza virus; RSV, respiratory syncytial virus.

of 23 samples) than among samples containing single coinfections (19 [9%] of 204 samples). In total, 23 (43%) of the 53 HBoV-positive samples contained other viruses. This frequency was higher than that observed for other viruses (47 [17%] of 271 samples [excluding HBoV coinfections]) (table 3).

Clinical associations of HBoV infections. Sample and study subject information was recorded for each sample tested and was available in anonymized form for analysis of the epidemiological profil of the virus and clinical associations. The analysis was based on total numbers of study subjects, rather than total numbers of samples, to prevent overrepresentation of individuals from whom multiple samples were referred.

The frequency of HBoV infection varied substantially over the study period (figu e 1*A*), with high prevalences of HBoV detection noted in December and January and with decreases noted before and after these midwinter months. The pattern of prevalence of HBoV infection resembled that of RSV infection; in contrast, adenovirus and parainfluenz virus infections showed much less evidence for seasonal variation. The highest frequencies of influenz virus infections were noted later than those of HBoV and RSV infections.

Similar age profile were noted for individuals with HBoV and RSV infections, with infections almost completely confine to infants and young children (figu e 1*B*), although RSV infections tended to occur more frequently in neonates (18% vs. 2%, for HBoV infection among infants <1 month of age). Other viral infections were far less associated with age, although the highest frequencies of adenovirus infection also occurred among infants and children who were 6 months to 2 years of age. HBoV infections were detected in 4 samples from 3 individuals 50–65 of age who were hospitalized in adult respiratory wards, at least 2 coming from immunosuppressed individuals, as were all 3 adults with RSV infections.

Frequencies of HBoV infections were higher among individuals with reported respiratory disease than among individuals without symptoms (figu e 2*A*), particularly those with LRTIs. The association of HBoV with respiratory disease was comparable to that observed for RSV and influenz viruses. In contrast, the highest frequencies of detection of PIVs and adenoviruses were noted for individuals without clinically apparent respiratory disease. In common with most of the viruses analyzed, samples referred from the pediatric accident and emergency department were frequently found to be positive for HBoV (frequency, 15%) and typically were obtained from individuals with acute respiratory symptoms (figu e 2*B*).

DISCUSSION

The present study reports a relatively high frequency of infection with the newly discovered HBoV, predominantly among young children. The overall prevalence of HBoV infection was

Table 3. Frequency of virus codetection.

Virus codetection status	Codetection, no. (%) of samples	No codetection, no. (%) of samples
HBoV codetected	23 (43)	30 (57)
Codetection excluding HBoV		
RSV	19 (17)	96 (83)
Influenza A virus	0	4
Influenza B virus	2 (5)	42 (95)
PIV-1	2 (29)	5 (71)
PIV-2	3 (11)	24 (89)
PIV-3	0	2
Adenovirus	21 (29)	51 (71)
Total	47 (17)	224 (83)

NOTE. HBoV, human bocavirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

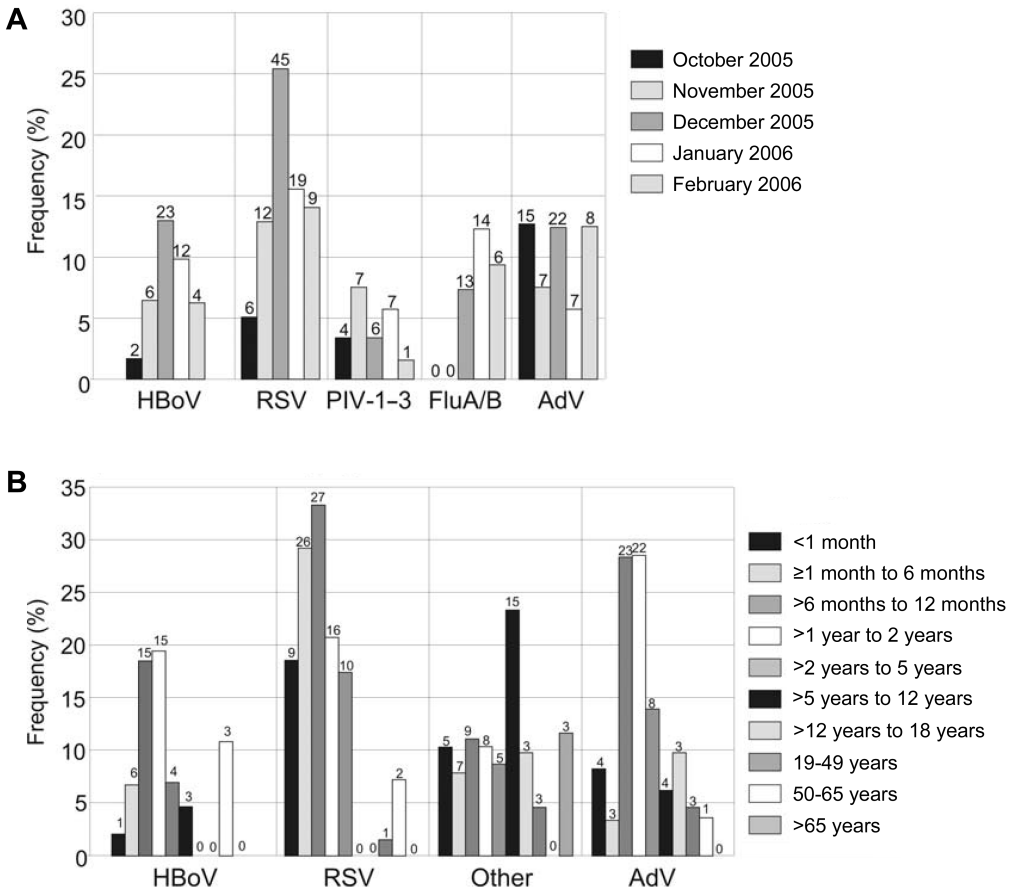

Figure 1. Frequencies of human bocavirus (HBoV) and other viral infections among individuals, according to different calendar months and years of the study *(A)* and different age groups *(B)*. The frequency is the percentage of positive samples among the total no. of samples tested in each category. Nos. above the bars denote the nos. of individuals positive for infection. AdV, adenovirus; FluA/B, influenza A and B viruses; PIV-1–3, parainfluenza virus types 1–3; RSV, respiratory syncytial virus.

higher in the present study than in previous studies conducted in Sweden, Australia, Japan, Canada, and France [3, 5–8]. However, these studies differ in their design; some estimated the prevalence of HBoV infection among all samples referred to a diagnostic laboratory for respiratory virus testing (i.e., studies from Sweden [3] and Canada [7]), whereas other studies limited estimation of the prevalence of HBoV infection to samples obtained from individuals with respiratory disease (Australia [5]) or samples obtained from pediatric patients (patients <5 years of age in the French study [8]). The Japanese study [6] was restricted to assessment of samples obtained from children who had LRTIs and excluded samples that were positive for other respiratory viruses (RSV, influenz A and B viruses, and HMPV). The comparison of rates of HBoV detection in the current study therefore requires preselection of subsets of the data obtained.

The overall prevalence of HBoV infection in the present study (8.2%) was ~3 times greater than that noted in the original Swedish study during the calendar months of October to February (13 [3.5%] of 370 samples tested positive) [3], matching the testing interval of the current study. The overall prevalence of 1.1% (18 of 1019 samples were positive) in the Canadian study was not strictly comparable with our data, because a greater proportion of samples were collected from adult patients [7], with <25% of the samples obtained from children <5 years of age, compared with 62% in the current study. However, 3 (2.5%) of ~120 samples obtained from children <5 years of age were found to be positive during the calendar months of October to February, which, again, is a frequency considerably lower than that noted for the matching subset of study subjects in the study from Edinburgh (41 [12%] of 357 individuals were positive for HBoV]). In the French study [8], the overall frequency of HBoV (9 [3.4%] of 263 samples were positive) among children <5 years of age who had samples collected between November and January was similarly 4 times lower than the frequency of 14% (32 of 245 samples were positive) in the matching subset of samples from Edinburgh. Similarly, HBoV was detected in 17 (7%) of 259 study subjects <5 years of age during

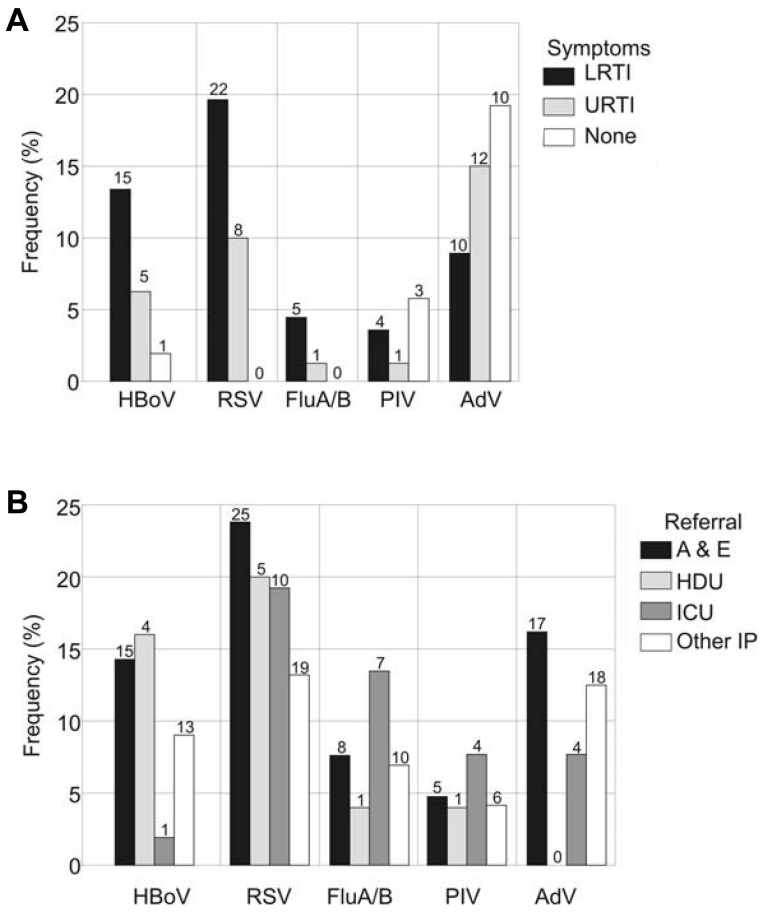

Figure 2. *A,* Frequencies of human bocavirus (HBoV) and other viral infections among individuals with symptoms and clinical descriptions denoting the presence of either lower respiratory tract infection (LRTI) or upper respiratory tract infection (URTI) or among individuals without respiratory symptoms. *B,* Frequencies of infection with each virus among individuals, according to different referral sources from the Royal Hospital for Sick Children (RHSC). A & E, accident and emergency department; AdV, adenovirus; FluA/B, influenza A and B viruses; HDU, high dependency unit; ICU, intensive care unit; IP, inpatients; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

the winter months in the Australian study [5], compared with the prevalence of 12% noted in the study from Edinburgh. Finally, in Japan, the year-round prevalence of HBoV among children with LRTIs who were found to be negative for other respiratory viruses was 5.7% (18 of 318 patients) [6]. The study does not include enough information for a precise, seasonally adjusted comparison of prevalence with that noted in the Edinburgh study; however, the frequency of HBoV infection among children in Edinburgh who had LRTIs and who were negative for other viruses in Edinburgh was again higher (11% [30] of 247 children were HBoV positive]).

Overall, after removal of the various compounding factors that originated from the different study designs, the frequencies of HBoV infection were 2–4 times higher in the Edinburgh study than in any of the previous studies. The increased rate of detection may have resulted from differences in the frequencies of HBoV infection in different parts of the world, as well as from different seasonal associations. However, all of the other studies involved the use of PCR assays with a single-round PCR rather than the potentially more-sensitive nested screening and confi matory PCR methods developed in the current study. Because assay sensitivity was not determined in any of the previously published studies, and because a non-optimized single-round PCR with a relatively large amplicon size would be unlikely to achieve the sensitivity of nested PCR, it is therefore probable that a proportion of HBoV-positive samples were missed in the earlier studies. Clearly, standardization of assay sensitivity will be important for comparative epidemiological studies in the future.

The present study was limited to the winter months of 2005–2006, and it therefore does not document any year-round changes in prevalence. However, the markedly increased frequencies of detection of HBoV in samples referred to the SVL in December and January were consistent with the peak incidence of HBoV infection in winter described in Australia [5] and Sweden [3]. However, estimates of seasonal prevalence are

complicated by the marked differences in the number of sample referrals between summer and winter, coinciding with changes in the incidence of respiratory disease. Indeed, the actual frequencies of HBoV detection per sample may be similar throughout the year. For example, dividing the year into 2 periods (April–September and October–March) yields a frequency of HBoV detection of 3.5% (3 of 85 samples were positive) in the summer in Stockholm, which is similar to the frequency of 3.1% noted in winter (14 of 455 samples were positive) [3]. A similarly constant frequency of detection on a per-sample basis was reported from Canada (8 of 617 samples obtained in summer were positive, and 10 of 592 samples obtained in winter were positive [7]). However, although not indicated in the latter study, monthly totals of samples were clearly adjusted to ~50 samples and did not reflec seasonal changes in the numbers of samples referred. Overall, the published data indicating a constant rate of HBoV detection in respiratory samples, irrespective of season, do not distinguish between HBoV being an incidental findin or being associated with seasonal respiratory disease. However, our own data documenting marked changes in rates of HBoV infection even within the 5-month study period (figu e 1*A*) provide the firs evidence of a seasonal epidemiological profil that is similar to that of RSV, distinct from that of other respiratory viruses, and independent of sample referral numbers.

Age associations of HBoV infections were more consistent between studies, with the studies from the United Kingdom (the present study), Sweden [3], and Australia [5] documenting infections almost entirely confine to children <2 years of age. However, these finding disagree with those of the Canadian study, in which there was much less difference in the prevalence of HBoV according to age; 7 (1%) of 768 samples obtained from study subjects >15 years of age were positive, compared with 11 (2.5%) of 441 samples obtained from study subjects <15 years of age [7]. In the present study, infection among adults was predominantly found in association with immunosuppression, consistent with long-term persistence and/or reactivation described for parvovirus B19 in these patient populations (reviewed in [13]).

On the basis of data available from the archive, our study clearly indicates an association between HBoV infection and LRTIs, on the basis of its frequent detection in samples obtained from individuals presenting at the accident and emergency department with acute respiratory illness (figu e 2). The preferential distribution of HBoV infections among study subjects with LRTIs was consistent in the Swedish, French, Japanese, and Australian studies [3, 5–7], which predominantly document disease presentations of bronchiolitis, pneumonia, bronchitis, and exacerbation of asthma, as well as frequent pyrexia in patients in whom a range of other coinfections with virus had been excluded. Again, these data are at variance with the disease pattern noted in the HBoV-infected study subjects in the Canadian study [7]; here, 14 of the 18 study subjects either displayed symptoms consistent with URTIs or presented with influenza-lik disease.

HBoV infections differ in at least one aspect from infections with other viruses: their frequency of coinfection. Nearly one-half (43%) of the HBoV-positive samples also contained other respiratory viruses, and this number may indeed have been higher if a more comprehensive respiratory virus screening test that included HMPV, human coronaviruses, enteroviruses, and rhinoviruses had been used. However, this frequency of coinfection is broadly similar to the frequencies of coinfection noted in other studies (10 coinfections [8 with RSV] in 18 samples in the Australian study [5], 3 coinfections [all with RSV] in 9 samples in the French study [8], and 3 coinfections in 17 samples in the Stockholm study [3]). The variation in frequencies from 18% to 56% may reflec differing viral incidences as well as differences in the range of viruses screened for in routine respiratory samples referred for diagnosis.

The frequency of HBoV coinfections in the present study (43%) was greater than that of coinfections involving other viruses (combined data of 47 [17%] of 271 samples) ($P = .00008$, by Fisher's exact test) (table 3). The additional observation that the frequency of HBoV infection was higher when another virus was present (table 2) suggests the interesting possibility that HBoV infection may be an exacerbating factor, particularly for RSV infections, with which it has frequently been associated in the present study and in other studies. Coinfections leading to more severe disease have been described for HMPV and RSV [14] and for RSV and coronaviruses with other respiratory pathogens [15]. It is possible that HBoV may similarly increase the severity of RSV and other viruses causing LRTIs, which may be particularly well represented in the current study group. Because of the nature of the diagnostic testing performed, the study group contains a large proportion of individuals with respiratory infections severe enough to require hospitalization. Investigation of the epidemiological profil of HBoV infections among other groups with less severe respiratory infections not requiring clinical referral would clearly be of value.

The data warrant future, nonanonymized investigation of HBoV infections in children, firs to provide more data on the course of infections and, second, to more clearly establish the role of HBoV in respiratory disease, independent of other pathogens. Diagnosis of HBoV may turn out to be clinically important, not least because it potentially differentiates a substantial proportion of LRTIs caused by HBoV from those caused by RSV for which specifi antiviral treatments are available. In summary, the present study documents frequent infections with the newly discovered parvovirus HBoV, but not with PARV4, in young children during the winter months in

the United Kingdom, with HBoV having a prevalence, epidemiological profile and association with LRTIs comparable to those of RSV, which is the main respiratory pathogen in young children.

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