M. setense is an emerging organism of the M. fortuitum group that must be added to the growing list of rapidly growing mycobacteria isolated from humans. The initial gram-positive rod appearance of M. setense may delay its accurate identification. Determination of antimicrobial drug susceptibility needs to be conducted by the reference broth dilution method. Further reports are warranted to characterize the role of M. setense in infection.

This report was supported by Unité des Rickettsies CNRS IRD UMR 6236.

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Human Bocavirus in Tonsillar Lymphocytes

To the Editor: We read with great interest the recent report by Longtin and colleagues (1) describing human bocavirus (HBoV) infection among Canadian children with acute respiratory tract illnesses (ARI). The authors identified HBoV by PCR in nasopharyngeal aspirates from 13.8% of young children hospitalized with ARI, an infection rate well within the range reported by other studies on children (2). However, these authors also detected an unexpectedly high rate of HBoV for their control group (43%), >3 times the rate for ARI case-patients.

In contrast, several similar studies did not detect HBoV in asymptomatic children. Kesebir et al. detected HBoV in 23 (5.2%) of 425 young children with ARI but in none of 96 children during routine well-child visits (3). Maggi et al. detected HBoV in 9 (4.5%) of 200 infants with ARI but in none of 30 healthy infants or 21 preadolescent healthy children without signs of ARI or history of asthma or wheezing (4). Finally, Allander et al. detected HBoV in 5 (19%) of 259 young children with acute expiratory wheezing but in none of 64 children who had not had respiratory symptoms during the preceding 4 weeks (5). In a recent study, we detected HBoV by PCR in 44 (12%) of 369 Thai children ≤4 years of age hospitalized with pneumonia but in only 2 (2%) of 85 asymptomatic age and temporally matched controls (6).

The inexplicably high rate of HBoV infection for patient controls reported by Longtin et al. (1) may reflect a unique feature of the children selected. The 100 controls were children without concomitant respiratory symptoms or fever at admission who were hospitalized during the study period for elective surgery, primarily of the ear, nose, and throat (71%). Most surgeries consisted of myringotomies, adenotomies, and tonsillectomies. The authors reported that these surgeries were more frequently performed on children found to be PCR positive for HBoV than on children negative for HBoV (84% vs. 61%). One possible explanation is that HBoV infection may directly induce inflammation of tonsillar tissues or facilitate bacterial superinfection prompting surgical intervention. Another possibility is that inflammation of mucosal lymphoid tissues enhances HBoV replication by recruitment of immune cells permissive for HBoV infection or by latent virus reactivation. Persistent infections and dependence on rapidly dividing cells are common features of the related parvoviruses, for example, human erythrovirus B19 (7). The presence of
low-level persistent HBoV infections may also help explain the exceptionally high rates of respiratory viral co-infections found with HBoV, as high as 90% in 1 study (average 42%) (2,6). Longtin et al. (7) found that 71% of the HBoV-positive patients were also co-infected with another respiratory virus. As we previously hypothesized (6), co-virus-induced cellular damage resulting in high levels of cellular division and differentiation may stimulate HBoV reactivation and replication, as has been shown for polyomavirus following induced kidney damage in newborn mice (8).

To assess whether HBoV is present in tonsillar lymphocytes and therefore possibly explain the high rate of PCR positives obtained by Longtin et al. (7) in their patient controls, we tested DNA extracts of lymphocytes separated by Coll-Paque from nasopharyngeal tonsils or adenoids (AL) and palatine/lingual tonsils (TL) from 164 patients (mostly children) undergoing routine adenoidectomies and tonsillectomies for HBoV DNA. Of these children, 21 had AL only, 57 had TL only, 18 had both AL and TL collected separately, and 68 had AL and TL combined in 1 container. Hyper tropic tonsil and adenoid tissues were removed because of clinical complications, generally obstructive sleep apnea, otitis media, or chronic tonsillitis. Data were not available on whether the children had concurrent respiratory tract illness, although surgeries would likely be postponed if symptoms were apparent. The median age of 162 children for whom age data were available was 5 years (range 1–19.7 years). HBoV real-time PCRs were performed as previously described, targeting 2 unique regions of the HBoV genome (9). All extractions were performed in a separate laboratory from PCR activities, and negative-assay controls were included in all PCR runs to monitor for DNA contamination.

HBoV DNA was detected by both PCRs in lymphocytes from 53 (32.3%) children (median age 3.7 years, range 1–7.6 years). A single assay target (nonstructural protein gene NS1 or NP-1) was positive in specimens from 6 additional children (3.7%), but these specimens were classified as HBoV negative because they did not meet our strict criteria for a positive test result. Children PCR negative for HBoV were significantly older (median age 5.5 years, range 1.8–19.7 years; p < 0.001). HBoV was more often detected in AL (56%) than TL specimens (16%; p < 0.001); the age distributions for children from each group were similar. Among 12 HBoV-positive children from whom both AL and TL were available and collected in separate containers, 6 were positive for HBoV in both specimens, 4 were positive for AL alone, and 2 were positive for AL but only for a single PCR assay target. Moreover, HBoV was present at a substantially higher load in AL (median 3.1 × 10^5 copies/10^7 cells; range 2.8 × 10^4 to 1.2 × 10^6 copies/10^7 cells) than TL (median 1.6 × 10^5 copies/10^7 cells; range 0.1 × 10^6 to 5.3 × 10^5 copies/10^7 cells) as measured by quantitative PCR (9). We have no clear explanation for the relative predominance of HBoV DNA in AL. The adenoids are located at the back of the nasopharynx in close proximity to inhaled pathogens and are covered with ciliated pseudostratified epithelium (respiratory epithelium) that may better support primary virus replication than the palatine and lingual tonsils, which are located in the lower pharynx and covered with nonkeratinized, stratified, squamous epithelium.

Our findings suggest that HBoV may establish latent or persistent infections of mucosal lymphocytes or contribute to tonsillar hyperplasia in young children. Further studies with appropriately matched controls will be necessary to fully understand the nature of HBoV infection and its role in human disease.

Acknowledgment

We thank Louis Villarreal for insightful discussions on polyomavirus replication.

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DOI: 10.3201/eid1408.080300

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Assessment of Reporting Bias for Clostridium difficile Hospitalizations, United States

To the Editor: Burckhardt et al. (1) recently reported on Clostridium difficile–associated disease (CDAD) in Saxony, Germany. In contrast to the observation by Wilcox and Fawley in the United Kingdom (2), the report from Germany argued against a reporting bias for gastroenteritides as a cause of the observed increase in the incidence of CDAD diagnoses from 2002 through 2006. To explore this issue further, I examined the potential influence of such reporting bias on the observed increase in the incidence of hospitalizations of patients with CDAD in the United States from 2000 through 2005.

In the 2000–2005 data from the National Inpatient Sample data from the Agency for Healthcare Research and Quality (3,4), I identified hospitalizations for gastrointestinal infections caused by C. difficile, Salmonella, rotavirus, and other unspecified infectious agents, using the corresponding diagnosis codes from the International Classification of Diseases, 9th Revision, Clinical Modification. I obtained censal and intercensal data on the numbers of the U.S. population from 2000 through 2005 from the U.S. Census Bureau (5). Based on these records, I calculated hospitalization incidence for each of the infectious causes.

Annual incidence of CDAD increased from 49.2 to 101.6 per 100,000 population within the period examined. Within the same time frame, the incidence of CDAD as the principal diagnosis also more than doubled, increasing from 11.6 to 25.8 hospitalizations per 100,000. Although the incidence of hospitalizations for Salmonella infections per 100,000 population remained stable, rotavirus infection showed a slight increase (from 10.8 to 14.5) as did other infectious gastroenteritides (from 38.9 to 49.9/100,000) (Figure). Thus, although a slight increase in the incidence was exhibited, a reporting bias for gastroenteric infections with organisms other than C. difficile does not appear to account fully for the observed doubling of the overall incidence of hospitalizations with CDAD in the United States from 2000 through 2005.

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DOI: 10.3201/eid1408.080446

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Figure. Annual incidence per 100,000 population of all hospitalizations for Clostridium difficile–associated disease (CDAD) compared with hospitalizations for a primary diagnosis of CDAD and with gastroenteritides caused by Salmonella, rotavirus, and other unspecified infectious agents, United States, 2000–2005.