Alkhurma Hemorrhagic Fever in Travelers Returning from Egypt, 2010

Fabrizio Carletti, Concetta Castilletti, Antonino Di Caro, Maria R. Capobianchi, Carla Nisii, Fredy Suter, Marco Rizzi, Alessandra Tebaldi, Antonio Goglio, Cristiana Passerini Tosi, and Giuseppe Ippolito

Two travelers returning to Italy from southern Egypt were hospitalized with a fever of unknown origin. Test results showed infection with Alkhurma virus. The geographic distribution of this virus could be broader than previously thought.

Alkhurma virus (ALKV) is a recently described member of the tick-borne hemorrhagic fever group of the genus Flavivirus. It was initially isolated in the late 1990s (1,2) and is today considered a variant of the Kyasun Forest disease virus, sharing 89% nt sequence homology (3,4). This emerging pathogen causes signs and symptoms such as fever, headache, joint pain, muscle pain, vomiting, and thrombocytopenia; severe cases may have hemorrhagic manifestations (epistaxis, ecchymoses, petechiae, hematemeses) and encephalitis, which can result in death (reported case-fatality rate as high as 25%) (5–8). Camels and sheep are thought to be the natural hosts of ALKV, but whether other mammals are also involved in its life cycle remains unknown. ALKV RNA was recently detected in an Ornithodoros savignyi tick collected near Jeddah, Saudi Arabia (9); on the Arabian Peninsula, these ticks have been associated with butchering of sheep and camels. No human-to-human transmission has been reported. ALKV has been detected only in Saudi Arabia, but the closely related Kyasun Forest disease virus has spread as far as India and the People’s Republic of China (4). We describe 2 cases of Alkhurma hemorrhagic fever in 2 travelers who returned to Italy from Egypt in 2010.

The Cases

The first patient, a 64-year-old man from Italy, spent 1 week (April 25–May 1, 2010) in a touristic village in southern Egypt, near the Sudan border. While visiting a camel and dromedary market in Shalatin on April 29, he was bitten on the foot by an unidentified arthropod (although not formally identified, was described as tick shaped). Soon after, a small, papular lesion developed. During his return flight to Italy, ≈48 hours after the bite, the patient experienced high fever, shaking chills, anorexia, malaise, nausea and vomiting, and blurred vision. During the next 5 days, these signs and symptoms worsened, and the man was admitted to the “Ospedali Riuniti di Bergamo” in northern Italy. His medical history was unremarkable, but he frequently traveled abroad and had been vaccinated against yellow fever in 1998.

Laboratory test results showed leukopenia (2,250 cells/mm³), thrombocytopenia (67,000 platelets/mm³), and increased liver enzymes (aspartate transaminase 469 U/L, reference 3–46 U/L; alanine transaminase 406 U/L, reference 3–46 U/L). The patient was given acetaminophen, and fever and general malaise progressively decreased over the next 5 days. He was discharged 11 days later, on May 17, in good general condition despite persistence of asthenia.

Acute-phase and convalescent-phase serum samples (collected on May 10 and 27, respectively) were sent to the virology laboratory of the “Lazzaro Spallanzani” National Institute for Infectious Diseases in Rome to be tested for dengue and West Nile viruses. Immunoglobulins (Ig) G and M for both viruses were detected by immunofluorescence of both samples; for each virus, IgG titer was ≥640 and IgM titer was ≥20. No evidence of rising antibody titers was found in the convalescent-phase specimen, raising suspicion of cross-reactivity to a previous Flavivirus infection or yellow fever vaccination. A genus-specific reverse transcription–PCR selective for the nonstructural protein (NS) 5 gene of flaviviruses (11) was positive for the acute-phase and negative for the convalescent-phase
samples. Sequence analysis of the amplicon (GenBank accession no. HM629507) showed high similarity with ALKV sequences in GenBank (BLAST [www.ncbi.nlm.nih.gov/blast/Blast.cgi] submission showed 97% identity with AF331718). This unexpected result called for further investigations to confirm the diagnosis of an ALKV infection. Thus, an ALKV-specific nested reverse transcription–PCR selective for a wider region of a different gene (E) was designed by using the following primers: outer forward 5′-TGGAAACCCCCACCGGTTGACT-3′; outer reverse 5′-ATGCCCACTGTCGTTGCGC-3′; inner forward 5′-CCCAACAGCAATCGAAAACGGCATC-3′; inner reverse 5′-GCCCAACATCACAGGTGACATGACC-3′.

All residual biological samples collected during the patient’s hospital stay were sent to the virology laboratory of the Spallanzani Hospital (Italy’s national reference laboratory for viral hemorrhagic fever viruses, BioSafety Level 4) in compliance with biosafety procedures. The new ALKV PCR result was positive, and the sequence of the amplicon (GenBank accession no. HM629508) showed high homology with ALKV (99% identity with AF331718). The phylogenetic trees based on partial sequences of NS5 (Figure 1) and E (Figure 2) genes confirmed the diagnosis of ALKV infection.

After submitting this article, we detected ALKV infection in a second patient. This patient had traveled to the same area ≈1 month later, visited the same camel market, and was affected by a milder disease. NS5 (HQ218942) and E (HQ218941) gene sequences obtained from this patient have been included in the phylogenetic tree, showing that they cluster together with those from the first patient (Figures 1, 2).

Conclusions

The 2 patients had traveled to an area of the world where ALKV had not been previously reported. Although viremia was demonstrated 10 days after symptom onset, and we can reliably suppose that it started when fever and chills appeared, the probability of a susceptible vector in Europe is small, and the infection seems not to be transmissible from human to human.

Laboratory diagnosis of this infection is not easy to obtain and requires a specialized laboratory because of antibody cross-reactivity with other members of the family Flaviviridae and because of the absence of commercially available serologic tests and reference biologic materials for their development. However, surveillance of travelers returning from areas where highly dangerous infectious diseases are endemic should be improved and should include ALKV. The finding that the distribution of this virus is wider than previously thought and that it includes the African continent is in line with the hypothesis that tick-borne flaviviruses originated in Africa (12). The low genetic distance between the Egypt and Saudi Arabia sequences supports the hypothesis of a recent divergence from Kyasanur Forest disease virus, i.e., the closest flavivirus (5), and a slow microevolution of ALKV, as for other tick-borne flaviviruses (13). The higher genetic divergence in the NS5 gene than in the E gene of ALKV strains confirms previous observations for viruses isolated from human samples after inoculation of suckling mice (5) and deserves more detailed evolutionary analysis.

The detection of 2 independent infection events for travelers who visited the same area in a restricted period strongly supports the hypothesis of sustained local ALKV circulation. Further veterinary and entomologic investigations are needed to expand understanding of the geographic distribution of ALKV and to assess the danger for local populations and visitors. It would be advisable to inform
travelers about the danger of coming into contact with infected animals in areas where the virus has been reported. Avoidance of or minimization of exposure to infected ticks should be recommended as the most effective prevention measure.

This work was conducted with the support of the following European Union–funded projects: RiViGene, European Network of P4 Laboratories, and the European Network for the Diagnosis of Imported Viral Diseases–Collaborative Network.

Dr Carletti is a scientist at the Virology Laboratory of the “Lazzaro Spallanzani” National Institute for Infectious Diseases in Rome. His research interests include emerging and reemerging infections, especially virologic aspects of host–pathogen interactions and development of tools and protocols for diagnosis of emerging viral diseases, including viral hemorrhagic fevers.

References

1. Zaki AM. Isolation of a flavivirus related to the tick-borne encephalitis complex from human cases in Saudi Arabia. Trans R Soc Trop Med Hyg. 1997;91:179–81. DOI: 10.1016/S0035-9203(97)90215-7

2. Qattan I, Akbar N, Abu Azmah S, Al-Khateeb T, Zaki A, et al. A novel flavivirus: Makkah region, 1994–1996. Saudi Epidemiology Bulletin. 1996;3:1–3.

3. Charrel RN, Zaki AM, Attoui H, Fakeeh M, Billoir F, Yousef AI, et al. Complete coding sequence of the Alkhurma virus, a tick-borne flavivirus causing severe hemorrhagic fever in humans in Saudi Arabia. Biochem Biophys Res Commun. 2001;287:455–61. DOI: 10.1006/bbrc.2001.5610

4. Mehla R, Kumar SR, Yadav P, Barde PV, Yergolkar PN, Erickson BR, et al. Recent ancestry of Kyasanur Forest disease virus. Emerg Infect Dis. 2009;15:1431–7. DOI: 10.3201/eid1509.080759

5. Charrel RN, Zaki AM, Attoui H, Fakeeh M, Billoir F, Yousef AI, et al. Low diversity of Alkhurma hemorrhagic fever virus, Saudi Arabia, 1994–1999. Emerg Infect Dis. 2005;11:683–8. DOI: 10.3201/eid1108.041109

6. Madani TA. Alkhurma virus infection, a new viral hemorrhagic fever in Saudi Arabia. J Infect. 1994–1999. Emerg Infect Dis. 2005;11:683–8.

7. Charrel RN, Zaki AM, Fagbo S, de Lamballerie X. Alkhurma hemorrhagic fever virus is an emerging tick-borne flavivirus. J Infect. 2006;52:463–4. DOI: 10.1016/j.jinf.2005.08.011

8. Alkhurma virus–Saudi Arabia: (Makkah). ProMED-mail [cited 2010 Jan 6]. http://www.promedmail.org, archive no. 20100106.0056.

9. Charrel RN, Fagbo S, Moureau G, Alqahtani MH, Temmam S, de Lamballerie X. Alkhurma hemorrhagic fever virus in Ornithodoros savignyi ticks. Emerg Infect Dis. 2007;13:153–5. DOI: 10.3201/eid1301.061094

10. Hoogstraal H. Argasid and nuttallielid ticks as parasites and vectors. Adv Parasitol. 1985;24:135–238. DOI: 10.1016/S0065-308X(86)0563-1
11. Scaramozzin N, Crance JM, Jouan A, DeBriel DA, Stoll F, Garin D. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription–PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. J Clin Microbiol. 2001;39:1922–7. DOI: 10.1128/JCM.39.5.1922-1927.2001

12. Grard G, Moureau G, Charrel RN, Lemasson JJ, Gonzalez JP, Gallian P, et al. Genetic characterization of tick-borne flaviviruses: new insights into evolution, pathogenetic determinants and taxonomy. Virology. 2007;361:80–92. DOI: 10.1016/j.virol.2006.09.015

13. Zanotto PM, Gould EA, Gao GF, Harvey PH, Holmes EC. Population dynamics of flaviviruses revealed by molecular phylogenies. Proc Natl Acad Sci U S A. 1996;93:548–53.

Address for correspondence: Giuseppe Ippolito, National Institute for Infectious Diseases “L. Spallanzani,” 292 Via Portuense, 00149 Rome, Italy: email: giuseppe.ippolito@inmi.it