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REVIEW

Alkhumra hemorrhagic fever virus infection

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Abstract

Objectives This review article summarizes what has been published on Alkhumra hemorrhagic fever virus (AHFV), a novel flavivirus that was discovered in Saudi Arabia in 1995.

Methods PubMed was used to search for studies published from January 1995 to June 2019 using the key words Alkhumra virus, Alkhurma virus, novel flavivirus, and tick-borne encephalitis virus. Additionally, records of the Saudi Ministry of Health were reviewed.

Results Thirty-two articles on AHFV were identified. Acute febrile flu-like illness, hepatitis, hemorrhagic manifestations, and, less commonly, encephalitis are the main clinical features. The virus seems to be transmitted from livestock animals to humans by direct contact with these animals or their raw meat, or perhaps by tick or mosquito bites. The ability of ticks and mosquitoes to serve as vectors for AHFV needs to be confirmed by biological studies. The exact role of animals such as sheep, goats, camels, and other mammals in the transmission and maintenance of the virus remains to be elucidated. Preventive measures require an interdisciplinary approach involving the human and veterinary health sectors, the municipality, the ministry of agriculture, the vector control sector, and academic and research institutes.

Conclusions AHFV has been well characterized; nevertheless, some aspects remain to be elucidated.

Introduction

A new virus causing hemorrhagic fever was reported in the Kingdom of Saudi Arabia (KSA) in 1995 [1]. A novel flavivirus was isolated and identified as the cause of this infection [1, 2]. The virus was named "Alkhumra hemorrhagic fever virus" (AHFV) after the Alkhumra district in Jeddah, where the first cases of infection with this virus were described [3]. From 2001 to 2003, 20 confirmed cases of AHFV infection were reported in Makkah, 75 km from its origin [3]. From 2003 to 2007, eight sporadic cases of AHFV infection were confirmed in the Najran region in southwestern KSA, bordering Yemen [4], followed by an outbreak in this region with 70 cases confirmed from 2008 to 2009 [4]. Subsequently, sporadic cases continued to be reported in Jeddah, Makkah, and Najran as well as in new southwestern regions, namely, Taif, Alqunfuda, and Jazan [5, 6]. Table 1 summarizes all AHFV cases reported in the KSA in the period 1995–2017.

Since 2010, evidence has been accumulating that the virus in fact exists beyond its original boundaries. In 2010, two Italian patients were confirmed to be infected with AHFV after travelling to southern Egypt [7]. Two more confirmed cases involving tourists coming from Egypt were also reported in Italy [8, 9]. In addition, results of a serosurveillance study of AHFV antibodies in humans in Djibouti [10] confirmed the presence of AHFV-neutralizing antibodies in the serum of a 13-year-old female child who lived near a slaughterhouse. Two years later, Horton *et al*. [11] showed that two ticks collected from cattle in Djibouti were infected with AHFV. These reports suggest that the geographic distribution of AHFV extends beyond the KSA.

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Table 1 Confirmed cases of Alkhumra hemorrhagic fever virus infection in Saudi Arabia (1995–2017)

Locality	Date	No. of confirmed cases	No. of fatal cases (%)	References
Jeddah (Alkhumra district)	Nov.–Dec. 1995	10	2 (20)	Zaki [2]
Makkah	8 Feb., 2001–9 Feb., 2003	20	5 (25)	Madani [3]
Najran	1 Aug., 2003–31 Dec., 2009	78	1 (1.3)	Madani et al. [4]
Najran	1 Jan., 2006–30 Apr., 2009	28	0	Alzahrani et al. [5]
Jeddah, Makkah, Jazan, Najran, Taif	Jan., 2009–Dec., 2011	233	2 (0.43)	Memish et al. [6]
Jeddah, Makkah, Jazan, Najran, Taif	2012–2015	248	4 (1.6)	MOH records
Algunfuda	April, 2017	3	0	MOH records
Total	Nov., 1995–April, 2017	620	14 (2.3)	

MOH Ministry of Health, Saudi Arabia

AHFV classification

AHFV is a member of the tick-borne encephalitis group, belonging to the genus *Flavivirus* of the family *Flaviviridae* [12–14]. AHFV is most closely related to Kyasanur Forest disease virus (KFDV). Table 2 shows a comparison of the characteristics of AHFV and KFDV. In many scientific publications, Alkhu**mr**a virus was mistakenly named 'Alkhu**rm**a' virus as a result of a typographical error in earlier publications [15]. The incorrect name, 'Alkhurma', is actually a small city 280 kilometers from Alkhumra district in Jeddah, and it is not associated with this virus in any way. The name was corrected by the International Committee on Taxonomy of Viruses (ICTV)

Table 2 Characteristics of Alkhumra hemorrhagic fever virus (AHFV) and Kyasanur Forest disease virus (KFDV)

Characteristic	AHFV	KFDV
Date discovered	1995	1957
Genus	*Flavivirus*	*Flavivirus*
Reservoir	Unknown	Monkeys
Vector	Unknown (Ticks?, mosquitoes?)	Ticks
Transmission	Direct contact with livestock animals or their blood or meat Tick bites? (*Ornithodoros savignyi* and *Hyalomma dromedarii* ticks) Mosquito bites?	Tick bites (*Haemaphysalis* ticks) Contact with monkeys
Ecosystem	Urban and rural	Forest
Geographic distribution	Southwestern Saudi Arabia, Southern Egypt, and possibly other African countries	Endemic to South India; first reported from the Kyasanur Forest of Karnataka in India
Incubation period (days)	Unknown (2–4?)	2–8
Case fatality rate	‹ 1– 25%	3–10%
Diagnosis	Molecular detection by PCR and sequencing Virus isolation Serological identification: serum neutralization test, and ELISA	Molecular detection by PCR and sequencing Virus isolation Serological identification: serum neutralization test, and ELISA
Laboratory biosafety level	3–4	3–4
Treatment	No specific treatment Supportive care	No specific treatment Supportive care
Prevention	No vaccine is available Avoidance of direct contact with live animals or their byproducts without personal protective equipment Use of mosquito repellants Avoidance of contact with ticks, where the disease is endemic	A vaccine is used in endemic areas of India Control of ticks Wearing protective clothes where ticks are endemic and avoidance of contact with monkeys

ELISA enzyme-linked immunosorbent assay

in 2011, and the name 'Alkhumra' was approved as the correct name of the virus [14]. However, some authors continue to use the wrong name, 'Alkhurma', in their publications.

Studies on AHFV characteristics

Molecular characteristics

Several studies have been conducted to study the molecular characteristics of AHFV isolates. Charrel *et al*. [16] reported the sequence of the complete open reading frame (ORF) of the prototype strain 1176, which was recovered from a patient's blood in 1995. The complete sequence of that strain was 10,248 nucleotides long and included a single ORF that encoded a 3,416 amino-acid-polyprotein (GenBank accession no. AF331718). Analysis of that polyprotein, the envelope protein, structural genes, genetic distances, and evolutionary relationships indicated that AHFV belongs to the group of tick-borne flaviviruses and is most closely related to the KFDV. It was concluded that AHFV and KFDV are likely to be descendants from a common ancestor and represent two genetic subtypes of the same virus species. In another report, Charrel *et al*. [17] studied the genetic heterogeneity of 11 AHFV isolates from hospitalized patients in Makkah and Jeddah, KSA, between 1994 and 1999. In that study, detailed genetic analysis of partial envelope, NS3, and NS5 gene sequences for each isolate showed low diversity, indicating slow microevolution. The authors suggested that the ancestral lineage of AHFV and KFDV diverged 66–177 years ago, and that the diversity observed among AHFV strains was a result of an evolution period ranging from 4 to 72 years. In 2007, Charrel *et al*. [18] reported sequence analysis of the genome of an AHFV strain isolated from an *Ornithodoros savignyi* tick, sand tampan (tick JE7), collected from southeast of Jeddah, KSA. The sequence analysis results revealed 99.7% nucleotide sequence identity to AHFV strain 1176 (GenBank accession no. AF331718) in the homologous region of the envelope gene.

In 2014, Madani *et al*. [19] reported the whole genome sequence of an AHFV strain isolated from a patient in Najran and compared it with the sequences of 18 other AHFV strains previously isolated from Jeddah and Makkah, and with dengue virus (DENV), KFDV, Langat virus, Omsk hemorrhagic fever virus (OHFV), and tick-borne encephalitis virus (TBEV). The RNA of this strain had 10,546 nucleotides encoding a single 5,410-amino-acid polyprotein, whereas the previously reported AHFV strains were composed of 10,685–10,749 nucleotides. Phylogenetic analysis of these viruses (Fig. 1) showed that the Najran strain shared 99% sequence identity with the other 18 AHFV strains, whereas the KFDV, Langat virus, TBEV, and OHFV isolates formed a distinct cluster with more sequence variability. The most important variations were observed in the core protein and NS4a gene sequences of two AHFV isolates. It was concluded that the observed difference in the number of nucleotides and phylogenetic position between the Najran strain and the other AHFV strains could have resulted from recombination of circulating virus strains.

Growth of AHFV in mammalian cell lines

Madani *et al*. [20] investigated the ability of AHFV to grow and propagate in four commonly used mammalian cell culture lines, namely, LLC-MK2, MDCK, Vero, and HEp-2, and determined the characteristics of viral growth curve in each. Mean titers were calculated and expressed as the median tissue culture infective dose per mL ($TCID_{50}$/mL). AHFV grew to different titers in the four cell lines, but LLC-MK2 cells were superior to the others for AHFV culture.

Growth of AHFV in mosquito cell culture

Madani *et al*. [21] investigated the ability of AHFV to replicate in mosquito cells. Serum and plasma from seven patients with clinically suspected acute AHFV infection were inoculated onto *Aedes albopictus* mosquito cells (C6/36). The cells were subsequently examined using an AHFV-RNA-specific reverse transcription polymerase chain reaction (RT-PCR) and indirect immunofluorescence assay (IFA) using AHFV-specific polyclonal antibodies. LLC-MK2 cells were employed to titrate the isolated virus. Five of the seven specimens were RT-PCR and culture positive, with cytopathic effects evident on the third day post-inoculation and syncytia eventually appearing on the eighth day post-inoculation. The presence of AHFV RNA in the cell culture was confirmed by RT-PCR and IFA, with a viral titer of 3.2×10^6 $TCID_{50}$/mL. Three additional viral passages were successfully made in C6/36 cells. This successful propagation of ALKV in C6/36 mosquito cells to a high titer supported the possibility of mosquito-borne transmission of this virus from animals to humans, as was also suggested previously by epidemiological data. However, biological studies are still needed to confirm that mosquitoes actually serve as vectors of this presumably tick-borne virus.

Growth of AHFV in tick cell lines

Madani *et al*. [22], examined three tick lines for propagation of AHFV. The first was derived from the soft tick *Ornithodoros moubata* (OME/CTVM 24), and the other two were derived from hard ticks: one from *Rhipicephalus*

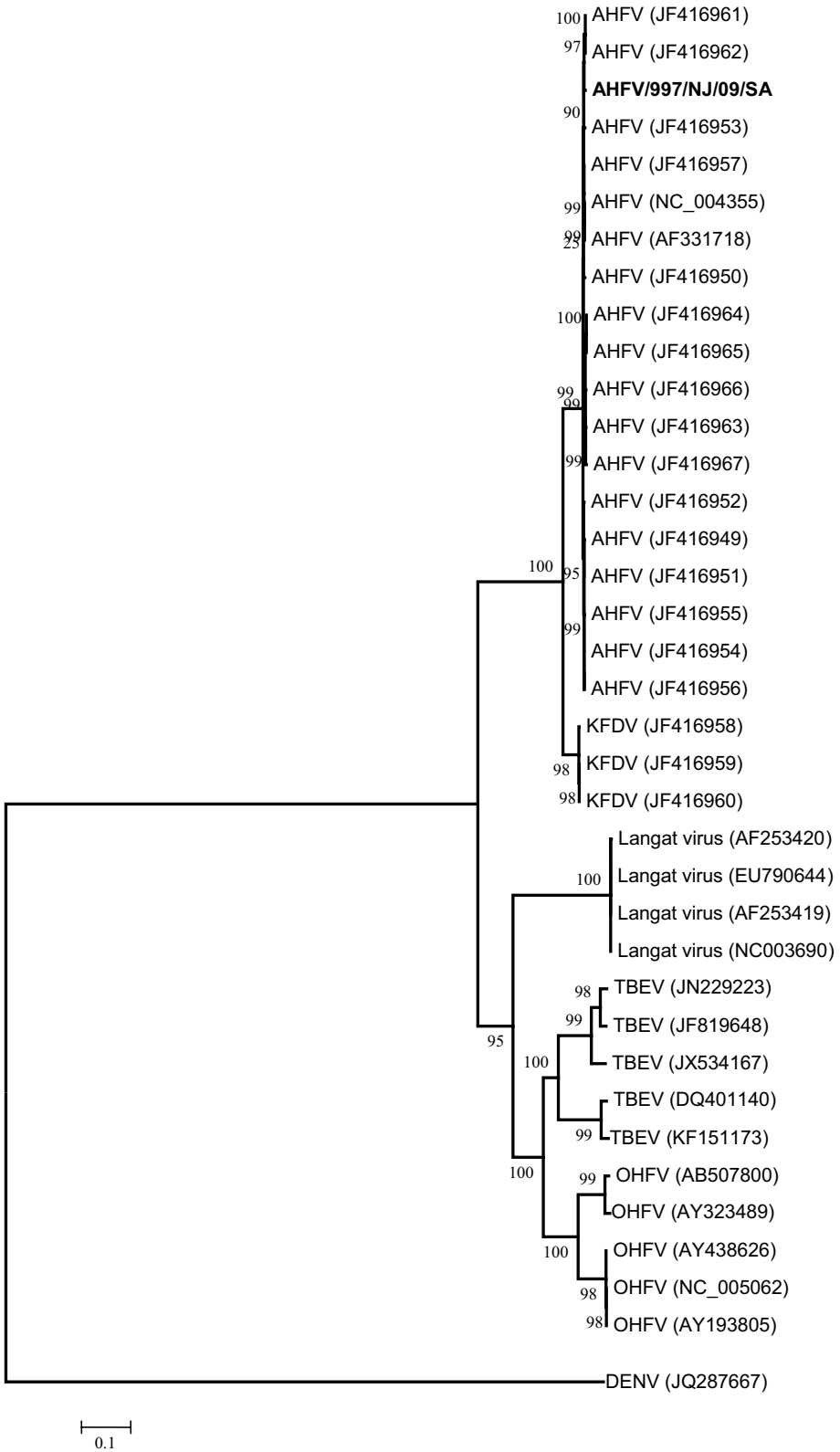

Fig. 1 A phylogenetic tree based on the complete genome sequences of Alkhumra hemorrhagic fever virus (AHFV) strain AHFV/997/NJ/09/SA (in bold), eighteen previously isolated AHFV strains, one strain of dengue virus (DENV), three strains of Kyasanur Forest disease virus (KFDV), four strains of Langat virus, five strains of Omsk hemorrhagic fever virus (OHFV), and five strains of tick-borne encephalitis virus (TBEV). Each isolate is indicated by a GenBank accession number [19].

appendiculatus (RAE/CTVM 1) and the other from *Hyalomma anatolicum* (HAE/CTVM 9). The OME/CTVM 24 and RAE/CTVM 1 cell lines both supported the growth of AHFV to high titers, whereas the HAE/CTVM 9 cell line failed to support propagation of the virus. It was concluded from this study that ticks might be important in the

epidemiology of AHFV. Since previous epidemiological studies indicated that mosquitoes were more likely than ticks to be vectors transmitting the virus from animals to humans, the role of ticks as reservoirs or vectors is not yet clear. The exact roles of ticks and mosquitoes in the epidemiology and transmission of AHFV in nature remain to be elucidated.

Propagation of AHFV in rodents

Adult and baby mice were used for primary isolation of AHFV. Mice were confirmed to be highly susceptible to infection with AHFV [2]. AHFV was successfully propagated to high titers in the brains of newborn Wistar rats [23]. The inoculated rats developed neurological manifestations in the form of irritability, tremor, opisthotonos, convulsions, and spastic paresis ascending from the hind limbs to involve the whole body. All infected rats died 3–7 days after inoculation with AHFV. Postmortem histopathological examination of the brains confirmed meningoencephalitis, and the virus was isolated from the brains of all infected rats at high titers ($10^{9.4}$ median rat lethal dose per milliliter [RLD_{50}/ml]). In another study, Dodd *et al.* infected C57BL/6J mice with AHFV and reported that the syndrome in mice was similar to the disease seen in human cases [24].

Hemagglutination of red blood cells (RBCs) by AHFV

Madani *et al*. investigated the ability of AHFV to agglutinate RBCs of mammalian and avian species, including human group O, camel, chicken, cow, duck, goat, goose, guinea pig, mouse, rabbit, rat, sheep, and turkey, with and without trypsin treatment [25]. AHFV failed to agglutinate RBCs of any of the examined species if the RBCs were not pre-treated with trypsin. After treatment with trypsin, AHFV agglutinated RBCs of five species, namely, goose, human group O, rat, guinea pig, and mouse, in descending order of sensitivity. The ability of this virus to induce hemagglutination after treatment with trypsin has potential for use as a serological and functional test for the diagnosis of AHFV.

Thermal inactivation of AHFV

Madani *et al*. studied the thermal stability of AHFV at different temperatures for different periods [26]. Titers of the residual virus were determined in units of median tissue culture infective dose ($TCID_{50}$/mL), and the rate of loss of infectivity was determined at various temperatures. The infectivity of AHFV was completely lost when the virus was heated at 60°C for 3 minutes or at 56°C for 30 minutes, but the virus maintained 33.2 % of its titer when heated at 45°C for 60 minutes and 32 % of its titer when heated at 50°C for 60 minutes. Thus, AHFV was confirmed to be thermo-labile, and its thermal inactivation followed first-order kinetics.

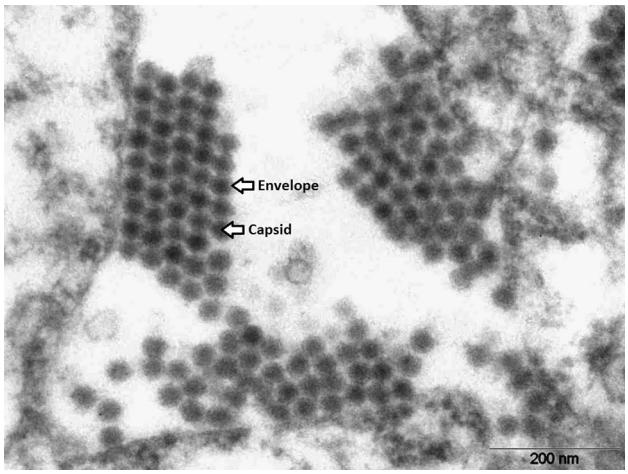

Fig. 2 Electron micrograph showing arrays of AHFV particles in a section of rat brain inoculated with AHFV, with a dark core representing the capsid and a translucent envelope [27]

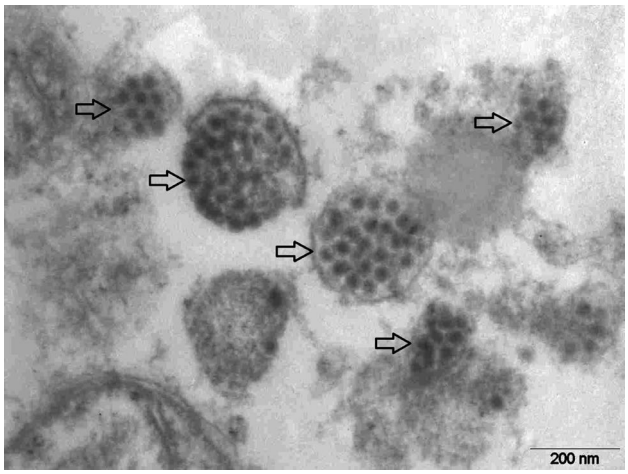

Fig. 3 Electron micrograph showing the presence of numerous virus particles (arrows) in intact and ruptured cytoplasmic vesicles in a section from AHFV-inoculated LLC-MK2 cells [27].

Electron microscopy (EM) of AHFV

EM images of the AHFV have been published recently [27]. The virus particles have a dark core (capsid) and a translucent envelope (Fig. 2). Numerous virus particles within intact and ruptured cytoplasmic vesicles were observed in AHFV-inoculated LLC-MK2 cells (Fig. 3). The mean

diameter of the virus is 40.59 ± 1.29 nm, which is within the diameter range (40 -70 nm) of members of the genus *Flavivirus* reported by the ICTV [12]. Other EM studies of AHFV-infected cells *in vivo* (rat brain cells) and *in vitro* (LLC-MK2 cell line) showed that the virus produces a severe cytopathic effect in both types of cells [28]. In those studies, multiplication of the virus was observed in the cytoplasm and involved the rough endoplasmic reticulum (RER), which had undergone hypertrophy and dilatation of its cisternae. The nucleus and mitochondria were devoid of virus particles, and the mitochondria showed swelling in their lumen, suggesting their involvement in supplying the virus with its requirements [28]. Cell lysis and apoptosis were seen in the infected rat brain as well as LLC-MK2 cells [28]. Precisely arranged virus particle arrays were seen, predominantly in the infected rat brain cells. These features of intracellular growth of AHFV are similar to those of other members of the genus *Flavivirus*.

Clinical manifestations and management of AHFV infection in humans

The spectrum of clinical manifestations of AHFV infection ranges from subclinical or mild infection to self-limited dengue-like illness to severe rapidly fatal infection. Clinical and laboratory manifestations usually include fever, headache, retro-orbital pain, arthralgia, myalgia, anorexia, vomiting, leukopenia, thrombocytopenia, and elevated liver enzymes, creatine kinase, and lactate dehydrogenase. The frequency of various clinical and laboratory features of AHFV infection were described in the outbreaks that occurred in Makkah, Jeddah, and Najran, KSA [3–7], and included fever (100%), headache (85.9%), malaise (85.9%), arthralgia (83.3%), anorexia (82.1%), myalgia (82.1%), backache (71.8%), nausea and vomiting (71.8%), chills (60.3%), retro-orbital pain (55.1%), diarrhea (51.3%), abdominal pain (48.7%), hemorrhagic manifestations (25.6%), central nervous system manifestations (23.1%), leucopenia (87.7%), elevated liver enzymes (85.7%), prolonged partial thromboplastin time (52.6%), thrombocytopenia (46.2%), elevated creatine kinase level (45.7%), and elevated lactate dehydrogenase (25.0%). In addition to these typical manifestations, Ravanini *et al.* reported a case of AHFV in which the patient presented with atypical manifestations in the form of rhabdomyolysis and severe muscle weakness [8]. Memish *et al*. reported gastroenteritis-like illness as a prominent clinical feature in all of the AHFV cases they studied [6].

There is no specific antiviral agent to treat AHFV. In previous reports, patients received supportive care, including intravenous fluids, and when indicated, ionotropic support, blood and fresh frozen plasma transfusions, mechanical ventilation, and anti-microbial therapy for secondary infections. Since there is no evidence of human-to-human transmission of AHFV, hospitalized patients need only to be under standard infection control precautions.

Laboratory diagnosis

Both immunological and molecular techniques have been employed in the early laboratory diagnosis of AHFV [2, 4]. The flavivirus-specific monoclonal antibody 4G2 has been used successfully for detection by immunofluorescence assay (IFA). Polymerase chain reaction (PCR) amplification of a 220-bp genome fragment that exhibits 89% nucleotide sequence identity to the KFDV NS5 is also used for the identification of AHFV. Madani *et al*., showed that real-time PCR was more sensitive than viral culture [29]. Madani *et al*. [30] compared the efficiency of detection of AHFV RNA in serum, plasma, and the buffy coat of seven patients with suspected AHFV infection in the first seven days of illness, using standard real-time RT-PCR. AHFV RNA was detected directly by RT-PCR in the buffy coat in five AHFV culture-positive cases (100%), while viral RNA was detected in plasma or serum in only four (80%) of the five patients. Thus, higher sensitivity was achieved by using the buffy coat than by using plasma or serum samples for the detection of AHFV RNA by real-time RT-PCR.

Serosurveillance for AHFV antibodies in humans

Serosurveillance data on AHFV antibodies in humans in the KSA or elsewhere are scarce. Serological surveillance conducted in the KSA showed that none of the samples were positive for AHFV IgM antibodies, and the prevalence of AHFV-IgG was 1.3% among 1024 soldiers from different regions of the KSA, indicating a wider geographic distribution than previously believed [31]. The distribution of the positive sera by locality showed that 61.5% were from Tabouk (Northern region), 23.1% from the Eastern region, 7.7% from Asir (South Western region), and 7.7% from Jazan (South Western region). Interestingly, the AHFV-IgG-positive individuals were from areas where no AHFV cases had been reported, suggesting that they had had a mild or subclinical infection with AHFV in the past. In another serosurveillance study in Djbouti [10] involving 893 individuals, AHFV-neutralizing antibodies were detected in a 13-year-old girl who lived near a slaughterhouse.

Transmission

Several studies have addressed the possible mode of transmission and risk factors for infection with AHFV in KSA, including the age, gender, occupation of patients, seasonality

of the disease, exposure to live animals and their by-products such as raw milk, raw meat, secretions and blood, and exposure to ticks and mosquitoes. The very first eight cases of this infection were described by Qattan *et al*., [1], who reported that all affected patients had contact with sheep and that six of them were butchers by profession. Madani *et al*. [3, 4, 21] reported that AHFV was likely transmitted from livestock animals or their products to humans by direct contact, and perhaps also by mosquito bites, and suggested that ticks may well be reservoirs of the virus or perhaps vectors for transmission. Mosquitoes were epidemiologically suspected to be important vectors for transmitting AHVF because of the common observation that many patients acquired the infection without having direct contact with animals or their products and without having tick bites. All such patients had exposure to mosquitoes and lived or worked in close proximity to livestock animals or livestock marketplaces, suggesting that mosquitoes were likely the vectors transmitting the virus from the animals in the vicinity to the patients [3, 4]. Alzahrani *et al*. [5] reported that, after multivariate modeling, animal contact, neighboring farms, and tick bites were significant risk factors for infection with AHFV and that drinking raw milk and handling raw meat products were not significant risk factors.

Human-to-human transmission of AHFV in the community or healthcare settings has not been reported. However, it has been observed that many cases of AHFV infection have occurred as clusters in the same families. Madani *et al*. [4], reported that 32.1% of their investigated cases were part of clusters. Alzahrani *et al*. [5] also reported that 71.4% of the cases occurred in clusters of two to seven members of the same family. Since droplets, airborne transmission, or contact with patients or their body fluids are not known to be modes of transmission of AHFV, clustering of cases is likely to be due to exposure to a common source of infection or vectors such as mosquitoes or ticks. Only a minority of patients who denied direct contact with animals recalled tick bites as a potential source of infection, whereas many of them recalled mosquito bites. Whether mosquitoes can be vectors for transmission of this presumably tick-borne virus remains to be proven, however [4, 21]. The fact that AHFV was successfully propagated in mosquito cell lines may support the hypothesis that mosquitoes could be vectors for transmitting this virus [4, 21, 22]. On two occasions, different species of mosquitoes were collected and examined for AHFV. In the first case, Mahdi *et al*. [32] failed to isolate AHFV from 237 mosquitoes collected from Najran. In the second case, Madani *et al*. (unpublished data, 2017) examined more than 1000 mosquitoes of various species from the Alkhumra area (south of Jeddah) and Najran by rtRT-PCR. All of the collected mosquitoes were negative for AHFV RNA.

Memish *et al*. [6] also reported that the transmission of AHFV from animals and their by-products to humans was by direct contact and proposed that ticks could also be involved in the transmission, especially in agricultural regions where the disease had been reported. In that context, seven reports were published examining ticks in relation to the transmission of AHFV between 2007 and 2017. The first report was by Charrel *et al*. [18], who detected AHFV RNA in the camel tick *Ornithodoros savignyi* (sand tampan) from Jeddah, KSA. The second report was by Mahdi *et al*. [32], who isolated AHFV from *Hyalomma dromedarii* and from sand tampan ticks (*O. savignyi*) collected in Najran, KSA. The third report was by Carletti *et al*. [7], who reported that two Italian tourists returning from Egypt developed clinical AHFV infection and that one of them had a bite on the foot by an arthropod described as tick-shaped, although not formally identified. The fourth and fifth reports were by Ravanini *et al*. [8] and Musso *et al*. [9], respectively, who also reported AHFV cases involving Italian tourists returning from Egypt who were exposed to an unidentified arthropod that was described as tick-shaped. The sixth report was by Horton *et al*. [11], who reported the detection of AHFV RNA in *Amblyomma lepidum* ticks from imported cattle in Djibouti. The seventh report was in April 2017, when AHFV RNA was detected in three patients who were also bitten by unidentified ticks in Algunfuda, KSA (personal communication, Ministry of Health, Jeddah, KSA). Therefore, from the foregoing reports, ticks may indeed play an important role in the epidemiology of AHFV, possibly as vectors and/or reservoirs of the virus.

No data reporting natural overt AHFV infection of any wild or domestic animal species have been published to date, and no serological surveys have been conducted to detect AHFV antibodies in animals. Examination of sera from camels and sheep by real time rtRT-PCR did not reveal the presence of AHFV RNA (Madani *et al*., unpublished data). Figure 4 shows a proposal of how AHFV might circulate between possible animal reservoirs and vectors and be transmitted to humans.

From the aforementioned studies, the role of mosquitoes and ticks could not be conclusively determined. Therefore, more research and proper vector capability studies are required to define the exact role of mosquitoes and ticks in the transmission cycle of AHFV to humans and animals. It is essential to establish biosafety level 3 laboratories for handling the AHFV research, and also animal biosafety level 3 laboratories for studies involving animal experimentation with AHFV and other hemorrhagic fever viruses. Such laboratories will enable establishment of animal models for studies on AHFV, including vaccine development and testing.

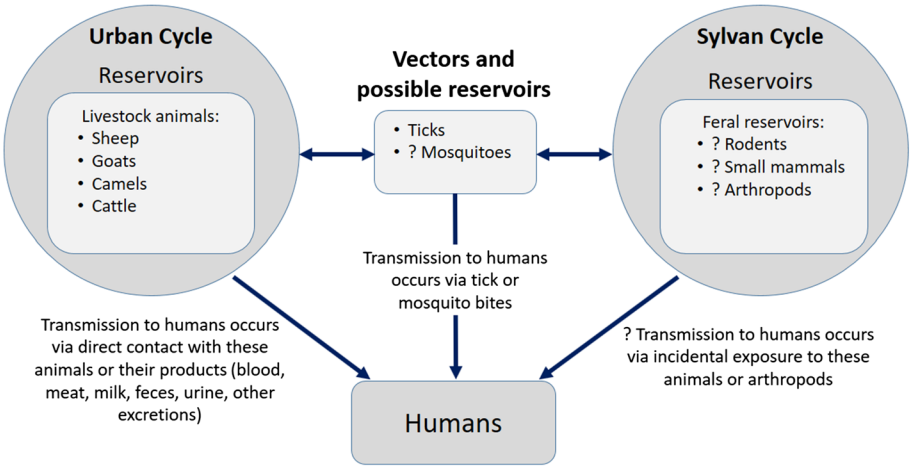

Fig. 4 Possible mode of circulation of AHFV between potential animal reservoirs and vectors and its transmission to humans

Epidemiology of AHFV infection

Infection with AHFV occurs year-round with peaks in the summer [4]. The peaking of AHFV outbreaks in the summer may be due to the optimal breeding conditions for the arthropod vectors (mosquitoes and ticks). This holds true in the epidemiology of arboviral diseases in general, where optimal meteorological conditions are conducive to interactions between vectors and their hosts.

AHFV infection is most commonly reported among young people between 10 and 40 years of age. Madani *et al.* [4] reported that about 80% of patients were 10 to 40 years old. Infection is rare among children younger than 10 years of age [4, 6]. The clinical, laboratory, and epidemiological features of AHFV infection described in Najran were similar to those observed in Makkah [3, 4]. Important differences between the Najran and Makkah infections included a larger proportion of females (38% versus 10%), more children younger than 10 years of age (five patients versus none), more patients with a family history of AHFV-like illness (25% versus 5%), and notably lower mortality (1.3% versus 25%) reported in Najran. These differences were attributed to differences in the lifestyle of the people inhabiting these two regions. Najran is a rural area where extended families usually live together in large houses with back gardens to keep sheep and goats. All family members, including women and older children, are usually involved in feeding, milking, slaughtering, and butchering the animals and cleaning the premises. The markedly low mortality reported in the Najran outbreak was perhaps attributed to an established endemicity of AHFV in this region, leading to some herd immunity among humans as a result of recurrent exposure to the virus [4].

Prevention and control

Based on the currently available epidemiological data related to the possible modes of transmission of AHFV, infection with this virus can be prevented by avoiding direct contact with livestock animals and their products and avoiding tick and mosquito bites. When human infection occurs in a specific slaughterhouse or animal marketplace, the health and veterinary medicine authorities as well as the municipality in the locality should be notified immediately to assess the risk factors for infection and to educate all workers about measures to protect themselves, including, most importantly, wearing personal protective equipment when handling livestock animals or any of their products. Measures to control ticks and mosquitoes should also be undertaken by the concerned authorities, usually the municipality. It is important to establish a national program to control ticks and mosquitoes. Many countries have established well-equipped national organizations to control ticks and have succeeded in minimizing tick-borne diseases [33]. Veterinary assessment of livestock animals is recommended to determine whether the animals have any clinical manifestations that could be attributed to AHFV infection. Collection of blood, urine, and fecal samples from these animals for AHFV PCR and, if possible, viral culture, and collection of sera for AHFV IgM and IgG serological testing are recommended. To date, there is no human or animal vaccine available to prevent AHFV. Thus, preventive measures require interdisciplinary teamwork that involves the human and veterinary health sectors, the municipality in charge of slaughterhouses, the ministry of agriculture in charge of farms and farm-related measures, the vector control sector, and the academic and research institutes for virus-related research activities and development of an effective vaccine.

Emergence of AHFV

Emergence of novel viruses in specific geographical regions of the world has always been associated with factors leading to their emergence or re-emergence. These factors have been extensively discussed by Sellers [34] and Liang *et al.,* [35]. Virus mutations, environmental and ecological conditions, and demographic factors are among the important determinants that can aid in virus emergence. Transmission of viruses by arthropods has also provided an efficient channel for the evolutionary development and emergence of many viruses.

The factors that led to the emergence of AHFV in KSA remain unclear. KSA is a cosmopolitan country where millions of pilgrims from all over the world visit the holy city of Makkah year-round for Omra and also annually for Hajj through the sea- and air-port of Jeddah, in the western part of the country. In addition to the pilgrims, hundreds of thousands of foreigners enter KSA year-round for work. Additionally, KSA imports millions of livestock animals year-round, primarily from African countries. Historically, many viral diseases have been introduced into KSA through people coming from different parts of the globe to practice their religious rituals or for work. Most of these introduced viruses were known in their home countries before introduction into KSA. Examples of these viruses are the dengue and influenza viruses. Others were introduced by infected animals or wind-driven mosquitoes, e.g., Rift Valley fever virus [36]. As for AHFV, the situation is different. It was reported for the first time in KSA and was not known elsewhere in the world. To trace the emergence of AHFV in KSA, some salient points have to be considered. AHFV infection was not reported anywhere else in the world before its discovery in KSA. The possibility that AHFV has evolved or mutated from a closely related virus needs to be investigated in extensive molecular and serological studies. In this context, the only closely related flavivirus is KFDV. In spite of the close sequence relationship (89%) between these two viruses, the epidemiology of the two diseases they cause is different. Furthermore, KFDV infection has never been reported in KSA. The epidemiology of KFDV has been examined in great detail [37–41], while most of the epidemiological features of the AHFV remain to be uncovered. Since it is strongly suggested that AHFV is a zoonotic arbovirus, much work should be directed towards identification of the possible vector(s) and reservoir(s) of this virus in the KSA ecosystem. This may include domestic livestock, wildlife, rodents, bats, other small wild mammals, ticks, and mosquitoes. Indeed, this may explore a zoonotic pool for the virus in the country and possibly a sylvan environment. When addressing the concept of the sylvan cycle, it should be considered that an emerging virus might have existed in the sylvatic environment without causing epidemics [35]. More biological and field research is required to define the exact modes of transmission of AHFV to humans. In this respect, it is advisable to keep the mind open for all possible modes of transmission of this virus, including those that have not been reported for similar viruses. Such a situation has been reported for other viruses, such as Crimean Congo hemorrhagic fever virus, which is transmitted by both tick bites and contact with infected animals or humans [42]. Based on recent reports, it is obvious that the geographic distribution of AHFV goes beyond KSA and that ticks may have a role to play in the epidemiology of AHFV [7–9, 11]. It is likely that AHFV was introduced into KSA via importation of livestock animals from the Horn of Africa. Millions of livestock animals are imported every year from Sudan, Somalia, and other African countries into KSA. The main seaport that receives these animals in KSA is Jeddah, where all imported animals are initially kept in quarantine in the Alkhumra district, after which the virus was named and where the first cases of AHFV were reported. It is also possible that AHFV was introduced into KSA through wind-driven infected mosquitoes as was described previously for other viruses [34]. Wind systems operate over the Arabian peninsula year-round, carrying different species of mosquitoes [43, 44] and Culicoides midges infected with various viruses [45–49].

Mortality of AHFV infection

The first epidemiological studies on AHFV in KSA after its discovery 24 years ago showed that the mortality among the cohorts described initially in Jeddah and Makkah was 20-25% [2, 3]. Subsequently, several large studies reported a mortality of about 1% during an outbreak of this virus in Najran that extended from 2003 to 2009 [4–6]. Subsequent limited outbreaks in Taif and Jazan between 2010 and 2015, and more recently in Algunfuda in April 2017, also had a low mortality rate (<0.5%). The high AHFV-related mortality initially reported for the cohorts in Jeddah and Makkah was likely an overestimation of the real mortality due to selection bias, as it represented mortality among a small sample of sick patients who were all hospitalized. The notably lower mortality subsequently reported from other regions in the southwest of KSA was likely due to the larger sample size and inclusion of patients with mild to moderate illness that was managed in an outpatient setting as well as due to early diagnosis and improved healthcare. Other published data have confirmed a similar spectrum of illness that ranged from asymptomatic infection to severe and fatal disease [5, 6]. The low mortality reported in these regions suggests that AHFV has established endemicity that has led

to partially protective herd immunity among the inhabitants due to recurrent exposure to the virus [4].

In summary, AHFV is a new hemorrhagic fever flavivirus that has mostly been reported in KSA. It was originally reported in 1995 in the Alkhumra district in Jeddah, from which the virus derived its name, then from Makkah in 2001-2003, and lately from Najran, from 2003 to date. Acute fever with a flu-like illness, hepatitis, hemorrhagic manifestations, and, less commonly, encephalitis are the most common manifestations. The virus seems to be transmitted to humans primarily from sheep and goats, and rarely from camels, by direct contact with these animals or their raw meat, and perhaps tick or mosquito bites. The AHFV vector capability of ticks and mosquitoes needs to be elucidated in biological studies. The exact role of animals such as sheep, goats, camels, and other mammals in the transmission and maintenance of the virus remains to be explored. Preventive measures require an interdisciplinary plan that involves the human and veterinary health sectors, the municipality in charge of slaughterhouses, the ministry of agriculture in charge of farms and farm-related measures, the vector control sector, and the academic and research institutes for virus-related research activities. Research activities should be aimed at defining the role of arthropods such as mosquitoes and ticks as vectors of the virus, the role of animals in the epidemiology and transmission of the virus, defining the natural reservoir(s) of the virus, determining the seroprevalence of AHFV antibodies among humans and sedentary and imported livestock animals, and development of an effective vaccine. It is essential to establish biosafety level 3 laboratories for handling the AHFV research and also animal biosafety level 3 laboratories for studies involving animal experimentation with AHFV and other hemorrhagic fever viruses. Such laboratories will enable establishment of animal models for studies on AHFV, including vaccine development and testing.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest statement.

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