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Kyasanur Forest Disease Virus Alkhurma Subtype in Ticks, Najran Province, Saudi Arabia

To the Editor: The lineage of Kyasanur Forest disease virus (KFDV) found in the Kingdom of Saudi Arabia is commonly referred to as Alkhurma hemorrhagic fever virus (AHFV). This virus was first isolated from a specimen collected in 1994 from a butcher living in Makkah Province, who was hospitalized for a hemorrhagic fever from which he died (1). The virus was assigned to the genus *Flavivirus* on the basis of reactivity with genus-specific monoclonal antibodies and sequencing of a fragment of the nonstructural 5 (NS5) gene, which

showed >89% identity with KFDV. Ten other cases were confirmed among patients who had leukopenia, thrombocytopenia, and elevated liver enzymes. Observations of patients in the original study or in a subsequent analysis (2) suggested that Alkhurma hemorrhagic fever (AHF) disease was associated with contact with blood from infected animals, bites from infected ticks, or the drinking of raw milk. However, the exact mode of transmission to humans has still not been fully elucidated. More recently, AHFV RNA was detected in a single pool of sand tampans (*Ornithodoros savignyi*, soft ticks), collected in western Saudi Arabia (3), which suggests a link with these ticks.

To analyze the virus association with arthropods further, we collected and identified ticks and mosquitoes in Najran Province, southern Saudi Arabia, during May and June 2009 from different sites close to where human AHF cases had been recently confirmed (4,5). Camel ticks (*Hyalomma dromedarii*) (130 adults) were collected while they fed on camels, and *O. savignyi* sand tampans (243 adults) were collected from the ground in camel resting places (except 1 collected while feeding on a camel). Mosquitoes were collected by using light traps (203 *Culex decens* females) or as larvae that were then raised in the laboratory (9 *Culiseta* sp. females). Ticks and mosquitoes were stored at room temperature and killed by overnight freezing the day before shipping to the Centers for Disease Control and Prevention (Atlanta, GA, USA). All arthropods were processed in the BioSafety Level 4 laboratory by injecting Vero E6 cells and by intracerebrally inoculating suckling mice with ground pools of either 5 ticks or 10 mosquitoes. All the tick material was used for the tested pools. Isolates of AHFV were obtained from 1 of 13 pools of *H. dromedarii* ticks and 1 of 6 pools of *O. savignyi* sand tampans, both from Al Mishaaliya

district, and from 5 of 8 pools of *O. savignyi* sand tampans from the Al Balad Magan camel market. Virus identity was confirmed by sequencing a 390-nt fragment from the virus core protein C and preM genes. No virus was isolated from any mosquito suspensions.

Phylogenetic analysis of the 7 tick isolates and the available homologous sequences of AHFV are presented in the Figure. The tick AHFV sequences are closely related but distinct from previously reported AHFV sequences from human isolates or from the only sequence reported from ticks collected in 2004 in Jeddah Province. The observed sequences are clustered by site of collection but not by tick species.

In this report, we confirm that the sand tampa (*O. savignyi* tick) is a vector and reservoir of AHFV in Saudi Arabia. Of all arthropods, this tick is one of the most highly adapted to the desert. It can be found in the shade of trees, beside rock fences, on livestock, and in livestock yards, particularly camel yards (6). It can feed rapidly during the day or night on camels, goats, sheep, wild mammals, and humans. Sand tampans

can survive for long periods without feeding, fulfilling perfectly the role of reservoir for AHFV. This tick has been reported in arid biotopes of northeastern, eastern, and southern Africa (7) and from Arabia to India and Sri Lanka, which suggests a potential wide distribution of AHFV or related viruses. In India, KFDV has been isolated from *Ornithodoros* spp. ticks collected in a bat-inhabited cave (8), and experimental transtadial and transovarial transmission of KFDV in *O. crossi* ticks has been reported (9).

The isolation of AHFV from the camel tick (*H. dromedarii*) also has public health implications. The capital city of Najran serves as a market for camels and other livestock from Saudi Arabia and Yemen. Adult camel ticks infest mainly camels, and infected ticks can feed on and infect animals just before sale or slaughter. AHF in persons working in the Najran market has been described (5). Unfortunately, no AHFV sequence is available from those cases.

The genetic diversity of the isolated viruses from ticks is quite low. Previous analysis of KFDV and AHFV suggested slow evolution with

divergence ≈ 33 years ago (10). The data reported here clearly strengthen the position of AHFV in the tick-borne flavivirus complex, although the numbers and species of mosquitoes tested were limited. Expanded epidemiologic and molecular studies should provide insight into the distribution and evolution of the virus and identify at-risk regions within Saudi Arabia. Laboratory infection and transmission studies in colonized ticks should clarify the role of *O. savignyi* and *H. dromedarii* ticks in the ecology of AHFV. Currently, public health messages are being developed for the community at risk and local health care workers.

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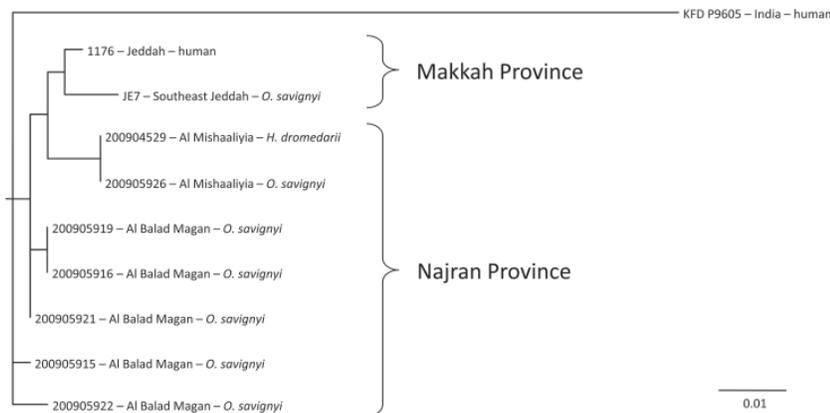


Figure. Phylogenetic analysis of Alkhurma viruses isolated from *Ornithodoros savignyi* and *Hyalomma dromedarii* ticks in Najran Province, Kingdom of Saudi Arabia. A 390-bp region of the core protein C and preM genes was amplified and sequenced for each of the isolates (HQ443410–6) by using primers ALK244S (5'-GTGTTGATGCGCATGATGGG-3') and ALK665R (5'-TGCAGAAACAGTCCACATCA-3'). A maximum-likelihood analysis was conducted with available sequences in GenBank for ALK (NC_004355; 3) by using Kyasanur Forest disease (AY323490) as the outgroup and the default settings in GARLI version 0.96b8 (www.phylo.org/pdf_docs/zwicklDissertation.pdf). Scale bar indicates substitutions per site.

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

West Nile Virus Infection, Assam, India

To the Editor: West Nile virus (WNV) is a mosquito-borne flavivirus. Sporadic infections with this virus have been found in Africa, Europe, Asia, and the United States. In humans, most infections with WNV cause subclinical or a mild influenza-like illness; encephalitis occurs in some (1). In India, antibodies against WNV were first detected in humans in Bombay in 1952 (2). Virus activity has been reported in southern, central, and western India. WNV has been isolated in India from *Culex vishnui* mosquitoes in Andhra Pradesh and Tamil Nadu, from *Cx. quinquefasciatus* mosquitoes in Maharashtra, and from humans in Karnataka State (3).

Assam (26°–27°30'N, 89°58'–95°41'E) is the most populated state in northeastern India; it contains ≈50% of the 38.8 million inhabitants of northeastern India. Japanese encephalitis virus (JEV) has caused sporadic epidemics in Assam since 1976. Studies conducted during 2000–2002 in Assam showed that 187 (53.7%) of 348 persons with acute encephalitis syndrome were infected with JEV (4). JEV-negative persons also showed symptoms of neurotropic viral infection.

Suspecting the presence of some other closely related flavivirus in this region, we screened samples from persons with acute encephalitis syndrome for WNV in 2006. To our knowledge, no study has been conducted on the prevalence of WNV in this region. We report WNV activity in the state of Assam in northeastern India. Ethical approval for this study was obtained from the institutional ethical committee, Regional Medical Research Center, Dibrugarh, India.

A JEV vaccination campaign (SA14-14-2 vaccine) was started in Assam during May 2006. During

its first phase, children 1–15 years of age in Dibrugarh and Sivasagar Districts were vaccinated. Mosquito surveillance in the study area and in an earlier study (5) identified *Cx. vishnui* mosquitoes.

During the study period, 103 serum samples and 88 cerebrospinal fluid samples were obtained from 167 patients with acute encephalitis syndrome admitted to the Assam Medical College and Hospital in Dibrugarh, which administers to the health needs of ≥7 districts of Upper Assam and neighboring states of Arunachal Pradesh and Nagaland. Among the 167 patients, 124 (74.2%) were children ≤15 years of age.

Among the 103 serum samples, 80 were positive for immunoglobulin (Ig) M against JEV (IgM monoclonal antibody–capture ELISA; National Institute of Virology, Pune, India) and 12 (11.6%) were positive for IgM against WNV (IgM antigen–capture ELISA; Panbio, Sinnamon Park, Queensland, Australia). These samples were from persons in 4 districts in Assam (Dibrugarh, Golaghat, Sivasagar, and Tinsukia) and negative for IgM against JEV (Table). Follow-up was conducted for 9 patients; 3 died, and 1 was lost to follow-up.

Virus-neutralizing antibody titers against JEV and WNV were estimated in pig kidney epithelial cells by using JEV (isolate 733913) and WNV (isolate 68856) and a cytopathic-effect assay in 96-well tissue culture plates (6). Mouse polyclonal antibodies against JEV and WNV and nonimmune serum samples were included in the assay. Of 9 paired serum samples, 6 showed neutralizing antibody for WNV, of which 4 showed a 4-fold increase in antibody titer. The remaining 3 paired samples showed cross-reactivity with WNV (titer ≤80) and JEV (titer ≤40).

All 12 WNV-infected patients had high fever and headache. Convulsions (6 patients), altered sensorium (7 patients), vomiting (5 patients), and