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Close Relative of Human Middle East Respiratory Coronavirus in Bat, South Africa

To the Editor: The severe acute respiratory syndrome (SARS) outbreak of 2002–03 and the subsequent implication of bats as reservoir hosts of the causative agent, a coronavirus (CoV), prompted numerous studies of bats and the viruses they harbor. A novel clade 2c betacoronavirus, termed Middle East respiratory syndrome (MERS)—CoV, was recently identified as the causative agent of a severe respiratory disease that is mainly affecting humans on the Arabian Peninsula (1). Extending on previous work (2), we described European *Pipistrellus* bat-derived CoVs that are closely related to MERS-CoV (3). We now report the identification of a South Africa bat derived CoV that has an even closer phylogenetic relationship with MERS-CoV.

During 2011–2012, fecal pellets were collected from 62 bats representing 13 different species in the KwaZulu-Natal and Western Cape Provinces of South Africa and stored in RNALater solution (Life Technologies, Carlsbad, CA, USA). Details about the bat sample are available in the online Technical Appendix Table (wwwnc.cdc.gov/EID/article/19/10/13-0946-Techapp1.pdf). RNA was extracted by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Screening for CoVs was done by nested reverse transcription PCR using broadly reactive oligonucleotide primers targeting a conserved region in the *RNA-dependent RNA polymerase (RdRp)* gene (online Technical Appendix). PCR results were positive for 5 (8%) of the 62 specimens. PCR amplicons for 4 positive specimens yielded alphacoronavirus sequences related to

recently described bat alphacoronaviruses from South Africa (4). The other positive specimen, termed PML/2011, was from an adult female *Neoromicia cf. zuluensis* bat sampled in 2011; the specimen yielded a novel betacoronavirus (GenBank accession no. KC869678). Online Technical Appendix Figure 1 shows the distribution of this bat species.

To obtain better phylogenetic resolution, we extended the 398-nt *RdRp* fragment generated by the screening PCR to 816 nt, as described (5). PML/2011 differed from MERS-CoV by only 1 aa exchange (0.3%) in the translated 816-nt *RdRp* gene fragment. Thus, PML/2011 was much more related to MERS-CoV than any other known virus. The amino acid sequence of the next closest known relatives of MERS-CoV, from European *Pipistrellus* bats (3), differed from MERS-CoV by 1.8%. The amino acid sequences of viruses from *Nycteris* bats in Ghana (3) and the 2c prototype bat CoVs, HKU4 and HKU5, from China (6) differed by 5.5%–7.7% from MERS-CoV. The smaller 152- to 396-nt *RdRp* fragments of 2c bat CoVs from a *Hypsugo savii* bat in Spain (7), bat guano in Thailand (8), and a *Nyctinomops* bat in Mexico (9) showed no or only partial overlap with the 816-nt fragment generated in this study; thus, a direct comparison could not be done. However, in their respective *RdRp* fragments, these CoVs yielded amino acid sequence distances of 3.5%–8.0% and were thus probably more distant from MERS-CoV than the virus described here.

A Bayesian phylogenetic analysis of the 816-nt *RdRp* sequence confirmed the close relationship between PML/2011 and MERS-CoV (Figure). Their phylogenetic relatedness was as close as that of SARS-CoV and the most closely related bat coronavirus known, Rs672 from a *Rhinolophus sinicus* bat (Figure). Like PML/2011 and MERS-CoV, Rs672 and SARS-CoV showed only 1 aa exchange in

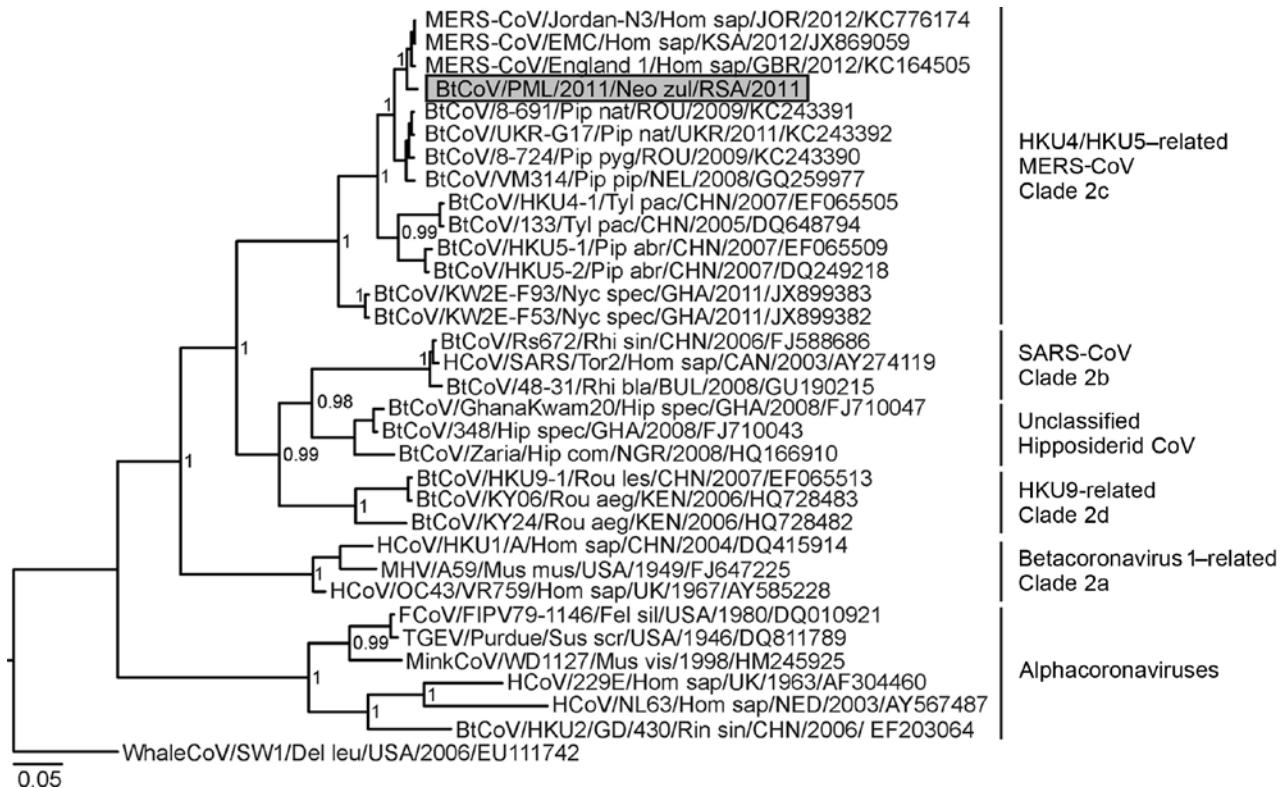


Figure. Partial RNA-dependent RNA polymerase (*RdRp*) gene phylogeny, including the novel betacoronavirus from a *Neoromicia zuluensis* bat in South Africa (GenBank accession no. KC869678 for both partial *RdRp* and *spike* gene sequences). The Bayesian phylogeny was done on a translated 816-nt *RdRp* gene sequence fragment, as described (5). MrBayes V3.1 (<http://mrbayes.sourceforge.net/>) was used with a WAG substitution model assumption over 2,000,000 generations sampled every 100 steps, resulting in 20,000 trees, of which 25% were discarded as burn-in. A whale gammacoronavirus was used as an outgroup. The novel *N. zuluensis* bat virus is highlighted in gray. Values at deep nodes represent statistical support from posterior probabilities. Only values >0.9 are shown. Coronavirus clades are depicted to the right of taxa. Scale bar represents genetic distance. MERS-CoV, Middle East respiratory syndrome coronavirus; SARS, severe acute respiratory syndrome; Bt-CoV, bat coronavirus; HCoV, human coronavirus, MHV, mouse hepatitis virus; FCoV, feline coronavirus; TGEV, transmissible gastroenteritis coronavirus.

the translated 816-nt *RdRp* fragment. To confirm this relatedness, we amplified and sequenced a short 269-nt sequence encompassing the 3'-terminus of the *spike* gene for PML/2011 (oligonucleotide primers available upon request from the authors). A partial *spike* gene-based phylogeny using this sequence yielded the same topology as that using the partial *RdRp* sequence (online Technical Appendix Figure 2). Again, PML/2011 was most closely related to MERS-CoV, showing only a 10.9% aa sequence distance in this gene, which encodes the glycoprotein responsible for CoV attachment and cellular entry. This distance was less than the 13.3% aa sequence distance between

MERS-CoV and the European *Pipistrellus* CoVs (3) and less than the 20.5%–27.3% aa sequence distance between MERS-CoV and HKU5 and between MERS-CoV and HKU4 (6) in the same sequence fragment.

Our results further support the hypothesis that, like human CoV-229E and SARS-CoV, ancestors of MERS-CoV might exist in Old World insectivorous bats belonging to the family Vespertilionidae, to which the genera *Neoromicia* and *Pipistrellus* belong (3). Knowledge of the close relatedness of PML/2011 and MERS-CoV, which contrasts with the more distant relatedness of CoVs in bats from the Americas and Asia, enables speculations of an African origin for bat

reservoir hosts of MERS-CoV ancestors. This hypothesis is limited by a global sampling bias, the small sample size, and the single clade 2c betacoronavirus detection in this study. Still, a putative transfer of MERS-CoV ancestors from Africa to the Arabian Peninsula would parallel the transfer of other viruses (e.g., the exportation of Rift Valley fever virus from East Africa, which led to a severe outbreak in Saudi Arabia in 2000) (10).

Studies of Vespertilionidae bats and potential intermediate hosts (e.g., carnivores and ungulates, such as camels) are urgently needed to elucidate the emergence of MERS-CoV. Such studies should focus on the Arabian Peninsula and Africa.

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Multidrug-Resistant *Escherichia coli* Bacteremia

To the Editor: Extraintestinal pathogenic *Escherichia coli* (ExPEC) bacteria have the ability to cause diverse and serious diseases, such as urinary tract infections (UTIs) and bacteremia (1–3); incidence of bacteremia is increasing globally (4). The emergence of multidrug resistance in *E. coli* is also becoming a global concern, with particular emphasis on *E. coli* sequence type (ST) 131, which is being increasingly reported in UTIs. Drug resistance is mediated by extended-spectrum β -lactamases (ESBLs), mainly of the CTX-M family, particularly CTX-M-15 and 14, and less frequently of the SHV and OXA families (5,6). Few studies are available regarding the characterization of *E. coli* strains causing bacteremia.

We characterized 140 *E. coli* isolates from bacteremia patients treated at Nottingham University Hospital (Nottingham, UK) over a 5-month period, with the aim of developing an epidemiologic profile of the population of ExPEC that causes bacteremia. For context, we compared the isolates with 125 *E. coli* isolates from urine samples collected during the same period. Cases were selected to include isolates from a diverse patient group: patient ages ranged from 1 month to 90 years; patient sex was evenly divided between male and female; infections were community- and hospital-associated; and suspected sources of infection varied. Antimicrobial drug susceptibility tests, PCR detection of ESBL genes multilocus sequence typing using the Achtman scheme (<http://mlst.ucc.ie/mlst/dbs/Ecoli>), and virulence-associated gene (VAG) carriage screening by PCR were performed on isolates as described (7).

Significantly more bacteremia *E. coli* isolates than urine *E. coli* isolates were resistant to ciprofloxacin (25.7%

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Technical Appendix

Sampling

Bats were sampled between November 2010 and mid-2012 at caves in Table Mountain National Park and in Millwood forest, Garden Route National Park (permit no. 11LB_SEI01), at Phinda Private Game Reserve in KwaZulu Natal (permit no. OP2021/2011) and in Greyton, Western Cape (permit no. AAA007-00373-0035).

Bats were captured during emergence from roof roosts or cave entrances using a harp trap, hand-net or mist nets. Animal handling and sample collection was done in accordance with accepted international guidelines for mammals as set out in Sikes et al. (1).

Individuals were then placed in individual cloth bags for up to 3 hours to collect faecal pellets. Faecal pellets were removed from each bag using sterile forceps and suspended in 1.0ml of RNAlater in a 2ml cryovial before transport to the laboratory in Tygerberg and virological testing under institutional clearance (ref. SU-ACUM12-00001).

In the Western Cape, all bats were released unharmed. In Phinda, they were euthanized with halothane and retained as accessioned voucher specimens in the mammal collection of the Durban Natural Science Museum. Associated specimen derivatives (e.g. faecal pellets) were obtained through a museum loan (loan no. M201011_1).

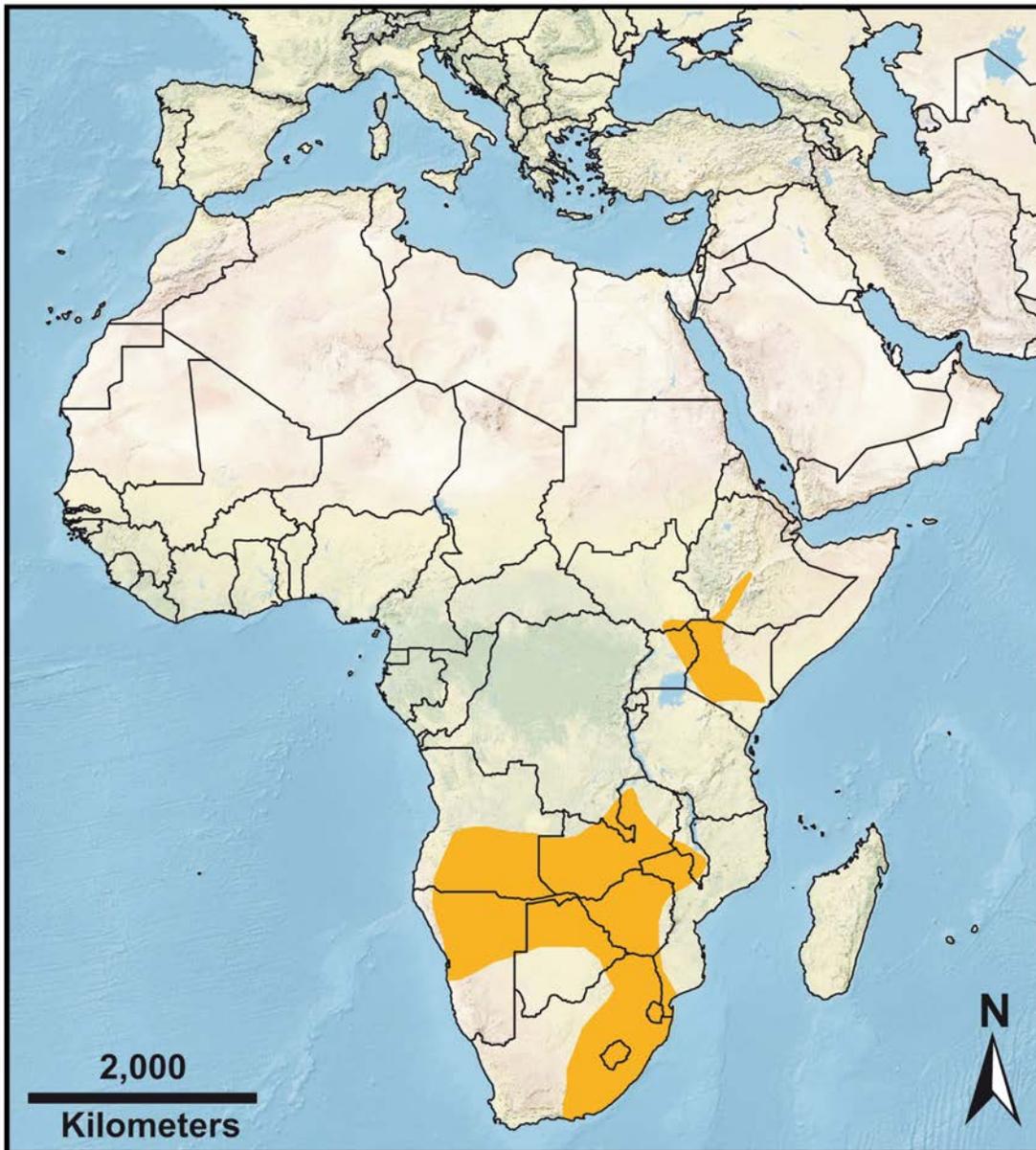
Species were determined based on morphological features following current systematics (2). Forearm length and body mass were measured and the age (adult or juvenile) was determined by assessing the presence of cartilaginous epiphyseal plates in the finger bones (3). The sex and reproductive status of each individual was also recorded.

References

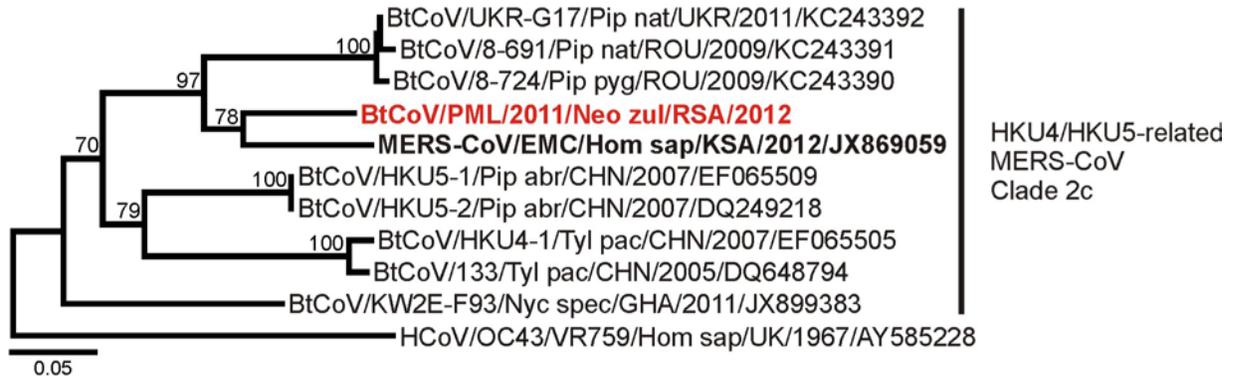
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Technical Appendix Table. South African bat species screened

Family	Species	Number of specimens
Insectivorous bats		
Molossidae	<i>Chaerephon pumilus</i>	5
	<i>Mops condylurus</i>	1
	<i>Tadarida aegyptiaca</i>	3
Hipposideridae	<i>Hipposideros caffer</i>	4
Miniopteridae	<i>Miniopterus natalensis</i>	13
Nycteridae	<i>Nycteris thebaica</i>	1
Rhinolophidae	<i>Rhinolophus clivosus</i>	2
	<i>Rhinolophus darlingi</i>	2
Vespertilionidae	<i>Neoromicia capensis</i>	10
	<i>Neoromicia nana</i>	6
	<i>Neoromicia</i> cf. <i>zuluensis</i>	1
	<i>Scotophilus viridis</i>	3
Fruit bats		
Pteropodidae	<i>Rousettus aegyptiacus</i>	12
Total		62



Technical Appendix Figure 1. Distribution map of *Neoromicia zuluensis*, Africa. Adapted from the IUCN Red List of Threatened Species. Version 2012.2 (www.iucnredlist.org) using ArcGIS software by Esri.



Technical Appendix Figure 2. Partial *Spike* gene phylogeny of the 2c betacoronavirus clade including the novel bat virus. The neighbor-joining phylogeny of the partial *Spike* gene of clade 2c coronaviruses including the novel South African *Neoromicia* coronavirus was done using a nucleotide percentage distance substitution model and the complete deletion option in MEGA5. The novel bat virus is shown in bold and red, Middle East respiratory syndrome (MERS)–CoV in bold only. Scale bar represents percentage nucleotide distance. The analysis comprised a total of 138 nucleotides corresponding to positions 25,379-25,517 in MERS-CoV. HCoV-OC43 was used as an outgroup. Oligonucleotide sequences of primers used for amplification of partial *Spike* gene sequences are available upon request. Of the larger 269 nucleotide fragment which was amplified, only the 3'-terminus of the *Spike* gene was included into the phylogenetic analysis to avoid a bias from potential recombination known to occur frequently at the borders of the *Spike* gene. Values at deep nodes represent statistical support of grouping by percentage of 1,000 bootstrap replicates.