Middle East Respiratory Syndrome Coronavirus in Bats, Saudi Arabia

Ziad A. Memish, Nischay Mishra, Kevin J. Olival, Shamsudeen F. Fagbo, Vishal Kapoor, Jonathan H. Epstein, Rafat AlHakeem, Mushabab Al Asmari, Ariful Islam, Amit Kapoor, Thomas Briese, Peter Daszak, Abdullah A. Al Rabeeah, and W. Ian Lipkin

The source of human infection with Middle East respiratory syndrome coronavirus remains unknown. Molecular investigation indicated that bats in Saudi Arabia are infected with several alphacoronaviruses and betacoronaviruses. Virus from 1 bat showed 100% nucleotide identity to virus from the human index case-patient. Bats might play a role in human infection.

Since Middle East respiratory syndrome (MERS) was described in September 2012, over 90 cases have been reported worldwide, 70 from Saudi Arabia. The incidence of infection with the causative agent, a betacoronavirus (MERS CoV) (1), has not been determined; however, the mortality rate among those who received clinical care is ≈65% (2). Although instances of human-to-human transmission have been documented between case-patients and others in close contact (including hospital patients sharing rooms, family members, and medical personnel), the sources of infection for most patients remain unknown. Because of sequence similarities between β-CoVs identified in bats and those of MERS CoV isolated from humans, a bat reservoir has been posited (3–5). Although neither detection of MERS CoV in bats nor contact of human MERS patients with bats have been reported, a role for bats in human infection cannot be excluded because contact can be indirect (mediated through another animal vector or fomites).

Author affiliations: Ministry of Health, Riyadh, Saudi Arabia (Z.A. Memish, S.F. Fagbo, R. AlHakeem, A.A. Al Rabeeah); Columbia University, New York, New York, USA (N. Mishra, V. Kapoor, A. Kapoor, T. Briese, W.I. Lipkin); EcoHealth Alliance, New York (K.J. Olival, J.H. Epstein, P. Daszak); Ministry of Health, Bisha, Saudi Arabia (M. Al Asmari); and EcoHealth Alliance, Dhaka, Bangladesh (A. Islam)

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Figure 1. Bat sampling sites and locations of home and workplace of index case-patient with Middle East respiratory syndrome, Bisha, Saudi Arabia.
All samples were stored in liquid nitrogen and conveyed to Riyadh for storage at –80°C before being transported to Columbia University in New York in dry nitrogen. The October 2012 shipment was inadvertently opened at customs in the United States and sat at room temperature for 48 hours before transfer to Columbia University; at arrival, all samples had thawed. The April 2013 samples arrived intact.

Total nucleic acid was extracted from samples by using the NucliSENS easyMAG system (bioMérieux, Durham, NC, USA) and subjected to 8 PCRs with primers and protocols designed to amplify regions within the helicase, RNA-dependent RNA polymerase (RdRp), and nucleocapsid or envelope proteins of CoVs (6–9). Products were sequenced and analyzed for similarity to GenBank database entries by using the BLASTn and BLASTx programs (www.ncbi.nlm.nih.gov/blast/Blast.cgi). Primer sequences are shown in Table 1. The identity of bat species yielding specific viral products was determined by amplifying and sequencing a fragment of the cytochrome B gene (10). All visual classifications of species were confirmed except for that of T. perforatus bats. There is no reference sequence for T. perforatus bats in GenBank. However, because the closest reference sequence was from T. nudiventris bats, at 84% identity we presume that the product represents bona fide T. perforatus bat cytochrome B gene sequence. Representative cytochrome B sequences have been uploaded to GenBank (accession nos. KF498635–KF498641).

Table 1. PCRs and primers used in CoV detection

| PCRs (reference) | Primers (5′→3′) | Nested fragment size, region (primer locations on the reference genome)† | Type of CoV (no.) |
|------------------|-----------------|---------------------------------------------------------------------|------------------|
| Nested pan-CoV-I  | PLQ-F1, CGTGGTACWAAYBTVCCWYTICARBRGG PLQ-R1, GTTTCATAGCTATGCTACCA CGTGGTACWAAYBTVCCWYTICARBRGG PLQ-R2, GGWAVCCWCTACCACTTGCTACCA | ≈400 nt, RdRp (18310–187450) | α-CoV (8), β-CoV (1) |
| (6)              |                 |                                                                     |                  |
| Nested pan-CoV-II | WT-CoV-F1, GTTTCATAGCTATGCTACCA CGTGGTACWAAYBTVCCWYTICARBRGG WT-CoV-R1, GTTTCATAGCTATGCTACCA CGTGGTACWAAYBTVCCWYTICARBRGG WT-CoV-R2, GGWAVCCWCTACCACTTGCTACCA | ≈430 nt, RdRp (15260–15700) | α-CoV (5), β-CoV (2) |
| (7)              |                 |                                                                     |                  |
| Hemi-nested RdRp-sequence assay (9) | EM-SeqNestF1-EMC/2012, complete genome (GenBank accession nos. KF498635–KF498641) EM-SeqNestF2-EMC/2012, complete genome (GenBank accession nos. KF498635–KF498641) | ≈230 nt, RdRp (15048–15290) | α-CoV (2), β-CoV (1) |
|                  |                 |                                                                     |                  |
| Hemi-nested N-sequence assay (9) | EM-SeqNestF1-EMC/2012, complete genome (GenBank accession nos. KF498635–KF498641) EM-SeqNestF2-EMC/2012, complete genome (GenBank accession nos. KF498635–KF498641) | ≈280 nt, N seq (29,549–29,860) | NA |
|                  |                 |                                                                     |                  |
| Nested CII-pan-CoV-I | NM-CoV-2F1, ACWGTTCAGCCGATCCCGGG NM-CoV-2F2, GTTTCATAGCTATGCTACCA CGTGGTACWAAYBTVCCWYTICARBRGG NM-CoV-2R1, GTTTCATAGCTATGCTACCA CGTGGTACWAAYBTVCCWYTICARBRGG NM-CoV-2R2, GGWAVCCWCTACCACTTGCTACCA | ≈355 nt, helicase (17,060–17,410) | β-CoV (2) |
|                  |                 |                                                                     |                  |
| Nested CII-MERS-RdRp | NM-HCoV-F1, GTTTCATAGCTATGCTACCA CGTGGTACWAAYBTVCCWYTICARBRGG NM-HCoV-F2, AGAGCTCGACTCACTTGCGGACNM-HCoV-F2, AGAGCTCGACTCACTTGCGGACNM-HCoV-R1, ACCCTAAAGATGCGGATTTACACNM-HCoV-R2, TGCGGATTATACACCACTTGCTACCA | ≈190 nt, RdRp (15068–15249) | β-CoV (1, MERS CoV) |
|                  |                 |                                                                     |                  |
| Hemi-nested CII-MERS N sequence | NM-SeqNestF1-EMC/2012, complete genome (GenBank accession nos. KF498635–KF498641) NM-SeqNestF2-EMC/2012, complete genome (GenBank accession nos. KF498635–KF498641) | ≈170 nt, N seq (29545–29713) | NA |
|                  |                 |                                                                     |                  |
| upE and ORF1b real-time assays (8) | upF1b-Prb: FAM-CTCTCCATATACTGCGCAGCTCG-TAMRA upF1b-Prb: FAM-CTCTCCATATACTGCGCAGCTCG-TAMRA | Upstream of E gene and ORF 1b |                    |
|                  |                 |                                                                     |                  |
| † Primer locations are based on human β-CoV 2c EMC/2012, complete genome (GenBank accession no. JX869059). |
Table 2. CoVs detected in bats, Saudi Arabia*

| Bat family, genus, species | Location            | No. bats | No. samples tested (no. positive) | Total no. samples, n = 1,003 | Total no. positive samples, n = 227 (closely related CoVs)† |
|----------------------------|---------------------|----------|---------------------------------|-------------------------------|----------------------------------------------------------|
|                            |                     |          | Throat swab | Fecal pellets | Urine | Serum | Roost feces |                                      |                                            |
| October 2012               |                     |          |              |               |       |       |            |                                      |                                            |
| Emballonuridae             | Taphozous perforatus| Bisha ruins | 29 | 29 (0) | 25 (2) | 8 (0) | 22 | 10 (1) | 94 | 1 β-CoV (1 MERS novel CoV) and 2 α-CoVs (1 bovine respiratory CoV, 1 Kenya bat CoV BIKY86) |
| Pteropodidae               | Eidolon helvum      | Bisha town center | 25 | 25 (0) | 25 (5) | 13 (0) | 19 | NA          | 82 | 1 β-CoV (1 Eidolon bat CoV-HKU1) and 4 α-CoVs (4 Kenya bat CoV BIKY86) |
| Roussettidae               | Rh. hardwickii      | Old Naqi and Naqi | 36 | 36 (0) | 35 (0) | 4 (0) | NA | 15 (0) | 90 | NA |
| Rhinopomatidae             | Rh. microphyllum    | Old Naqi | 1 | 1 (0) | 1 (0) | NA | NA | NA | 2 | NA |
| Vespertilionidae           | Eptesicus bottae    | Bisha ruins | 1 | 1 (0) | 1 (0) | 1 (0) | NA | NA | 32 (0) | 35 | NA |
| Pipistrellus kuhlii        | Bisha ruins         | 1 | 1 (0) | 1 (0) | NA | NA | NA | 2 | NA |
| April 2013                 |                     |          |              |               |       |       |            |                                      |                                            |
| Rhinopomatidae             | Rh. hardwickii      | Greater Bisha area | NA | NA | NA | NA | NA | 209 (93) | 209 | 2 β-CoVs (2 canine respiratory CoVs) and 9 α-CoVs (5 canine CoVs, 2 Miniopterus bat CoVs, 84 Chaerephon bat CoV) |
| Vespertilionidae           | P. kuhlii           | Greater Unaizah area | 9 | 9 (0) | NA | NA | NA | 263 (126) | 277 | 126 α-CoVs (69 alphaCoV P.kuh-Spain, 3 canine CoVs, 37 bat CoV P.pyg/Germany, 1 human CoV NL63, 2 Rousettus bat CoV HKU10, 11 porcine epidemic diarrhea virus, 2 Cardioderma bat CoVs, 1 Hipposideros bat CoV HKU10) |
|                            |                     |          |              |               |       |       |            |                                      |                                            |

*CoV, coronavirus; MERS, Middle East respiratory syndrome; NA, not applicable.
†Based on BLASTn (www.ncbi.nlm.nih.gov/blast/Blast.cgi).

CoV sequences were amplified from 220 of 732 roost feces samples and 7 of 91 rectal swab samples or fecal pellets. A product obtained by PCR amplification of nucleic acid from a fecal pellet of a T. perforatus bat captured in October 2012 in Bisha showed 100% nt identity to the human β-CoV 2c EMC/2012 cloned from the index case-patient in Bisha. A phylogenetic analysis of CoVs obtained in this study is shown in Figure 2. CoV sequences have been uploaded in GenBank (accession nos. KF493884–KF493888).

Conclusions

A wide range of CoV species are circulating among bats in Saudi Arabia. Although the prevalence of CoVs was high (~28% of fecal samples), MERS CoV was found in only 1 bat. A 3.5% MERS CoV infection rate (n = 29; 95% CI 0–20%) in T. perforatus bats is low compared with that for severe acute respiratory syndrome–like CoV in rhinolophid bats in China (10%–12.5%) but consistent with CoV prevalence among bats in Mexico (4). Furthermore, the sensitivity for viral nucleic acid detection in samples collected in October 2012 was probably reduced because of failure in cold chain transport. Whereas 219 (32%) of 675 of fecal pellets collected in April revealed a CoV sequence by PCR, only 8 (5%) of 148 of rectal swab samples or fecal pellets collected in October were positive by the same assays. We were unable to recover additional sequences beyond the 190-nt RdRp fragment represented in Figure 2 but are confident in the fidelity of the finding. First, although RdRp is a conserved portion of the CoV genome, there is no precedent for 100% identity of a bat sequence with a human MERS CoV sequence. Second, when this work began we did not have cultured MERS CoV, human MERS samples, or MERS CoV cDNA in the
laboratory at Columbia University where samples were removed directly from the tubes in which they were collected in the field for nucleic acid extraction, PCR, and sequence analysis. Third, the only MERS-positive signal was obtained in PCR analysis of the T. perforatus bat captured in Bisha near the home and workplace of the MERS index case-patient used to generate the human β-CoV 2c EMC/2012 sequence.

Bats are reservoirs of several viruses that can cause human disease, including rabies, Hendra, Nipah, Marburg, severe acute respiratory syndrome CoV, and probably Ebola viruses (11–14). Cross-species transmission from bats to humans can be direct, through contact with infected bats or their excreta, or facilitated by intermediate hosts (15). Bat CoVs are typically host specific; however, MERS-related CoVs have reportedly been found in many bat families, including Vespertilionidae, Molosidae, Nycteridae, and now Emballonuridae (sheath-tailed bats) in Africa, the Americas, Asia, and Europe. We sampled only a small sample of bats in Saudi Arabia. Nonetheless, given the rarity of MERS CoV sequences detected by our survey and the broad distribution of MERS cases throughout the Middle East, we speculate that there are probably other hosts. Future work should investigate additional bat and other wildlife species and domestic animals for CoV infection and potential linkage to human disease.

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Dr Memish is deputy minister for public health, director of the WHO Collaborating Center for Mass Gathering Medicine in the Ministry of Health, and professor in the College of Medicine of Alfaisal University in Riyadh. His research interests include emerging infectious diseases, infection control, and preventive medicine.
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Address for correspondence: W. Ian Lipkin, Center for Infection and Immunity, Columbia University, 722 West 168th St, New York, NY 10032, USA; email: wil2001@columbia.edu