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Instructions for Authors

Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have one Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Human Bocavirus in Infants, New Zealand

To the Editor: In 2005, a parvovirus, subsequently named human bocavirus (HBoV), was discovered in respiratory samples taken from infants and children hospitalized at Karolinksa University Hospital, Sweden, with lower respiratory tract infection (*1*). HBoV has since been identified in infants and children with respiratory illness in >17 countries, at frequencies ranging from 1.5% to >18.0%.

In the past decade New Zealand has experienced increasing bronchiolitis hospitalization rates, currently >70 admissions per 1,000 infants. To determine the contribution of HBoV to New Zealand's bronchiolitis disease prevalence, we tested samples collected from infants hospitalized with community-acquired bronchiolitis (*2*) during 3 consecutive winter epidemics (June to October, 2003; July to October, 2004; and June to October, 2005) in Wellington, NZ, for HBoV by PCR. The Central Regional Ethics Committee approved the study. Written, informed consent was obtained from the parent or guardian.

Demographic, clinical, and laboratory data were collected during hospitalization. Ethnicity of those who ascribe to >1 group was determined by using a national census method that prioritizes ethnicity as follows: Māori>Pacific>Other>New Zealand European. Oxygen requirement was determined to be the best measure of bronchiolitis severity (*2*). Infants who needed assisted ventilation or continuous positive airway pressure were classified severe; those who required oxygen supplementation moderate; and infants who were hospitalized but did not require supplemental oxygen mild.

Nucleic acid was extracted from thawed nasopharyngeal aspirates (stored at 80°C) by using a High Pure Viral Nucleic Acid kit (Roche Diagnostics, Auckland, NZ). The HBoV nonstructural protein (NP-1) gene was amplified by using primers 188F (5′-GAGCTCTGTAAGTACTATTAC-3′) and 542R (5′-CTCTGTGTTGACTGAATACAG-3′) (*1*) with Expand High Fidelity DNA Polymerase (Roche Diagnostics, Basel, Switzerland) for 35 cycles. Products (354 bp) were purified and sequenced from primers 188F and 542R on an ABI3730 Genetic Analyzer by using a BigDye Terminator version 3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequences were submitted to GenBank under accession nos. EF686006–13.

Alignments of NP-1 gene sequences from nucleotides (nt) 2410–2602, and NP-1 predicted amino acid sequences from amino acids (aa) 1–97 were constructed by using ClustalW version 1.83 (available from www.ebi.ac.uk/tools/clustalw/index.html) and compared with HBoV prototype sequences from GenBank (DQ00495-6). Nasopharyngeal aspirates were also screened for respiratory syncytial virus (RSV) by reverse transcription–PCR (RT-PCR) and nested PCR (*3*) and for human metapneumovirus (*4*), influenza A (H1, H3), and influenza B by RT-PCR (*5*).

Eight (3.5%) of 230 samples, collected from infants hospitalized with bronchiolitis during the 2003–2005 winter epidemic seasons, were positive for HBoV. In 5 HBoV-positive infants no other pathogens were identified, but RSV was detected in 3 (Table). The 8 HBoV-positive infants had a median age of 9.5 months, and the male:female ratio was 1:1. The median length of hospital stay was 5.5 (range 1–16) days.

As expected, because HBoV NP-1 is highly conserved, sequence variation among New Zealand isolates and the prototype Stockholm ST-1 and ST-2 (*1*) NP-1 sequences was limited. Alignments of the partial NP-1 sequence (nt 2410–2602) of New Zea-

Table. Summary of 8 infants with human bocavirus infection hospitalized with bronchiolitis, New Zealand, 2003–2005*

Infant no.	Date admitted	Sex/ age, mo	Ethnicity	Attended daycare?	Length of hospital stay, d	Illness severity	Apnea	Underlying conditions/ comorbitities	RSV subtype	Highest temp., °C	Enteritic symptoms
1	Jul 2003	M/9	Pacific	No	16	Mod	–	–	A	40.1	Diarrhea
2	Aug 2003	F/4	Pacific	No	6	Sev	–	–	B	38.4	Diarrhea
3	Sep 2003	F/11	NZ European	No	1	Mod	–	–	–	38.1	–
4	Sep 2003	F/10	Pacific	No	4	Sev	–	33 weeks' gestation	–	38.3	Diarrhea
5	Aug 2004	M/8	Pacific	No	2	Mod	–	*Haemophilus influenzae* conjunctivitis	–	37.7	–
6	Jul 2005	M/10	Chinese	No	10	Mod	–	34 weeks' gestation, repaired esophageal atresia and tracheomalacia	–	37.7	–
7	Aug 2005	F/9	Pacific	No	9	Sev	+	30 weeks' gestation	A	39.2	–
8	Sep 2005	M/13	NZ European	Yes	5	Mod	–	Hydronephrosis, *Pseudomonas aeruginosa* urinary tract infection	–	37.4	–

*Temp., temperature; Mod, moderate; Sev, severe; –, absent; NZ, New Zealand; +, present.

land isolates with those of ST-1 and ST-2 were identical, except for a G→A change at nt 176 in 2 New Zealand isolates (from infants 5 and 8 years of age), which resulted in a predicted amino acid exchange of S→N at aa 59. In addition, an A→T change at nt 274 in all 8 NZ isolates resulted in a predicted amino acid substitution of T→S at aa 92, a change that has been reported previously in Japanese isolates (*6*).

This study reaffirms previous reports of finding HBoV in a subset of infants with bronchiolitis (*7*). It is also, to our knowledge, the first study of its kind in New Zealand infants, confirming wide distribution of HBoV. In the northern hemisphere, HBoV circulates primarily during the winter months, although it continues circulating until early summer, later than most other seasonal respiratory viruses (*8*). Therefore, this study may underestimate the percentage of New Zealand infants with bronchiolitis whose HBoV test results were positive because sample collection ceased in October (southern hemisphere spring) at the end of the bronchiolitis epidemic. The small number of HBoV-positive infants prevents conclusions concerning ethnicity, coinfection, and bronchiolitis severity.

Although detection of viral nucleic acid by PCR in infants with bronchiolitis does not prove that the virus is the cause of the disease, it raises a hypothesis worthy of investigation. Further studies are required to determine the role of HBoV as a human pathogen. Although coinfection is common, HBoV detection appears to be infrequent in asymptomatic controls (*9*). In our study RSV was detected in 3 (37.5%) HBoV-positive samples. We may have underestimated additional coinfection because we did not test for several respiratory agents, including parainfluenza viruses, rhinoviruses, or the newly discovered coronaviruses.

Finally, HBoV has recently been detected in fecal samples (*10*). Because 3 HBoV-positive infants had diarrhea in addition to bronchiolitis, knowing prevalence of HBoV in fecal specimens from asymptomatic New Zealand children and in those with acute gastroenteritis would be of interest.

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Lyme Disease in Urban Areas, Chicago

To the Editor: Lyme disease is a multisystem illness caused by infection with the tickborne spirochete *Borrelia burgdorferi*. Most infections in the United States occur in the Northeast and upper Midwest, and the midwestern focus now includes Illinois (*1*,*2*). Previously, the greatest risk of contracting Lyme disease in the Midwest was confined to the northernmost states (Wisconsin and Minnesota) and did not encroach into heavily populated areas around the city of Chicago. However, we showed recently that *B. burgdorferi*–infected *Ixodes scapularis* ticks were recovered from sites in Cook and DuPage counties (*3*), but the percentages of infected ticks were low (≤5%). Since that time, however, reports of Lyme disease in Cook County have been reviewed and individual *I. scapularis* tick submissions from Lake County, north of Chicago, have been received. We therefore surveyed new areas north of Chicago (closest was <1 mile from the city limits; farthest was ≈25 miles from the city limits) and examined additional ticks for infection with *B. burgdorferi*.

From December 2006 to May 2007, we collected 172 adult *I. scapularis* ticks from sites to the north and northwest of Chicago (Figure). Adult ticks were collected because nymphal ticks are more difficult to obtain, and the infection rate in adult ticks is similar (*1*). The tick midguts were removed aseptically, inoculated into tubes containing 1 mL of modified Barbour-Stoenner-Kelly medium (*4*), incubated at 35ºC, and examined for spirochetes for up to 3 weeks. Spirochetes were recovered from 21 (32%) of 65 ticks and 40 (37%) of 107 ticks collected from sites in Cook and Lake counties, respectively. In addition, PCR using primers specific for outer surface protein A (*5*) confirmed that the spirochetes were *B. burgdorferi*.

The findings demonstrate that the midwestern endemic focus of

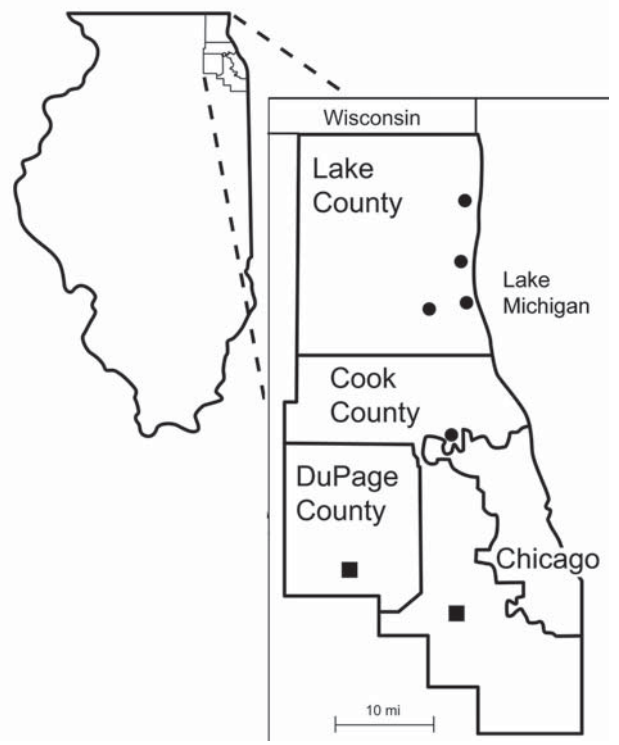

Figure. Sites surrounding Chicago from which *Borrelia burgdorferi*–infected *Ixodes scapularis* ticks were recovered in 2005–2006 (■) and 2006–2007 (●).