surveillance for coronaviruses in bats, Lebanon and Egypt, 2013–2015

Mahmoud M. Shehata,1 Daniel K.W. Chu,1 Mokhtar R. Gomaa, Mounir AbiSaid, Rabeh El Shesheny, Ahmed Kandeil, Ola Bagato, Samuel M.S. Chan, Elie K. Barbour, Houssam S. Shaib, Pamela P. McKenzie, Richard J. Webby, Mohamed A. Ali, Malik Peiris, Ghazi Kayali

Author affiliations: National Research Centre, Giza, Egypt (M.M. Shehata, M.R. Gomaa, R. El Shesheny, A. Kandeil, O. Bagato, M.A. Ali); The University of Hong Kong, Hong Kong, China (D.K.W. Chu, S.M.S. Chan, M. Peiris); Lebanese University, Al Fanar, Lebanon (M. AbiSaid); Animal Encounter, Aley, Lebanon (M. AbiSaid); King Abdulaziz University, Jeddah, Saudi Arabia (E.K. Barbour); American University of Beirut, Beirut, Lebanon (E.K. Barbour, H.S. Shaib); St. Jude Children’s Research Hospital, Memphis, Tennessee, USA (P.P. McKenzie, R.J. Webby, G. Kayali)

DOI: http://dx.doi.org/10.3201/eid2201.151397

To the Editor: Coronaviruses (CoVs) in bats are genetically diverse, and evidence suggests they are ancestors of Middle East respiratory virus CoV (MERS-CoV), severe acute respiratory syndrome CoV, and human CoVs 229E and NL63 (1–4). We tested several bat species in Lebanon and Egypt to understand the diversity of bat CoVs there.

Samples were collected during February 2013–April 2015. A total of 821 bats were captured live in their caves; 5.5% of the bats tested positive.

In Lebanon, we sampled 4 bat species. Four Rhinolophus hipposideros bats and 6 Miniopterus schreibersii bats tested negative. One of 3 Rhinolophus ferrumequinum bats sampled was positive. We sampled 438 Rousettus aegyptiacus bats from 10 different locations and detected HKU9-like viruses in 24 rectal swab specimens (prevalence 5.5%). Overall, 5.5% of the bats tested positive.

In Egypt, we sampled 3 bat species (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/22/1/15-1397-Techapp1.pdf). Eighty-two Egyptian tomb bats (Taphozous perforatus) tested negative for CoV. We also sampled 31 desert pipistrelle bats (Pipistrellus deserti) and detected an HKU9-like betacoronavirus (b-CoV) in the liver of 1 bat (prevalence 3.2%). From 257 specimens from Egyptian fruit bats (Rousettus aegyptiacus), we detected b-CoV in 18 samples from 18 different bats (prevalence 7%). A murine hepatitis virus–like CoV was detected in the lung of 1 bat. HKU9-like viruses were detected in 5 oral, 2 lung, 5 liver, and 5 rectal samples. Overall, 5.1% of the bats tested positive.

Address for correspondence: Hiroshi Nishiura, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; email: nishiurah@m.u-tokyo.ac.jp

5. Nishiura H. Determination of the appropriate quarantine period following smallpox exposure: an objective approach using the incubation period distribution. Int J Hyg Environ Health. 2009;212:97–104. http://dx.doi.org/10.1016/j.ijeh.2007.10.003

6. Mizumoto K, Endo A, Chowell G, Miyamatsu Y, Saitoh M, Nishiura H. Real-time characterization of risks of death associated with the Middle East respiratory syndrome (MERS) in the Republic of Korea. 2015. BMC Med. 2015;13:228. http://dx.doi.org/10.1186/s12916-015-0468-3

7. Mizumoto K, Saitoh M, Chowell G, Miyamatsu Y, Nishiura H. Estimating the risk of Middle East respiratory syndrome (MERS) death during the course of the outbreak in the Republic of Korea, 2015. Int J Infect Dis. 2015;39:7–9. http://dx.doi.org/10.1016/j.ijid.2015.08.005

O. Bagato, M.A. Ali); The University of Hong Kong, Hong Kong, China (M.M. Shehata,1 Daniel K.W. Chu,1 Malik Peiris, Ghazi Kayali

Address for correspondence: Hiroshi Nishiura, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; email: nishiurah@m.u-tokyo.ac.jp

The authors contributed equally to this article.

These authors contributed equally to this article.
The b-CoVs HKU9 and HKU10 were detected in Chinese fruit bats. All but 1 of the detected viruses were HKU9-like. However, there was enough genetic variability within the sequenced RdRp fragments to suggest the circulation of at least 3 diverse groups comprising 3 different CoV species.

Our detection of CoVs in oral, rectal, lung, and liver samples suggests that CoV infection in those bats was systemic, although the bats were apparently healthy. One bat had a murine hepatitis virus–like infection. This bat was captured from a brood that inhabited the windowsills of a historic building in urban Cairo. This infection might...
have been a cross-species infection from mice to bats in the same habitat.

Although bats rarely come in direct contact with humans, humans can come into more frequent contact with bat urine and feces and, in the case of fruit bats, bat saliva through partially eaten fruits. Bats in the Middle East are not eaten for food but are occasionally hunted. In this study, HKU9-related viruses were detected in apparently healthy fruit bat species from Egypt and Lebanon and appear to cause systemic infection. HKU9-related viruses are not known to cause human disease. MERS-CoV was not detected in bats sampled in this study. More surveillance for bat CoVs in the Middle East is needed, and the zoonotic potential for bat-CoVs requires further study.

This work was funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, US Department of Health and Human Services, under contract no. HHSN272201400006C; and supported by the American Lebanese Syrian Associated Charities.

References

1. Drexler JF, Corman VM, Drosten C. Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. Antiviral Res. 2014;101:45–56. http://dx.doi.org/10.1016/j.antiviral.2013.10.013
2. Ithete NL, Stoffberg S, Corman VM, Cottontail VM, Richards LR, Schoeman MC, et al. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. Emerg Infect Dis. 2013;19:1697–9. http://dx.doi.org/10.3201/eid1910.130946
3. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis. 2013;19:1819–23. http://dx.doi.org/10.3201/eid1911.131172
4. Yang L, Wu Z, Ren X, Yang F, Zhang J, He G, et al. MERS-related betacoronavirus in Vespertilio superans bats, China. Emerg Infect Dis. 2014;20:1260–2. http://dx.doi.org/10.3201/eid2007.140318
5. Hulva P, Maresova T, Dundarova H, Bilgin R, Benda P, Bartonicka T, et al. Environmental margin and island evolution in Middle Eastern populations of the Egyptian fruit bat. Mol Ecol. 2012;21:6104–16. http://dx.doi.org/10.1111/mec.12078
6. Towner JS, Amman BR, Sealy TK, Carroll SA, Corder JA, Kemp A, et al. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog. 2009;5:e1000536. http://dx.doi.org/10.1371/journal.ppat.1000536
7. Kalunda M, Mukwawa LG, Mukuye A, Lule M, Sekyalo E, Wright J, et al. Kasokero virus: a new human pathogen from bats (Rousettus aegyptiacus) in Uganda. Am J Trop Med Hyg. 1986;35:387–92.
8. Amman BR, Albarino CG, Bird BH, Nyakarahuka L, Sealy TK, Balinandi S, et al. A recently discovered pathogenic paramyxovirus, Sosuga virus, is present in Rousettus aegyptiacus fruit bats at multiple locations in Uganda. J Wildl Dis. 2014;51:774–9. http://dx.doi.org/10.7589/2015-02-044
9. Woo PC, Wang M, Lau SK, Xu H, Poon RW, Guo R, et al. Comparative analysis of twelve genomes of three novel group 2c and group 2d coronaviruses reveals unique group and subgroup features. J Virol. 2007;81:1574–85. http://dx.doi.org/10.1128/JVI.02182-06

Ebola Virus Disease Complicated by Late-Onset Encephalitis and Polyarthritis, Sierra Leone

Patrick Howlett, Colin Brown, Trina Helderman, Tim Brooks, Durodamil Lisk, Gibrilla Deen, Marylou Solbrig, Marta Lado

Author affiliations: Kings Sierra Leone Partnership, Freetown, Sierra Leone (P. Howlett, M. Lado); University College London Hospital, London, UK (C. Brown); Medair, Ecublens, Switzerland (T. Helderman); Public Health England, Porton Down, UK (T. Brooks); Connaught Hospital, Freetown (D. Lisk, G. Deen); University of Kansas, Lawrence, Kansas, USA (M. Solbrig)

DOI: http://dx.doi.org/10.3201/eid2201.151212

To the Editor: Ebola virus (EBOV) disease is usually an acute illness, but increasing evidence exists of persistent infections and post-Ebola syndromes. We report a case of EBOV encephalitis.

A 30-year-old woman with no known EBOV contact sought treatment at an Ebola isolation unit in Freetown, Sierra Leone, on January 1, 2015 (day 7 of illness). She was afebrile and weak, but ambulatory, with a history of fever, vomiting, diarrhea, headache, and muscle and joint pain. According to local protocol, she was given oral antimalarial, antimicrobial, and antiemetic drugs and oral rehydration therapy. On day 8 of illness, after testing EBOV PCR–positive (cycle threshold [Ct] value of 23.5) (1), she was given intravenous ceftriaxone (2 g) for 7 days, artesunate (180 mg) for 3 days, and Ringer’s lactate (4–6 L) with supplemental KCl for 5 days.

During days 13–15, the patient improved, moving independently and talking. On day 16, she became confused; by day 20, she was unresponsive to voices. Intravenous ceftriaxone (2 g) and artesunate (180 mg) were administered for an additional 7 and 3 days, respectively. On days 28 and 29, she was still unconscious; serum PCR test results on both days were negative for EBOV. On day 29, she was transferred to Connaught Hospital in Freetown, where she had a Glasgow Coma Scale score of 9/15 (E3, V1, M5) but no localizing or focal signs. She was

Addresses for correspondence; Malik Peiris, School of Public Health, The University of Hong Kong, 21 Sassoon Rd, Pokfulam, Hong Kong Special Administrative Region, China; email: malik@hku.hk; Ghazi Kayali, Department of Infectious Diseases, St. Jude Children’s Research Hospital, 262 Danny Thomas Pl, Memphis, TN 38105 USA; email: ghazi.kayali@stjude.org

150 Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 22, No. 1, January 2016
Surveillance for Coronaviruses in Bats, Lebanon and Egypt, 2013–2015

Technical Appendix 1

Laboratory Methods

Screening

Viral RNA was extracted by using the Qiaamp viral RNA minikit (QIAGEN, Hilden, Germany). The RNA was eluted in 60 μL AVE buffer and was used as a template for further detection by a pan-coronavirus nested PCR targeting the RNA-dependent RNA polymerase (RdRp) gene. First-round reverse transcription PCR (RT-PCR) was conducted by using forward primer 5′-GGKTG–GGAYTAYCCKAARTG-3′ and reverse primer 5′-TGYTGTSWRCA-RAAYTCRTG-3′ and QIAGEN 1-step RT-PCR kit. A 25-μL reaction mixture contained 5 μL of 5X reaction buffer, 1 μL dNTPs, 1 μL enzyme mix, 1.5 μL (10 Pmole) forward primer, 1.5 μL (10 Pmole) reverse primer, 10 μL ddH₂O, and 5 μL of sample RNA. The PCR cycler conditions for the amplification were 50°C for 30 min (reverse transcription) then 95°C for 15 min, 45 cycle of 94°C for 15 s (denaturation), 48°C for 30 s (annealing), 72°C for 40 s (extension), then 72°C for 10 min (final extension). The PCR product was then put through a second round PCR by using a new set of primers (forward primer 5′-GGTTGG-GACTATCCTAAGTGTA-3′, reverse primer 5′-CCATCATCAGATAG-AATCATCAT-3′) which amplify a final PCR product of 440 bp. Using Phusion High Fidelity PCR Master Mix Kit (Thermo Scientific, Waltham, MA, USA), a 25-μL reaction contained 12.5 μL of 2X phusion master mix, 1.5 μL (10 Pmole) forward primer, 1.5 μL (10 Pmole) reverse primer, 7.5 μL H₂O, and 2 μL of PCR product. The PCR cycler conditions were 98°C for 2 min then 45 amplification cycles (98°C for 15 s, 48°C for 15 s, 72°C for 30 s), then 72°C for 2 min. The final PCR amplicons were gel purified using the
QIAquick gel purification kit (QIAGEN) and analyzed by sequencing (1). The *upE* quantitative reverse transcription PCR was performed as previously described (2).

**Sequencing**

The second round forward and reverse primers were to sequence the purified DNA amplicons using a BigDyeR Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer’s instructions and were further amplified for 26 cycles at 95°C for 30 s, 50°C for 15 s, and 60°C for 4 min. The reaction product was purified by exclusion chromatography in CentriSep columns (Princeton Separations, Adelphia, NJ, USA). The recovered materials were sequenced by using a 96-capillary 3730xl DNA Analyzer (Applied Biosystems). Sequences were assembled by using SeqMan DNA Lasergene 7 software (DNASTAR, Madison, WI, USA). Sequence analysis was performed by using BioEdit 7.0 and MEGA 6.0 for multiple sequence alignment and phylogenetic tree construction of applying the neighbor-joining method with Kimura’s 2-parameter distance model and 1,000 bootstrap replicates (3,4).

An RT nested PCR designed specifically for this study was used for amplifying N gene sequences of HKU9-like viruses. Reaction conditions of this assay were same as the RdRp assay above except for the 1-step RT-PCR with outer forward primer 5-ATGTCTGGAMGGAAATAAGCCCG-3 and inner reverse primer 5-TTATTAGGATTACGDTGCCCAT-3, and nested PCR with inner forward primer 5-GTTCAAGCAAGAATCTGACGGTT-3 and inner reverse primer 5-ACCTTCTTCACCCACCCGAGTATA-3. The expect size of the second PCR product was 400 bp.

GenBank accession nos.: KT220528–KT220562, KT368821, KT581588–KT581603.

**Serology**

A pseudo-particle neutralization assay was used to test bat serum against MERS-CoV as previously described (5).

**Ethical Statement**

Ethics approval was obtained from St. Jude Children’s Research Hospital Institutional Animal Use and Care Committee (Memphis, TN, USA). Swabs and tissues were tested by RT-PCR, and positive samples were sequenced.
References

1. Chu DK, Leung CY, Gilbert M, Joyner PH, Ng EM, Tse TM, et al. Avian coronavirus in wild aquatic birds. J Virol. 2011;85:12815–20. PubMed [http://dx.doi.org/10.1128/JVI.05838-11](http://dx.doi.org/10.1128/JVI.05838-11)

2. Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17:20285. PubMed

3. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. PubMed [http://dx.doi.org/10.1093/molbev/msr121](http://dx.doi.org/10.1093/molbev/msr121)

4. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–8.

5. Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, et al. Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. Euro Surveill. 2013;18:20574. PubMed [http://dx.doi.org/10.2807/1560-7917.ES2013.18.36.20574](http://dx.doi.org/10.2807/1560-7917.ES2013.18.36.20574)

Technical Appendix 1 Table 1. Screening for coronaviruses in bats, Egypt, 2013–2015

| Species                  | No bats | No. samples | type          | Results                                | Location                           | Date     |
|--------------------------|---------|-------------|---------------|----------------------------------------|------------------------------------|----------|
| Taphozous perforatus     | 5       | 5 Serum     | 5 Oral        |                                        | Abu-Rawwash, 13 km west of Cairo   | Feb 2013 |
|                          |         | 5 Lung      |               |                                        |                                    |          |
|                          |         | 5 Liver     |               |                                        |                                    |          |
| T. perforatus            | 52      | 52 Serum    | 52 Oral       |                                        | Abu-Rawwash, 13 km west of Cairo   | Aug 2013 |
|                          |         | 52 Rectal   |               |                                        |                                    |          |
| T. perforatus            | 25      | 24 Serum    | 25 Oral       |                                        | Abu-Rawwash, 13 km west of Cairo   | Oct 2013 |
|                          |         | 25 Rectal   |               |                                        |                                    |          |
| Pipistrellus deserti     | 29      | 29 Serum    | 29 Oral       | 1 (HKU9-like)                          | Abu-Rawwash, 13 km west of Cairo   | Feb 2013 |
|                          |         | 29 Lung     |               |                                        |                                    |          |
|                          |         | 29 Liver    |               |                                        |                                    |          |
| P. deserti               | 2       | 1 Serum     | 2 Oral        | 1 (murine hepatitis virus-like)        | Abu-Rawwash, 13 km west of Cairo   | Oct 2013 |
|                          |         | 2 Rectal    |               |                                        |                                    |          |
| Rousettus aegyptiacus    | 14      | 14 Serum    | 14 Oral       | 1 (murine hepatitis virus-like)        | Cairo                              | Feb 2013 |
|                          |         | 14 Lung     |               |                                        |                                    |          |
| A. aegyptiacus           | 24      | 24 Serum    | 24 Oral       | 3 (HKU9-like)                          | Abu-Rawwash, 13 km west of Cairo   | Feb 2013 |
|                          |         | 24 Lung     |               | 2 (HKU9-like)                          |                                    |          |
|                          |         | 24 Liver    |               | 5 (HKU9-like)                          |                                    |          |
| A. aegyptiacus           | 5       | 4 Serum     | 5 Oral        | 4 (HKU9-like)                          | Abu-Rawwash, 13 km west of Cairo   | Oct 2013 |
|                          |         | 4 Rectal    |               |                                        |                                    |          |
| A. aegyptiacus           | 102     | 101 Serum   | 102 Oral      | 2 (HKU9-like)                          | Abu-Rawwash, 13 km west of Cairo   | Oct 2014 |
|                          |         | 102 Rectal  |               |                                        |                                    |          |
| A. aegyptiacus           | 112     | 112 Serum   |               | 1 (HKU9-like)                          | Abu-Rawwash, 13 km west of Cairo   | Nov 2014 |
### Technical Appendix 1 Table 2. Screening for coronaviruses in bats, Lebanon, 2013–2015

| Species                       | No bats | Sample no., type | Result                | Location                | Date   |
|-------------------------------|---------|------------------|-----------------------|-------------------------|--------|
| Rhinolophus hipposideros      | 4       | 4 Serum          |                       | Zgharta, North Lebanon  | Oct 2013 |
| Rhinolophus ferrumequinum     | 1       | 1 Serum          | 1 (HKU9-like)         | Aley, Mount Lebanon     | Oct 2013 |
| Miniopterus schreibersii      | 6       | 6 Serum          |                       | Amchit, North Lebanon   | Oct 2013 |
| Rousettus aegyptiacus         | 50      | 50 Serum         | 4 (HKU9-like)         | Akkar, North Lebanon    | Oct 2013 |
| Rousettus aegyptiacus         | 1       | 1 Serum          | 1 (HKU9-like)         | Amchit, North Lebanon   | Oct 2013 |
| Rousettus aegyptiacus         | 51      | 51 Serum         | 1 (HKU9-like)         | Bisri, South Lebanon    | Oct 2013 |
| Rousettus aegyptiacus         | 34      | 34 Serum         | 3 (HKU9-like)         | Antelias, Mount Lebanon | Oct 2013 |
| Rousettus aegyptiacus         | 21      | 21 Serum         | 5 (HKU9-like)         | Ras Keefa, North Lebanon| Apr 2014 |
| Rousettus aegyptiacus         | 5       | 5 Serum          |                       | Berqayel, North Lebanon | Jun 2014 |
| Rousettus aegyptiacus         | 75      | 72 Serum         | 7 (HKU9-like)         | Tripoli, North Lebanon  | Jun 2014 |
| Rousettus aegyptiacus         | 34      | 34 Serum         | 3 (HKU9-like)         | Antelias, Mount Lebanon | Sep 2014 |
| Rousettus aegyptiacus         | 101     | 101 Serum        |                       | Bisri, South Lebanon    | Jun 2014 |
| Rousettus aegyptiacus         | 30      | 30 Serum         |                       | Jbeil, Mount Lebanon    | Jun 2014 |
| Rousettus aegyptiacus         | 32      | 32 Serum         |                       | Edde, Mount Lebanon     | Oct 2014 |
| Rousettus aegyptiacus         | 4       | 4 Serum          |                       | Karm Saddeh, North Lebanon| Apr 2015 |

Total 451  25 positive samples (5.54%)
Technical Appendix 1 Figure. Phylogenetic tree of the coronavirus N gene. This tree was constructed on the basis of sequence alignment of 400 bp of the N gene and neighbor-joining method. Sequences in red are those found in this study. Scale bar indicates nucleotide substitutions per site.