Health developed and implemented strict plans to prevent the appearance of this disease in the Hajj (pilgrimage) period. Annually, 2-3 million people from all over the world convene in the holy city of Makkah in the Western Province of Saudi Arabia to perform Hajj, part of which involves slaughtering of hundreds of thousands of livestock animals. As part of active surveillance for viral hemorrhagic fever (VHF) in Makkah between 8 and 23 February 2001, the Hajj period of year 2001, all patients hospitalized with acute febrile illness were reviewed and clinically assessed by the author. The author identified 4 patients with typical acute VHF who were imprecisely diagnosed as acute hepatitis and encephalitis. Laboratory tests for RVF, Crimean-Congo hemorrhagic fever (CCHF), and dengue were negative in the 4 patients. Blood specimens were therefore sent to the Centres for Disease Control and Prevention (CDC) for viral culture and testing for other hemorrhagic fever viruses. A new flavivirus closely related to the tick-borne Kyasanur forest disease virus (KFDV) was isolated. This new flavivirus, however, was originally isolated in Jeddah, Saudi Arabia in 1995 from 6 patients with Dengue-like hemorrhagic fever.[1] These 6 patients were from Alkhumra district, south of Jeddah, Saudi Arabia. Therefore, the author in this article refers to this virus as Alkhumra virus (ALKV). The name 'Alkhurma' used by Charrel et al. and others is not accurate as Alkhurma refers to a small city, 200 km east of Makkah, which is different from Alkhumra district in Jeddah, from which the original cases were identified.[2] After its first identification in Jeddah, ALKV was not reported until its reappearance in Makkah in 2001, following which a total of 37 cases, 20 of them laboratory-confirmed, were identified over a 24-months period. This study describes the epidemiological, clinical, and laboratory features of this new VHF.

Patients and methods

After the identification of the first 4 cases of ALKV infection in Makkah in 2001, a case definition was formulated for surveillance of this disease in Saudi Arabia. ALKV infection was suspected if a patient had an acute febrile illness of at least 2 days duration, negative RVF, CCHF, and dengue confirmatory tests, and at least two of the following four clinical or laboratory categories: (1) at least three-fold elevation of alanine transferase (AlT), or aspartate transferase (AsT), or clinical jaundice; (2) features of encephalitis such as confusion, disorientation, drowsiness, coma, neck stiffness, hemiparesis, paraparesis, or convulsions; (3) hemorrhagic manifestations such as ecchymosis, purpura, petechiae, gastrointestinal bleeding (hematemesis, melena, hematochesia), epistaxis, bleeding from puncture sites, or menorrhagia; (4) platelets count $<100\times10^9$/l, or lactate dehydrogenase (LDH) or creatine phosphokinase (CK) enzyme >2 times upper normal level.

Blood specimens were collected from all patients with suspect ALKV infection and tested for RVF (antigen, immunoglobulin M [IgM], immunoglobulin G [IgG], reverse-transcription polymerase chain reaction [RT-PCR], and culture), ALKV (IgM, IgG, RT-PCR, and culture), CCHF (IgM, IgG, antigen), Dengue (IgM, IgG, RT-PCR), and West Nile encephalitis (IgM, antigen, RT-PCR). All diagnostic laboratory tests with the exception of viral cultures were performed at the Central Laboratory in Riyadh, Saudi Arabia in collaboration with the Centres for Disease Control and Prevention (CDC), Atlanta, U.S.A., using previously described methods.[3-5] Anti-ALKV IgM antibody titres were determined by IgM antibody-capture ELISA, with ALKV virus-infected cell slurry prepared as described.[3] Anti-ALKV IgG antibody titres were determined by using ALKV virus-infected cell antigens in an ELISA format as described.[3] RT-PCR assay was used for detection of Tick-borne encephalitis (TBE) complex virus RNA. Virus isolation, performed at the CDC, was achieved with Vero E6 cells and passage in suckling mice.

Patients data were prospectively collected on standardized data collection forms. Information collected included patient demographics, risk factors for VHF infection, clinical manifestations, laboratory results, and outcome. All patients received supportive care with intravenous fluids and, when indicated, ionotropic support, blood and fresh frozen plasma transfusion, mechanical ventilation, and anti-microbial therapy for secondary bacterial or fungal infections. No specific antiviral medication was used for therapy. In addition to standard precautions, all patients were kept in neutral-pressure single rooms under contact and droplet precautions.

Data analysis

The Statistical Package for Social Science (SPSS) program (Release 7.5.1, 1996) was used for data analysis. Comparison of proportions (categorical data) was by Fisher's exact test because of the small number of cases. Two-tailed *P* value of 0.05 or less was considered significant.

Results

From 8 February 2001 to 9 February 2003, a total of 37 cases fulfilled the case definition of ALKV infection, 20 cases of which were laboratory confirmed. Tests for RVF (antigen, IgM, IgG, RT-PCR, culture), CCHF (IgM, IgG, antigen), Dengue (IgM, IgG, RT-PCR), and West Nile encephalitis (IgM, antigen, RT-PCR) were negative in the 37 patients. All 37 suspect cases were reported from Makkah. All cases either lived or visited districts in Makkah that had livestock marketplaces or slaughterhouses (Al-Sharaye, Al-Kakia, Al-Shemaisi, Al-Moaysem). Table 1 summarizes the demographic characteristics and possible risk factors for acquiring the disease in the laboratory-confirmed cases. The mean (± standard deviation) age of patients was 33.4 (±13.6). No cases were reported among children less than 10 years of age. The disease predominantly affected male patients with a male to female ratio of 9:1. None of the patients who had contact with animals reported animal abortion, disease, or death.

Table 2 shows the clinical features and complications in 20 patients with laboratory-confirmed ALKV infection. Eleven (55%) patients had one or more of the following hemorrhagic manifestations: epistaxis (5 patients, 25%), ecchymoses (4 patients, 20%), petechiae (4 patients, 20%), hematemesis (4 patients, 20%), bleeding from gum (3 patients, 15%), bleeding from puncture sites (3 patients, 15%), melena (1 patient, 5%), and purpura (1 patient, 5%). Seven (35%) patients had one or more of the following central nervous system manifestations: confusion (5 patients, 25%), drowsiness (5 patients, 25%), coma (4 patients, 20%), convulsion (1 patient, 5%), and irritability (1 patient, 5%).

Table 3 shows the laboratory characteristics on admission, and Table 4, the mean values and range of laboratory results on admission, for 20 patients hospitalized with laboratory-confirmed ALKV infection. Table 5 presents the confirmatory laboratory test results and the time period in days between the onset of illness and the collection of blood specimens for testing for each patient with laboratory-confirmed ALKV infection. None of the patients had the virus isolated from Vero E6 cells alone nor did any patient have a positive RT-PCR alone. None of the patients with positive IgG or IgM antibodies had a positive culture or RT-PCR. The mean duration between the onset of illness and the collection of blood specimens from patients who were positive for only IgM was 5 days, and from patients who were positive for only IgG was 10 days. For patients who were positive for only IgG, convalescent sera to test for a rising IgG titre were not obtained because patients were either deceased or discharged from the hospital and not available for testing. However, these cases were considered to have confirmed ALKV infection because of their typical clinical

Table 1 Demographic characteristics and risk factors for 20 patients with laboratory-confirmed Alkhumra virus infection in Saudi Arabia

Variable	Number of patients (%)
Age, mean±SD, range (year)	33.4±13.6, 11- 60
Age groups (year)	
<10	0
10-<20	3 (15)
20-<30	5 (25)
30-<40	6 (30)
40-<50	3 (15)
50-<60	2 (10)
≥60	1 (5)
Gender	
Male	18 (90)
Female	2 (10)
Nationality	
Saudi Arabia	8 (40)
Bangladesh	5 (25)
Other nationalities[a]	7 (35)
Occupation	
Laborer	10 (50)
Office employee	3 (15)
Student	3 (15)
Butcher	1 (5)
Driver	1 (5)
Housewife	1 (5)
Soldier	1 (5)
Risk factors	
Living in or visiting districts that have livestock marketplaces or slaughterhouses in Makkah[b]	20 (100)
Mosquito bites only	9 (45)
Direct contact with sheep or goat only	3 (15)
Mosquito bites and direct contact with sheep or goat	5 (25)
Tick bites	0
Drinking raw milk	0
Contact with a patient with a similar illness	1 (5)
Number of patients reporting abortion storms, disease, or extraordinary deaths among animals	0

[a] Egypt, 2 patients; Yemen, 2 patients; Burma, 1 patient; Ethiopia, 1 patient; Pakistan, 1 patient.
[b] Al-Sharaye, Al-Kakia, Al-Shemaisi, Al-Moaysem.

Table 2 Clinical features and complications in 20 patients with laboratory-confirmed Alkhumra virus infection in Saudi Arabia

Variable	Number of patients (%)
Clinical features	
Fever	20 (100)
Headache	15 (75)
Malaise	15 (75)
Myalgia	15 (75)
Hemorrhagic manifestations	11 (55)
Nausea and vomiting	10 (50)
Arthralgia	9 (45)
Central nervous system manifestations	7 (35)
Backache	5 (25)
Chills	5 (25)
Diarrhoea	5 (20)
Anorexia	4 (20)
Rash	3 (15)
Abdominal pain	2 (10)
Sore throat	2 (10)
Conjunctival injection	1 (5)
Dizziness	1 (5)
Jaundice	1 (5)
Retro-orbital pain	1 (5)
Complications[a]	6 (30)
Admission to ICU	4 (20)
Encephalitis	4 (20)
Gastrointestinal bleeding	4 (20)
Disseminated intravascular coagulation	3 (15)
Hemorrhagic shock	2 (10)
Mechanical ventilation	2 (10)
Hemodialysis	0
Mortality	5 (25)

[a] Some patients had more than one complication, hence total number adds up to >6 patients.

presentation and risk factors, and the fact that the time between the onset of illness and the collection of blood specimens was at least 7 days, a period that may generally be enough for IgG to become detectable. In fact, 1 patient was positive for both IgM and IgG on day 5 of his illness (Table 5), confirming that IgG may develop as early as 5 days after the onset of illness. Table 6 shows the result of bivariate analysis of important clinical complications and laboratory derangements and mortality.

Discussion

Viruses that cause hemorrhagic fever belong to four

Table 3 Laboratory characteristics on admission of 20 patients hospitalized with laboratory-confirmed Alkhumra virus infection in Saudi Arabia

Variable	Number of patients (%)
AsT>40 U/l	20 (100)
AlT>40 U/l	16 (80)
AsT>200 U/l	13 (65)
AlT>200 U/l	9 (45)
Bilirubin>17 μmol/l	6 (30)
Hemoglobin<110 g/l	1 (5)
Platelets<100×10^9/l	15 (75)
INR>1.2	9 (45)
PTT>45 s	15 (75)
Leukopenia<3.0×10^9/l	13 (65)
Creatinine>150 μmol/l	5 (25)
High LDH>500 U/l	17 (85)
High CK>400 U/l	19 (95)

Abbreviations: AlT, alanine transferase; AsT, aspartate transferase; CK, creatine phosphokinase; INR, international normalized ratio; LDH, lactate dehydrogenase; RT-PCR, reverse transcription polymerase chain reaction; PTT, partial thromboplastin time; SD, standard deviation.

major groups: Arenaviridae, which include Lassa, Junin (Argentine hemorrhagic fever), Machupo (Bolivian hemorrhagic fever), Guanarito (Venezuelan hemorrhagic fever), and Sabia (Brazilian hemorrhagic

Table 4 Mean values and range of laboratory results on admission for 20 patients hospitalized with laboratory-confirmed Alkhumra virus infection in Saudi Arabia

Laboratory test	Mean±SD	Range	Normal
AsT (U/l)	849±1287	56-5486	0-35
AlT (U/l)	397±447	15-1447	0-35
Bilirubin (μmol/l)	24.6±24.8	2.2-88.9	5.1-17
Hemoglobin (g/l)	135±24	84-174	120-180
Leukocytes×10^9/l	3.9±4.2	1.5-20.6	3.8-10.8
Platelets×10^9/l	84±68	12-275	130-400
Creatinine (μmol/l)	148±102	53-328	<120
LDH (U/l)	2071±2769	244-10 128	100-250
CK (U/l)	3337±6625	231-28 338	10-200
INR	1.9±1.6	1.0-5.5	0.9-1.2
PTT (seconds)	68±29	40-122	25-35

Abbreviations: AlT, alanine transferase; AsT, aspartate transferase; CK, creatine phosphokinase; INR, international normalized ratio; LDH, lactate dehydrogenase; PTT, partial thromboplastin time; SD, standard deviation.

Table 5 Confirmatory laboratory results for 20 patients hospitalized with laboratory-confirmed Alkhumra virus infection in Saudi Arabia

Test(s)	Number of patients (%)	Days between onset of illness and collection of blood for each patient
IgM only	3 (15)	3, 5, 7
IgG only	8 (40)	7, 8, 8, 9, 10, 10, 13, 17
IgM and IgG	4 (20)	5, 7, 8, 17
Isolation from suckling mice only	3 (15)	5, 6, 7
Isolation of the virus from Vero E6 cells and from suckling mice	1 (5)	3
Isolation of the virus from Vero E6 cells and from suckling mice, and a positive RT-PCR	1 (5)	3

fever) viruses; Bunyaviridae, which include RVF, CCHF, and the hantaviruses causing hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome; Filoviridae, which include Marburg and Ebola viruses; and Flaviviridae, which include yellow fever, dengue, tick-borne encephalitis, Omsk hemorrhagic fever, Kyasanur forest disease, and Alkhumra viruses. Most hemorrhagic fever viruses are zoonotic, with the possible exception of the four dengue viruses, which may continually circulate among humans.[6] Many VHF viruses are vector-borne (RVF, CCHF, yellow fever, dengue, tick-borne encephalitis, Omsk hemorrhagic fever, Kyasanur forest disease, and Alkhumra viruses), while others are not (Lassa, Junin, Machupo, Guanarito, Sabia, Hantaviruses, Marburg, and Ebola viruses).

ALKV is the fourth VHF identified in Saudi Arabia; the other three are CCHF, dengue fever, and RVF. CCHF caused an outbreak in Makkah in 1990, after which the disease has not been reported in Saudi Arabia.[7] Dengue fever caused an outbreak in Jeddah, Western Province, in 1994. Few more cases have since been sporadically reported from Jeddah.[8] Three of the four VHF diseases identified in Saudi Arabia, namely ALKV, CCHF, and Dengue, are thus confined to Makkah and Jeddah which are 80 km apart in the Western Province. The occurrence of ALKV and CCHF in these two cities is likely related to the importation of large numbers of livestock into Makkah city through the seaport, Jeddah, for the Hajj season. On the other hand, RVF the fourth VHF identified in Saudi Arabia, caused a major epidemic in 2000-2001 in three different areas in the southwest of Saudi Arabia, namely Jizan, Asir, and Alqunfuda, that are far from Makkah city.[9]

The epidemiological, clinical, and laboratory characteristics of ALKV are similar to RVF infection.[9] Both diseases seem to be transmitted to humans by the mosquito bites and/or direct contact with infected sheep and goat. No confirmed cases of either ALKV or RVF infection were reported in children less than 10 years of age. Men were predominantly affected in the two diseases largely because of animal-related occupation. Unlike RVF,

Table 6 Mortality in those with, vs. those without, selected complications in 20 patients with laboratory-confirmed Alkhumra virus infection in Saudi Arabia

Complication	Patients with the complication			Patients without the complication			*P*
	Total	Died	%	Total	Died	%	
Bleeding manifestations	11	4	36.4	9	1	11.1	0.32
CNS manifestations	7	5	71.4	13	0	0	< 0.01
Creatinine > 150 μmol/l	5	3	60	15	2	13.3	0.07
AsT > 200 U/l	13	5	38.5	7	0	0	0.11
AlT > 200 U/l	9	4	44.4	11	1	9.1	0.13
Platelets < 100×10^9/l	15	5	33.3	5	0	0	0.27
LDH > 500 U/l	17	5	29.4	3	0	0	0.54
CK > 400 U/l	19	5	26.3	1	0	0	1.0
Leukocytes < 3×10^9/l	13	2	15.4	7	3	42.9	0.29
Bilirubin > 17 μmol/l	6	4	66.7	14	1	7.1	0.01

Abbreviations: AlT, alanine transferase; AsT, aspartate transferase; CNS, central nervous system; CK, creatine phosphokinase; LDH, lactate dehydrogenase.

animal disease, death, and frequent abortion, characteristic features and usually the first signs of RVF epidemic, were not observed with ALKV infection. ALKV-infected cases described herein were reported from Makkah city only, whereas, RVF epidemic involved three regions far from this city. Contact with animals in the case of ALKV infection occurred in slaughterhouses and livestock marketplaces, whereas in the case of RVF, it occurred in the field. Clinical manifestations of both diseases are similar with a few noteworthy differences. Hemorrhagic manifestations (55 vs. 7%), thrombocytopenia (75 vs. 38%), and elevated creatine phosphokinase (CK) enzyme (95 vs. 27%) are more commonly observed with ALKV than with RVF infection. Case fatality of ALKV is higher than that of RVF infection in hospitalized patients (25 vs. 14%, respectively). Visual complications and severe hemolytic anemia (hemoglobin <80 g/l) noted in 1.5 and 15% of RVF patients, respectively, are not observed with ALKV infection. The mean AsT (1526 vs. 849 U/l) and AlT (1151 vs. 397 U/l) levels are higher in RVF than in ALKV infection. The frequency of renal impairment was similar in both diseases (28% for RVF vs. 25% for ALKV).

The complete genome sequence of ALKV has been determined.[2] The virus is composed of 10 248 nucleotides encoding for a single 3416 amino acid polyprotein. Independent analyses of the complete polyprotein and the envelope protein provided genetic and phylogenetic evidence that ALKV belongs to the tick-borne flavivirus group. Analysis of structural genes, genetic distances, and evolutionary relationship indicates that ALKV and KFDV are derived from a common phylogenetic ancestor and constitute two distinct genetic subtypes of the same virus species.[2]

KFDV, first recognized in 1957, causes acute VHF principally in the Shimoga and Kanara district of Karnataka, India.[10,11] The disease is common in young adults exposed during the dry season in the forest. Rodents, shrews, and monkeys are the reservoirs of this virus. It is transmitted by the bite of infective ticks (*Haemaphysalis spinigera*), especially nymphal ticks, and less commonly by direct contact with infected rodents. Human to human transmission has not been reported. After an incubation period of 3-8 days the disease manifests with sudden onset of fever and severe headache, followed by back pain, severe pain in the extremities, prostration, relative bradycardia, lymphadenopathy, conjunctival injection, petechiae, and other hemorrhagic signs. After this initial stage, a biphasic course with mild meningoencephalitis and fever developing after an afebrile period of 1-2 weeks is common. A small proportion of patients develop coma or bronchopneumonia prior to death. The case fatality from KFDV is 2-10%.[10,11] Compared to KFDV, this study suggests that ALKV is transmitted to humans by mosquito bites or direct contact with infected sheep or goat. Unlike KFDV, ticks do not seem to be important in the transmission of ALKV to humans. The role of ticks and rodents in the transmission of ALKV among livestock, however, remain to be elucidated. It is not yet clear whether transmission of ALKV from animals to humans occurs during acute clinical or subclinical infections or from asymptomatic carriers among sheep and goat. Compared to KFDV, the case fatality from ALKV infection is relatively high (2-10 vs. 25%, respectively). The presence of central nervous system manifestations, or elevated serum bilirubin in patients with confirmed ALKV infection was significantly associated with higher mortality. Bivariate analysis of other complications and laboratory derangement showed no statistically significant effect on outcome, perhaps due to the limited number of patients.

In conclusion, ALKV is a novel hemorrhagic fever flavivirus that has been isolated, until this writing, only from patients from Makkah and Jeddah in the Western province of Saudi Arabia. Acute febrile flu-like illness with hepatitis, hemorrhagic manifestations, and less commonly encephalitis are the main clinical features. The role of arthropods such as ticks and mosquitoes, and animals such as sheep, goat, and rodents in the transmission and maintenance of the virus remains to be elucidated.

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