

panel B). We performed *C. burnetii*-specific qPCR on the ticks; 14 (88%) were positive.

We genotyped *C. burnetii*-positive DNA from the feces and from 6 of the 16 ticks by using multispacer sequence typing as described (5). All samples were identified as MST17, the unique genotype circulating in Cayenne (5).

After obtaining the laboratory results, we confirmed that a local group in charge of the collection and treatment of injured animals usually released rehabilitated 3-toed sloths into Tiger Camp. Residents of Tiger Camp regularly observed and came into contact with the sloths, and ticks were frequently observed on the fur of the animals. Furthermore, 3 Q fever patients from Cayenne reported contact with sloths.

Feces from the sloth in this study were highly infectious for *C. burnetii*. Because sloths live in tall trees and can shed this bacterium in their feces, human contamination might occur through inhalation of infectious aerosols from feces. The high prevalence of *C. burnetii* infection in ticks also suggests possible transmission through tick bites or from aerosols of tick feces that have been deposited on the skin of animal hosts; such feces can be extremely rich in bacteria and highly infectious (10).

In this 2013 outbreak of Q fever, epidemiologic studies led to the identification of 3-toed sloths as a putative source of *C. burnetii* infection. Further investigations are needed to confirm the role of sloths as a reservoir for *C. burnetii* in French Guiana and to implement efficient measures to prevent transmission to humans.

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## Marburgvirus Resurgence in Kitaka Mine Bat Population after Extermination Attempts, Uganda

**To the Editor:** Marburg virus (MARV) and Ravn virus (RAVV), collectively called marburgviruses, cause Marburg hemorrhagic fever (MHF) in humans. In July 2007, 4 cases of MHF (1 fatal) occurred in miners at Kitaka Mine in southern Uganda. Later, MHF occurred in 2 tourists who visited Python Cave, ~50 km from Kitaka Mine. One of the tourists was from the United States (December

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2007) and 1 was from the Netherlands (July 2008); 1 case was fatal (1,2,3). The cave and the mine each contained 40,000–100,000 *Rousettus aegyptiacus* bats (Egyptian fruit bats).

Longitudinal investigations of the outbreaks at both locations were initiated by the Viral Special Pathogens Branch of the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA, and Entebbe, Uganda) in collaboration with the Uganda Wildlife Authority (UWA) and the Uganda Virus Research Institute (UVRI). During these studies, genetically diverse MARVs and RAVVs were isolated directly from bat tissues, and infection levels of the 2 viruses were found to increase in juvenile bats on a predictable bi-annual basis (4,5). However, investigations at Kitaka Mine were stopped when the miners exterminated the bat colony by restricting egress from the cave with papyrus reed barriers and then entangling the bats in fishing nets draped over the exits. The trapping continued for weeks, and the entrances were then sealed with sticks and plastic. These depopulation efforts were documented by researchers from UVRI, the CDC, the National Institute of Communicable Diseases (Sandringham, South Africa), and UWA during site visits to Kitaka Mine (online Technical Appendix Figure, <http://wwwnc.cdc.gov/EID/article/20/8/14-0696-Techapp1.pdf>). In August 2008, thousands of dead bats were found piled in the forest, and by November 2008, there was no evidence of bats living in the mine; whether 100% extermination was achieved is unknown. CDC, UVRI, and UWA recommended against extermination, believing that any results would be temporary and that such efforts could exacerbate the problem if bat exclusion methods were not complete and permanent (6,7).

In October 2012, the most recent known marburgvirus outbreak was detected in Ibanda, a town in southwest Uganda. Ibanda is ≈20 km from the

Kitaka Mine and is the urban center that serves smaller communities in the Kitaka area. This MHF outbreak was the largest in Ugandan history: 15 laboratory-confirmed cases occurred (8). In November 2012, an ecologic investigation of the greater Ibanda/Kitaka area was initiated. The investigation included interviews with local authorities to locate all known *R. aegyptiacus* colonies in the area. Although minor colonies of small insectivorous bats were found, the only identifiable colony of *R. aegyptiacus* bats was found inside the re-opened Kitaka Mine, albeit at much reduced size, perhaps 1%–5% of that found before depopulation efforts.

To determine whether the *R. aegyptiacus* bats that had repopulated Kitaka Mine were actively infected with marburgviruses, we tested 400 bats by using previously described methods (4,5). Viral RNA was extracted from ≈100 mg of liver and spleen tissue by using the MagMAX Total Nucleic Acid Isolation Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommended protocol. The Fisher exact test was conducted by using IBM SPSS Statistics, version 19.0 (IBM Corp., Armonk, NY, USA).

Of the 400 *R. aegyptiacus* bats collected, 53 (13.3%) were positive for marburgvirus RNA by quantitative reverse transcription PCR (32/233 [13.7%] adults and 21/167 [12.6%] juveniles; online Technical Appendix Table); marburgvirus was isolated from tissue samples from 9 of the 400 bats. The overall level of active infection was significantly higher than that found in Kitaka Mine during 2007–2008 (5.1%) (5) (Fisher exact test,  $p < 0.001$ ) and in other studies in Uganda (Python Cave [2.5%]) and Gabon (4.8%) (4,9). The reason for the increase is not clear, but it may be related to the effects of the extermination and subsequent repopulation. Increases in disease prevalence in wildlife populations after culling

are not unprecedented (6,7). We speculate that after the depopulation attempt, a pool of susceptible bats became established over time and was subjected to multiple marburgvirus introductions, as evidenced by the genetic diversity of viruses isolated from the bats (Figure). A pool of susceptible bats would have led to higher levels of active infection within the colony, thereby increasing the potential for virus spillover into the human population. A significant sex and age bias was not detected with respect to active infection during the breeding season (Fisher exact test,  $p > 0.5$  for both), and overall, the presence of virus-specific IgG among the bats was 16.5%, a finding consistent with that in previous studies (4,5).

Phylogenetic analysis of viral RNA genome fragment sequences in this study showed high marburgvirus genetic diversity, including the presence of RAVVs and MARVs. Sequences for isolates from 3 bats were nearly identical to those of the MARV isolates obtained from patients in the 2012 Ibanda outbreak (8), suggesting that bats from Kitaka Mine were a likely source of the virus.

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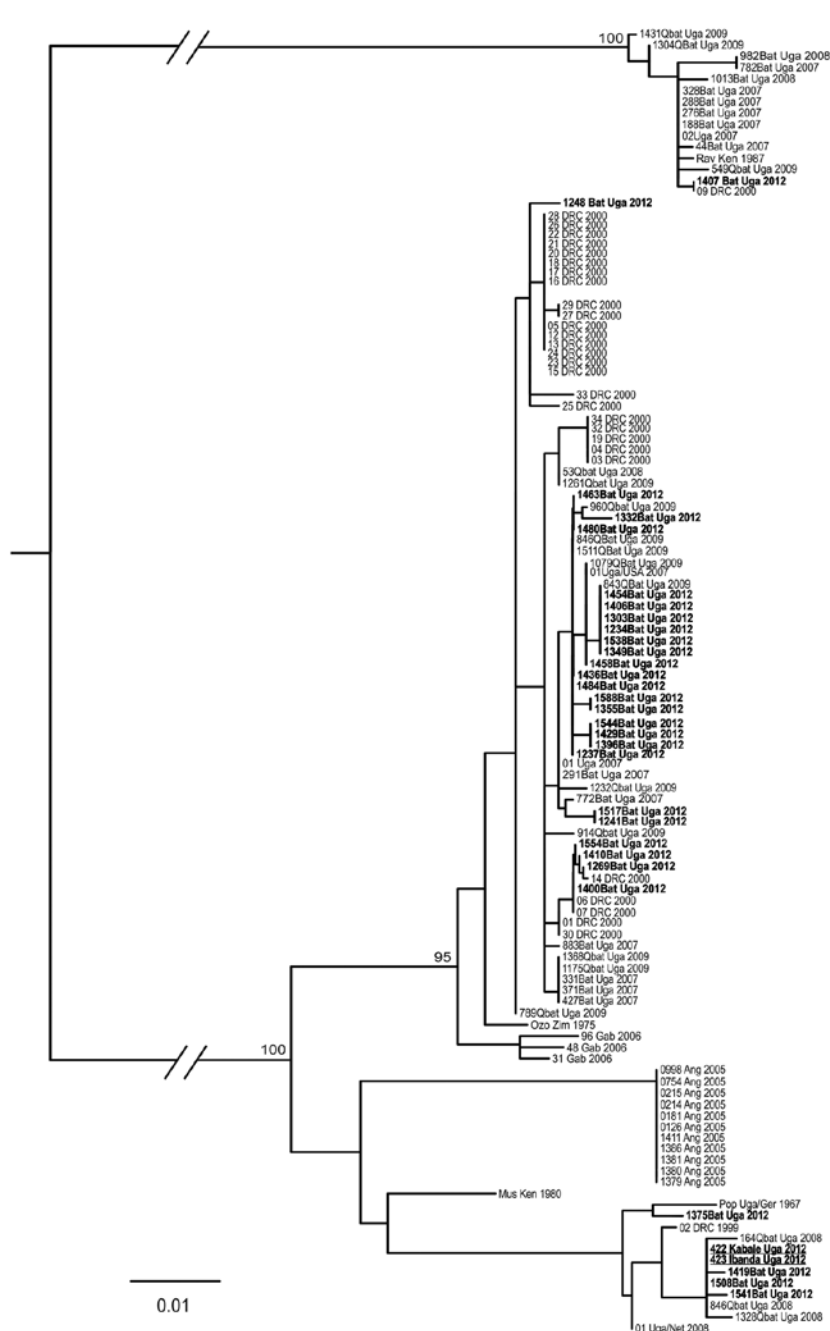


Figure. Phylogeny of concatenated marburgvirus nucleoprotein (NP) and viral protein 35 (VP35) gene fragments as determined by using the maximum-likelihood method. Sequences from the NP (289–372 nt) and VP35 (203–213 nt) genes were amplified and determined from viral RNA and then sequenced as described elsewhere (4). Sequence names in boldface represent those generated from samples collected from bats during the November 2012 outbreak investigation at Kitaka Mine, Uganda. Underlined sequence names represent those generated from samples obtained from marburgvirus-infected persons in Kabale and Ibanda, Uganda, in 2012. Multiple sequence alignments were generated, and a maximum-likelihood analysis was conducted on concatenated NP and VP35 (208–580 nt) sequences by using the PhyML method in conjunction with the GTR+I+G nucleotide substitution model implemented in SeaView version 4.2.12 (10). NP and VP35 gene sequences determined from samples in this study (in boldface) were submitted to GenBank (accession nos. KJ747211–KJ747234 and KJ747235–KJ747253, respectively). Bayesian posterior probabilities above 50 are shown at the nodes. Scale bar indicates nucleotide substitutions per site. Ang, Angola; DRC, Democratic Republic of Congo; Gab, Gabon; Ger, Germany; Ken, Kenya; Net, Netherlands; Rav, Ravn virus; Uga, Uganda; Zim, Zimbabwe.

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## Detection of Measles Virus Genotype B3, India

**To the Editor:** Molecular epidemiologic investigations and virologic surveillance contribute notably to the control and prevention of measles (1). Nearly half of measles-related deaths worldwide occur in India, yet virologic surveillance data are incomplete for many regions of the country (2,3). Previous studies have documented the presence of measles virus genotypes D4, D7, and D8 in India, and genotypes D5, D9, D11, H1, and G3 have been detected in neighboring countries (3,4).

Kerala, India's southernmost state, has high measles vaccination coverage compared with many other states in the country; however, the disease is still endemic in the region. Two districts, Thiruvananthapuram and Malappuram, report the highest numbers of cases (5). Baseline data on circulating measles virus genotypes are needed for measles elimination, but such data are not available for Kerala. In this context, we performed a pilot genetic analysis of the measles virus strains circulating in Thiruvananthapuram, the capital of Kerala. We used throat and nasopharyngeal swab and serum samples from children admitted to Sree Avittom Thirunal Hospital during measles outbreaks occurring March–August 2012.

We used the Vero/human-SLAM cell line (<http://www.phe-culturecollections.org.uk>) for isolation of measles virus from throat and nasopharyngeal swab samples. For serologic confirmation of cases, we used a commercial measles IgM ELISA kit (IBL International GmbH, Hamburg, Germany). Virus genotyping was based on the 450-nt coding sequence for the carboxyl terminus of nucleoprotein (N) of measles virus, as recommended by the World Health Organization (3,6). We extracted

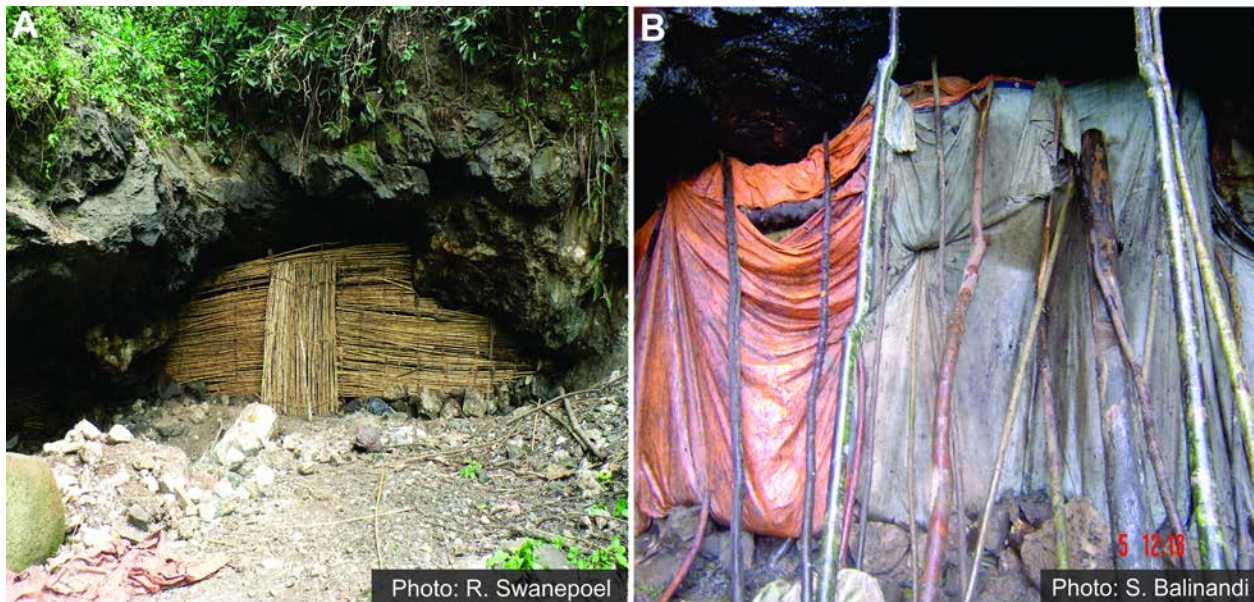
RNA from the samples using TRIzol reagent (GIBCO-BRL, Grand Island, NY, USA). We performed reverse transcription PCR using a SuperScript One-Step RT-PCR kit with a Platinum *Taq* system (Invitrogen, Carlsbad, CA, USA) and previously described primers (3,6). Amplicons were subjected to bidirectional sequencing using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). We edited and aligned nucleotide sequences using Bio Edit 7.1.11 software (7). Phylogenetic analysis was performed by using the maximum-likelihood method implemented in the MEGA5 program (8) to compare the determined N gene sequences with the World Health Organization reference sequences of the 24 known measles genotypes.

PCR products could be amplified from 16 of the 24 samples analyzed. Ten samples provided high quality sequence reads for the N gene coding region, which were used for further analysis. Clinical and demographic data for these 10 cases, virus isolation status, and GenBank accession numbers of the sequences are summarized in the Table.

Phylogenetic analysis revealed 1 of the 10 measles virus strains to be of genotype D8 (online Technical Appendix Figure 1, <http://wwwnc.cdc.gov/EID/article/20/10/13-0742-Techapp1.pdf>), a genotype previously found to be circulating in Kerala and in other regions of India (3,6,9,10). The other 9 virus strains were closely related to B3 genotype reference strains, indicating circulation of the B3 genotype in Kerala (online Technical Appendix Figure 1). The nucleotide sequences of 7 of the 9 strains were identical, indicating a single chain of transmission. The remaining 2 samples showed sequence divergence, indicating independent sources of infection. In a phylogenetic analysis comparing the Kerala B3 genotypes and a dataset of

# Marburgviruses in Bats in Mine after Extermination Attempts, Uganda

## Technical Appendix



Technical Appendix Figure. Photographs taken at Kitaka Mine, Uganda, between August 2008 and September 2009 showing local efforts to exterminate and exclude *Rousettus aegyptiacus* bats from the mine. A) Papyrus reed walls constructed over entrances and air shafts in the mine. B) Plastic tarps used to completely seal off all access to the mine. Photographs courtesy of R. Swanepoel and S. Balinandi.

Technical Appendix Table. Demographic characteristics of bats captured during a Marburg hemorrhagic fever outbreak investigation at Kitaka Mine, Uganda, November 2012\*

Characteristic	Total no.	No. (%) marburgvirus-positive bats	
		Reverse transcription PCR	IgG
Sex			
Female	203	25 (12.3)	30 (14.8)
Male	197	28 (14.2)	36 (18.3)
Age group			
Adult	233	32 (13.7)	46 (19.7)
Juvenile	167	21 (12.6)	20 (12.0)

\*All animal work was performed in accordance with protocols approved by the Animal Care and Use Committee, Centers for Disease Control and Prevention.