

developed 6 weeks later (6). Blood culture grew *B. longum* and *B. infantis*, which were probiotic strains. Apart from 1 case of sepsis caused by *B. longum* associated with acupuncture in a 19-year-old healthy patient (7), we did not find other reports of invasive *Bifidobacterium* spp. infections.

Because neutropenic episodes, even with bowel involvement, are common during treatment for cancer (8), no reason to promote therapeutic use of probiotics has been proven. Probiotics can cause substantial bacterial overgrowth when stimulating factors are present. In our opinion, avoiding fecal impaction is crucial for preventing colonic bacterial overgrowth and minimizes the chance that bacteria will translocate and cause invasive infection. Nutritional recommendations for a neutropenic diet for children are still debated. The problem is not probiotic therapy but rather fermented food products to which small amounts of probiotics are added. After we reviewed the literature, we did not find enough data to safely recommend the use of these products in children receiving chemotherapy (9). Nevertheless, probiotic therapy is recommended for many immunocompromised patients, such as preterm infants and persons with chronic inflammatory bowel disease (10). We believe that this case of *B. breve* sepsis in an oncology patient underscores the invasive potential of probiotics.

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## Filovirus RNA in Fruit Bats, China

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**To the Editor:** Filovirus-associated diseases, particularly those caused by Ebola and Marburg viruses, represent major threats to human health worldwide because they have extremely high death rates and antiviral therapies or vaccines against them are not available (1). Members of the family *Filoviridae* are classified into 3 genera: *Marburgvirus*, *Ebolavirus*, and the recently approved *Cuevavirus* (2,3). Marburg virus (MARV) and Ebola virus (EBOV) were initially isolated in Africa, but other filoviruses have been identified on other continents. The initial *Cuevavirus*, Lloviu virus (LLOV), was identified in Europe (Spain) (3), and Ebola-Reston virus has been found in pigs in Asia (the Philippines) (4).

Bats are natural reservoirs for filoviruses (5). Viral isolation and serologic studies indicate that filovirus infections have occurred in various bat species in central Africa countries (6), the Philippines (7), China (8), and Bangladesh (9). However, identification of these viruses in bats has been difficult; although isolates of MARV have been obtained (6) and the genome of LLOV has been fully sequenced (3), very short sequences of EBOV have been obtained from bats, and only in Africa (5). Reports of molecular detection or isolation of filoviruses in bats in Asia are lacking. We conducted a study to investigate the presence of filoviruses in bats in China.

In June 2013, twenty-nine apparently healthy *Rousettus leschenaultii* fruit bats were captured in Yunnan Province, China. All bats were humanely killed, and their intestines, lungs, livers, and brains were collected and subjected to viral metagenomic analysis by a previously described method (10). As a result, we obtained and reassembled *de novo*

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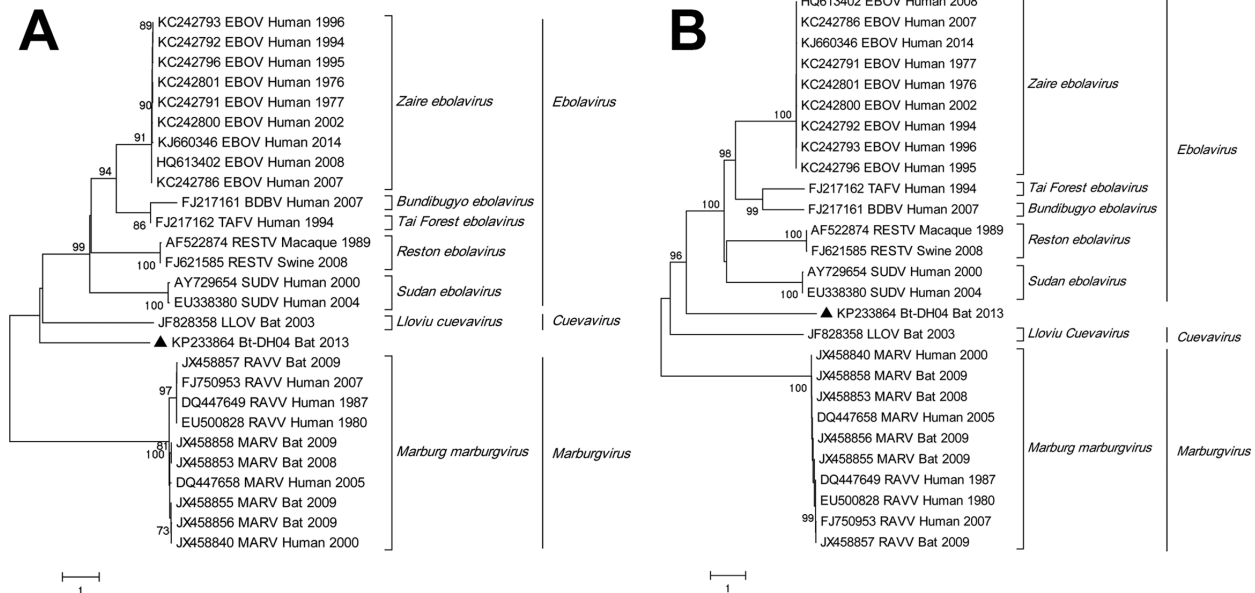
10 million reads into 590,010 contigs. Of these contigs, 3 (129–354 nt) were genetically close to filovirus, corresponding to the nucleoprotein gene of LLOV (74% nt identity), the viral protein 35 gene of Sudan Ebola virus (69% nt identity), and the L gene of Tai Forest Ebola virus (72% nt identity) (online Technical Appendix Table 1, <http://www-wnc.cdc.gov/EID/article/21/9/15-0260-Techapp1.pdf>).

For further screening, we used the longest contig as a template for design of specific seminested primers. Nested degenerate primer pairs were also designed and focused on the most conserved region of the L gene of all currently known filoviruses (online Technical Appendix Table 2). After screening, 2 reverse transcription PCRs of tissues from 1 bat (Bt-DH04) showed positive amplification in specimens from its lung but not from intestine, liver, or brain tissue. Moreover, 5 blind passages in Vero-E6 cells failed to isolate the virus from the lung homogenate. In an attempt to obtain its genomic sequence, 24 primer pairs covering the full genome were further designed by alignment of these contig sequences with the full genomes of representative filoviruses within the 3 genera. All amplifications used ddH<sub>2</sub>O as a negative control; positive controls were not available because filoviruses were not available in China. Two fragments of 2,750-nt (F1) and 2,682-nt (F2) were successfully amplified from lung tissue of Bt-DH04; attempts to amplify the remaining regions failed. Alignment with sequences of 26 representative filoviruses of 7 species from 3 genera revealed that F1 covered the 3' end of the nucleoprotein gene and almost the

entire viral protein 35 gene, and that F2 covered the middle region of the L gene, corresponding to nt 1,313–4,085 and nt 12,613–15,302 of the full genome of EBOV (GenBank accession no. HQ613402). The 2 fragment sequences were submitted to Genbank (accession no. KP233864), and the strain has been tentatively named Bt-DH04.

Phylogenetic analysis showed that the Bt-DH04 strain is placed, together with LLOV, at basal position and intermediate between EBOV and MARV (Figure). It is divergent from all known filoviruses, with F1 sharing the highest nucleotide identities (46%–49%) to members of the genus *Ebolavirus*, followed by 44% to LLOV and <40% to MARV (Figure, panel A). The L gene is the most conserved region of filoviruses, and F2 of Bt-DH04 strain shared relatively closer 66%–68% nt identities with members of the genus *Ebolavirus*, followed by 64% with LLOV and ≈60% with MARV (Figure, panel B). This sequence diversity is likely the main factor for unsuccessful amplification of the full genome of Bt-DH04.

Increasing PCR evidence has identified the existence of filoviruses in bats in Africa and Europe (3,5); however, although serologic studies have shown that filovirus antibodies are prevalent in bats in a few countries in Asia (e.g., the Philippines, Bangladesh and China [7–9]), filovirus or filovirus RNA have not been reported in bats in Asia. Our results show that the Bt-DH04 strain is likely a novel bat-borne filovirus in Asia and provide evidence that bats in Asia harbor more divergent filoviruses than previously thought.



**Figure.** Phylogenetic analysis of 2 fragments of filovirus Bt-DH04 and other filoviruses. Full genomes of representatives from the family *Filoviridae* were trimmed and aligned with F1 (partial nucleoprotein/viral protein 35 gene, panel A) and F2 (middle L gene, panel B) of filovirus strain Bt-DH04 by using ClustalW version 2.0 (<http://www.clustal.org>), then phylogenetically analyzed by using MEGA6 (<http://www.megasoftware.net>) by the maximum-likelihood method, resulting in a bootstrap testing value of 1,000. Sequences are listed by their GenBank accession numbers, followed by the virus name, host, and collection time. Triangles identify the novel filovirus strain Bt-DH04 (China). Scale bars indicate nucleotide substitutions per site.

Fruit bats in the genus *Rousettus* are widely distributed throughout Southeast Asia, South China, and the entire Indian subcontinent and have had positive serologic results for Ebola viruses in these regions (7–9), indicating that these bats play a role in the circulation of filoviruses in Asia. The possibility of new emerging filovirus-associated diseases in the continent emphasizes the need for further investigation of these animals.

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## Increase in Lymphadenitis Cases after Shift in BCG Vaccine Strain

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**To the Editor:** Bacillus Calmette-Guérin (BCG) vaccine is one of the most commonly used vaccines for tuberculosis (TB) worldwide (1). The original BCG strain was developed in 1921. Numerous strains have since been developed, and 5 strains, including Danish SSI 1331 (Statens Serum Institute, Copenhagen, Denmark), account for >90% of BCG vaccine used. Each strain has unique characteristics and a different reactogenicity profile (2). The most common severe adverse events related to BCG vaccination are nonsuppurative and suppurative lymphadenitis.

In the country of Georgia, BCG vaccine is administered routinely to infants (estimated coverage 96%); the National Center for Disease Control and Public Health receives its vaccine supply from the United Nations Children’s Fund and is responsible for countrywide distribution. Before 2012, Russian BCG-I (Bulbio, Sofia, Bulgaria) and Danish SSI 1331 strains were used (~50% each). Shortly after a change to exclusive use of the Danish 1331 strain during 2012–2013, an increasing number of BCG-related lymphadenitis cases were reported to the National Center for Tuberculosis and Lung Diseases (NCTLD). We aimed to quantify the increase in cases of BCG lymphadenitis and to evaluate clinical management of the cases. The Institutional Review Boards of Emory University (Atlanta, GA, USA) and the National Center for Disease Control and Public Health approved the study.

Medical chart abstraction was conducted for all infants with BCG lymphadenitis either reported to the NCTLD or found by inquiry of pediatricians at the largest children’s hospital in the country during January 2012–July 2013. We used national surveillance data to obtain the number of live-born infants.

BCG vaccine is given intradermally over the deltoid muscle on the left arm to infants within 5 days after birth at the maternity hospital. BCG lymphadenitis was clinically