academicJournals

Vol. 8(1), pp. 12-17, April 2013
DOI:10.5897/BMBR2013.0208
ISSN 1538-2273© 2013 Academic Journals
http://www.academicjournals.org/BMBR

Biotechnology and Molecular Biology Reviews

Review

Alkhumra virus: A zoonotic butcher in the Middle East? Concerns and consideration

Meerasahib Mohamed Fareez[1]*, Lubna Saeed Mahmood[2] and Abdulbasit I. I. Al-Sieni[3]

[1]Biomedical Sciences, Department of Health Sciences, Faculty of Arts and Sciences, Qatar University, Doha, Qatar.
[2]Human Nutrition and Food Science, Department of Health Sciences, Faculty of Arts and Sciences, Qatar University, Doha, Qatar.
[3]Department of Biochemistry, Faculty of Sciences King Abdulaziz University Jeddah, Kingdom of Saudi Arabia (KSA).

Accepted 05 April, 2013

New, emerging, and re-emerging infectious disease incidences have increased rapidly and frequently with significant human and financial costs. Most of the viral infectious diseases are of zoonotic nature, and public awareness of the human health risks of infections have grown in recent years, since viral epidemics such as severe acute respiratory syndrome, West-Nile virus, and Ebola virus diseases have emerged over the past two decades. The Alkhumra virus, which belongs to the flaviviruses family, discovered in Saudi Arabia in the mid-1990s causes hemorrhagic fevers among cattle farmers and butchers. Flaviviruses are transmitted through arthropods, and most of them are of zoonotic nature. Epidemiological data indicates that Alkhumra virus (ALKV) is transmitted from livestock animals to humans by direct contact with animals or by mosquito bites, but not by ticks. In the recent past the incidence of alkhumra virus infection has notably increased and to date, no specific treatment or containment strategies have been developed for Alkhumra virus infection, thus, there is a possibility of a major outbreak if appropriate prevention and control strategies are not adopted. This review presents current facts and future concerns of the disease around the Gulf region.

Key words: Alkhumra virus, hemorrhagic fever, Saudi Arabia, tick-borne infection.

INTRODUCTION

There are numerous new, emerging, and re-emerging diseases of humans that are caused by pathogens originating from both domestic and wild animals as well as products of animal origin (Meslin et al., 2000). These pathogens can emerge through introduction into a new population or when the interaction with the vector changes; emergence is also influenced by microbiological adaptation and change and other variants in human ecology as well as behaviours, such as deforestation and reforestation projects, that alter habitats of disease-carrying insects and animals. Most of the emerging and re-emerging viral infectious diseases are of zoonotic nature, and public awareness of the human health risks of infections has increased in recent years since viral epidemics, such as severe acute respiratory syndrome (SARS), West-Nile virus, and Ebola virus have emerged over the past several decades. Recently, concern of H5N1 flu transmission from migratory bird populations has also increased due to focus on fatal human cases (Heeney, 2006). These viral infections with zoonotic potential can become serious threats on life once they are able to establish the necessary adaptations for efficient animal-to-animal, animal-to-human, and human-to-human transmission under circumstances that are sufficient to reach epidemic proportions. Therefore, rapid and decisive action to identify and control novel pathogens is crucial in order to contain outbreaks and prevent further transmission.

Many emerging diseases are acquired from animals or transmitted by arthropods. The discovery of the Alkhurma hemorrhagic fever virus (AHFV) in the Makkah and Najran

*Corresponding author. E-mail: fareez@qu.edu.qa or fareez@live.com. Tel: 00974 70131633.

provinces of south-western Saudi Arabia is one such zoonotic virus. The virus was isolated in 1995 from the blood of a patient with a severe illness and has been characterized serologically and genetically as a variant genotype of Kyasanur Forest Disease virus (KFDV), which belongs to the family of tick borne *flaviviruses* (Charrel et al., 2005 and Memish et al., 2012). Flaviviruses comprise more than 70 different viruses, many of which are arthropod-borne and transmitted by either mosquitoes or ticks (Heinz and Karin, 2012)

Flaviviruses are responsible for causing many human encephalitic and hemorrhagic diseases, such as tick-borne encephalitis, dengue fever, West Nile, and yellow fever (Gould et. al., 2004). Despite a large geographical separation, there is a common ancestry of KFDV in India and AHFV in Saudi Arabia (KFDV and AHFV share 89% sequence homology), and it has been suggested that the movements of birds may carry this virus (Mehla et al., 2009). Similar to KFD, Alkhurma haemorrhagic fever causes acute febrile flu-like illness and vomiting with hepatitis. The fatality rate is 25 to 30% of all documented cases, which makes AHFV one of the most deadly flaviviruses (Charrel et al., 2005). There are currently no known treatments or specific therapies available for this hemorrhagic fever and therefore a suitable vaccine development should be seriously and urgently considered.

POSSIBLE HEMORRHAGIC FEVER OUTBREAK IN SAUDI ARABIA

To date, four types of viral hemorrhagic fevers, including Alkhumra Hemorrhagic Fever (AHF), Crimean–Congo Hemorrhagic Fever (CCHF) (EI-Azazy and Scrimgeour, 1997), Dengue Fever (Alzahrani et al., 2013), and Rift Valley Fever (RVF) (Al-Afaleq and Hussein, 2011) have been identified in Saudi Arabia. All of these viral hemorrhagic fever viruses, except AHFV, have caused outbreaks in Saudi Arabia during the last two decades. CCHF caused an outbreak in Makkah in 1990, but the disease has not been reported in Saudi Arabia (Madani, 2004). Dengue fever caused an epidemic in Jeddah in 1994, and more cases have since been sporadically reported in Jeddah (Fakeeh and Zaki, 2001, Ahmed, 2010; Alzahrani et al., 2013). RVF caused a major epidemic in 2000 to 2001 in three different areas in the southwest of Saudi Arabia, namely Jizan, Asir, and Alqunfuda, which are located far from Makkah city (Madani et al., 2003). Three of the four viral hemorrhagic fever diseases identified in Saudi Arabia, including AHFV, CCHF, and Dengue, are thus confined to the cities of Makkah and Jeddah, which are 80 km apart in the Western Province. Since the discovery of AHFV north of Jeddah in 1994 to 1995, it was also discovered in Makkah in 2001 to 2003. Furthermore, there were 58 cases AHFV infections reported in the Najran province bordering Yemen since 2003 (Madani et al., 2011).

There has also been a sharp increase in the number of reported cases from the Najran region since 2008, and the outbreak is currently still active (Alzahrani et al., 2010). A total of 148 suspected cases have been reported in Saudi Arabia, of which 78 (52.7%) cases were laboratory confirmed; two cases in 2003, one case in 2004, four cases in 2005, one case in 2007, 12 cases in 2008, and 58 cases in 2009. Importantly, the occurrence of AHFV and CCHF in these two cities is most likely related to the importation of large numbers of livestock into Makkah city through the Jeddah seaport for the Hajj season (Madani et al., 2011).

STRUCTURE OF FLAVIVIRUSES

Mature virions of Flavivirus are approximately 50 nm in diameter, and the RNA genome is packaged in a host-derived lipid bilayer containing two envelope glycoproteins E (envelope) and M (membrane). The RNA genome encodes one long open reading frame (ORF), which is cleaved co- and post-translationally into three structural (Capsid, C; Precursor membrane, prM; and envelope, E) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins (Figure 1) (adopted from Koraka et al., 2010). Intracellular (immature) virions contain a precursor membrane (prM) protein, and the cleavage of prM to M occurs during the exit of virions from cells (Gollins and Porterfield, 1986). The envelope protects the genome from cellular nucleases (Murphy, 1980). The single ORF is flanked by 5' and 3' un-translated regions (UTR), the structures of which are important in viral transcription and replication (Russell et al., 1980).

The envelope protein is the major surface protein of the viral particle. It interacts with cell receptors and mediates virus-cell membrane fusion. In mammalian hosts, it also induces virus neutralizing antibodies that play an important role in the establishment of protective immune responses (Koraka et al., 2010). The envelope protein is 496 amino acids (aa) long for all TB flaviviruses, including AHFV and KFDV, with the exception of POWV, which has 497 aa (Heinz and Allison, 2000). The E protein of flaviviruses is a homodimer and each monomer is divided into three domains (D I, II, and III). Studies of the B-cell repertoire upon flavivirus infection suggest that the human antibody response is predominantly directed to epitopes located in DII (Diamond et al., 2008). During the infectious cycle, the NS3 (helicase) and NS5 (RNA-dependent RNA polymerase) proteins form polymerase complexes, which are most likely associated with membranes through the non-structural protein NS1 and NS2A (Oliphant et. al, 2005). The NS1 protein induces protective immune responses against flaviviruses (Winkler et al., 1988; Cane and Gould, 1988). In addition, NS3, in association with NS2B, provides virus-specific serine protease activity for the cleavage of newly synthesized virus polyprotein. The non-structural proteins NS4A and NS4B most likely provide appropriate orientation of the polyprotein within intracellular membranes, thereby ensuring correct cleavage

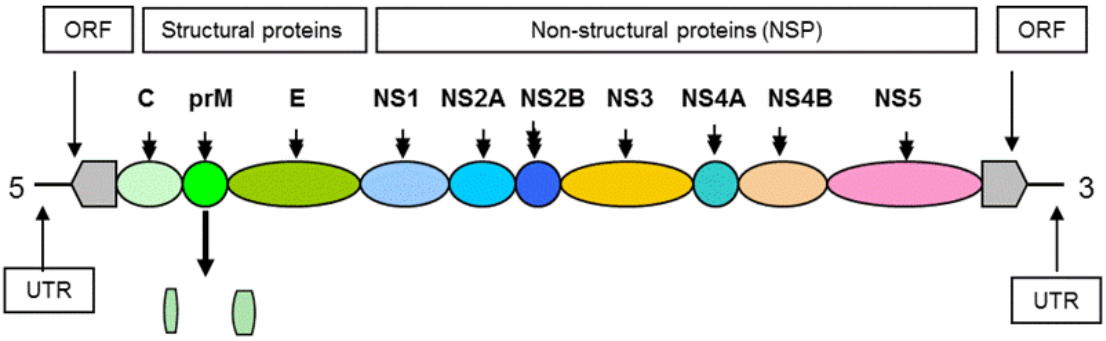

Figure 1. Structure of Flavivirus genomic RNA, which consists of an un-translated region (UTR), open reading frame (ORF), and structural and non-structural proteins. C, Capsid; PrM, pre-membrane; E, envelope (Koraka et al., 2010).

and functioning of polymerase complexes (Diamond et al., 2008). The NS5 region is responsible for methylation of the viral RNA cap structure (Jacobs et al., 1992).

ALKHUMRAH HEMORRHAGIC FEVER VIRUS (AHFV)

The Alkhumra virus consists of a 3,416 aa polyprotein that is highly conserved among other mammalian tick-borne flaviviruses. The putative cleavage sites of AHFV polyprotein C, PrM, M, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 are identical in length in all of the tick-borne flaviviruses (Diamond et al., 2008). The viral RNA encodes a serine protease (NS2B–NS3), which is essential for virus replication in infected cells (Pastorino et al., 2006). The relatedness of AHFV with tick-borne flaviviruses was also demonstrated through analysis of the E protein (Rauscher et al., 1997). The AHFV envelope protein (E) is 496 aa in length and has the common structure of flaviviruses. According to a study by Mohabatkar (Mohabatkar, 2011), which discussed the medically im-portant structural properties of protein E of AHFV and compared these features with two closely relates viruses [Kyasanur Forest disease virus (KFDV) and Tick-borne encephalitis virus (TBEV)], the evolutionary distance of the E protein from all three viruses is almost equal. Fur-thermore, several conserved patterns have been identified among the tick-borne flaviviruses including the 12 cysteine residues involved in intramolecular disulfide bond formation and the three potential N-glycosylation sites (positions 154–156, 361–363, and 473–475). Based on these similarities with other tick-borne flaviviruses, AHFV is more closely related to KFDV. Phylogenetic analysis of these two closely-related viruses suggests that the AHFV may be considered a genetic subtype of KFDV (Oliphant et al., 2005). However, the ecological conditions that determine virus survival and propagation are very different for KFDV compared to AHFV (Monath and Heinz, 1996). Human infection with KFDV is caused by contact with infected ticks or monkeys. In contrast, human infection with AHFV is a result of contact with infected cattle or mosquito bites, which is generally encountered in a semi-desert environment (Pialoux et al., 2007; Petersen and Marfin, 2005). A recent study by Madani et al. (2012) demonstrated that ALKV can be propagated in C6/36 mosquito cells.

Epidemiology of AHFV

Tick-borne flaviviruses are usually transmitted by hard ticks (tick bites) or through direct contact with infected blood (during the slaughter of animals) or tissues of viremic animals or humans. However, Alkhurma viral RNA has been detected in soft ticks (*Ornithodoros savignyi*) collected at a camel resting place in Jeddah (Carletti et al., 2010) However, no evidence substantiate that this virus is transmitted through tick bites but a study by Madani et al. (2012), provides evidence of this virus being transmitted from livestock animals to humans by direct contact with animals or by the mosquito bites. No documented animal reservoirs have been reported, but human infections have been linked to contact with small ruminants (sheep, goats) and camels, and consumption of un-pasteurized dairy products from infected animals (camel) has been reported as a mode of transmission (Mansfield et al., 2009). Several studies have hypothesized that mosquitoes could also be vectors (Charrel et al., 2007), but human-to-human transmission has not been reported. However, due to the paucity of data, these two modes of infections cannot be ruled out. The disco-very of the link between *O. savignyi*, tick, and AHFV has a wide range of implications in the spread of the disease through several routes, including movement and transport of livestock, infected meat products, and humans in the Persian Gulf region and beyond.

O. savignyi is a cryptic tick that is nocturnally active and usually attacks humans and other animals resting under trees. The lifestyle of *O. savignyi* supports its role as a vector and transmitter of AHFV (Charrel et al., 2007). In the arid ecosystems of Saudi Arabia and other parts of the Persian Gulf, *O. savignyi* has been associated with camels and their resting places, and to a lesser extent,

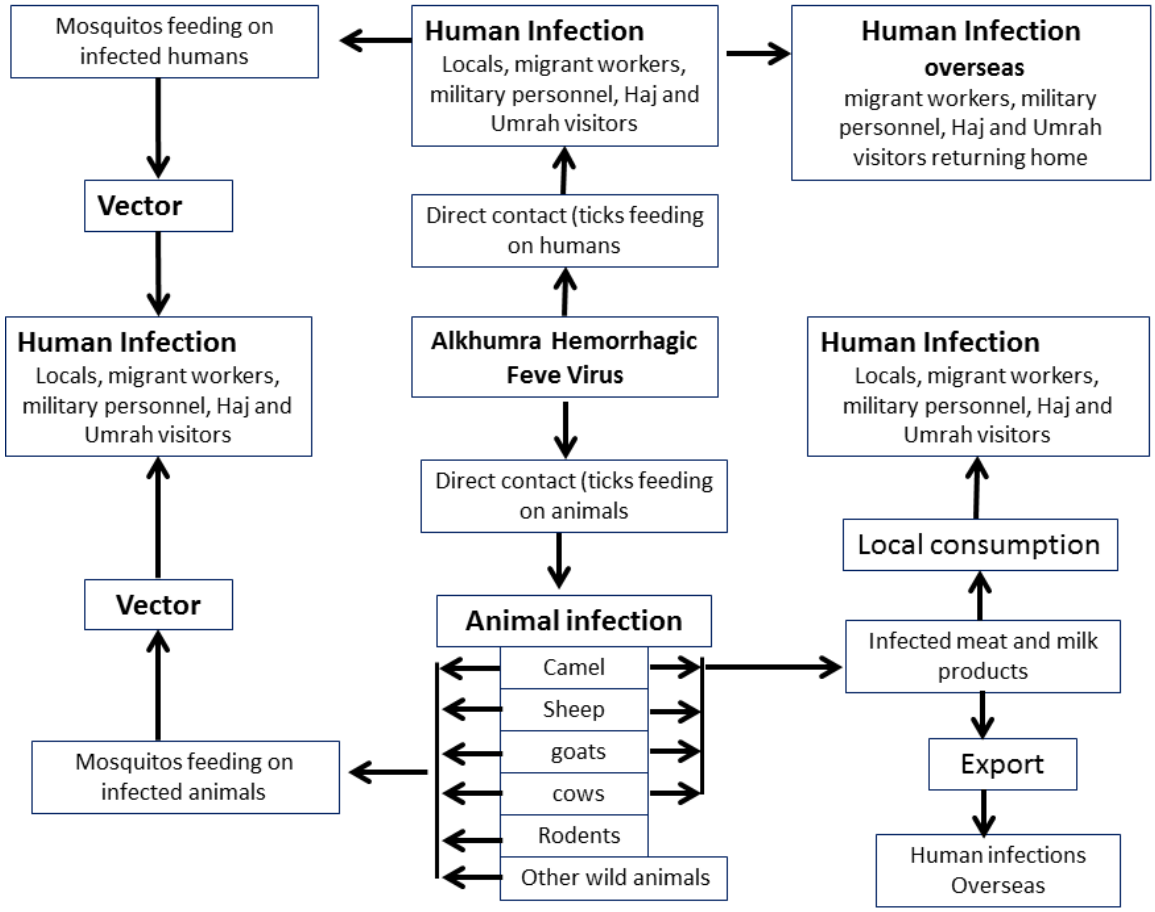

Figure 2. Schematic diagram of AHFV infection and a possible epidemic occurrence.

other domestic and wild animals found in camel resting places (Hoogstraal et al., 1981). Most of the AHFV cases reported in Saudi Arabia occur in butchers, who are likely to be either bitten by ticks or infected through animal products. The most important factors that contributes to the incidence of disease is the abundance of ticks containing a high dose of infectious AHFV that occur in the animal habitat (Sidi et al., 2005).

There are several factors that potentially influence the incidence of a global epidemic, including migrant workers, annual hajj and umrah pilgrimages, livestock trade between neighboring countries, and the presence of military personnel and their activities in this region (Figure 2). Two to three million people from all over the world convene in the holy city of Makkah annually to perform Hajj, part of which involves the slaughter of thousands of livestock animals. Furthermore, the distribution of meat around the world can also pose a threat of the spread of infection globally. If an outbreak were to occur, pilgrims could exacerbate the problem when they returned home by passing their infection on to others.

The relationship between *Ornithodoros* ticks and military activity-related tick-borne disease incidence has been recently documented in the Middle East (Sidi et al., 2005; Domenech et al., 2006). A large number of foreign military and civilian personnel have been recently based in the region, which provides the opportunity for exporting infected ticks. The association of the tick JE7 with camels further supports the role of camels in the AHFV transmission cycle as well as the zoonotic nature of the disease (Hoogstraal et al., 1981). The threat of this virus spreading to neighboring countries is a high possibility through cattle trade and migration. The recent epidemics of H5N1, swine fever, and foot-and-mouth diseases have served as a reminder of the existence of infectious diseases and the capacity of these diseases to occur unexpectedly in new locations and animal species. Moreover, the large number of migrant workers from South East Asia, Africa, Europe, and North America can act as carriers who pose a substantial threat of an outbreak of this disease.

BURDEN AND CONTROL OF THE DISEASE

The advancement of science and medical technologies has helped in combating certain infectious diseases. Nevertheless, numerous new infectious diseases continue to

emerge that cause deaths in humans and livestock as well as untold financial losses. It is a formidable challenge to prevent the spread of these new infections diseases; however, measures can be taken to reduce the burden on human and animal populations if rapid and concerted efforts are in place (Marsh Inc., 2008). The AHFV represents a serious threat among the farmers and butchers in Saudi Arabia. Monitoring the epidemiology and burden of AHFV infections in Middle Eastern countries is crucial because, as mentioned above, the AHFV does not just use cattle as a host for infections, but, mosquitoes, and others as well. Therefore, if an outbreak of AHFV were to occur, it would threaten the farming sector, which has a direct effect on the cattle trade and food supply in this region. This risk has the potential to severely disrupt supply chains and further harm human health and welfare as well as cause marked economic losses (WHO, 2007). In order to control the spread of AHFV successfully, improved evidence-based guidance and the implementation of preventive measures should be in place. The following recommendations would potentially reduce the impact of an AHFV infection:

1) Further investigation into areas of uncertainty: Although several investigations have been conducted to understand the nature of the virus, vectors, and incubation period, further investigation is required to:

a) Understand the life cycle of the Alkhurma virus and its mode of transmission
b) Determine if infected birds are capable of carrying the virus
c) Perform larger studies involving more tick species in the gulf region in order to better understand AHFV ecology and transmission dynamics
d) Obtain better knowledge of the geographic distribution of AHFV in countries near Saudi Arabia.

2) An effective screening system and vaccine development: To date, there is no treatment or screening system available for AHFV. The screening of bodily fluids of people who are at high risk (farmers and butchers) is essential to identify infection before the manifestation of symptoms. Furthermore, farmed animals should also be screened for viral detection. Despite the threat of an epidemic, no effective vaccine, antiviral drug for treating flaviviral infection, or for the viruses that are known to cause hemorrhagic fever have been developed to date. In the absence of effective antiviral treatment, prevention through vaccination would greatly reduce morbidity and mortality associated with flavivirus infections.

3) Strengthening prevention: Identification of the incidence of AHFV movements could help restrict its spread and prevent future movements of this virus. Random screening of a high-risk population as well as continued vector studies and analysis of specimens from wild and domestic animal populations should be conducted on a regular basis. Moreover, entomological studies should also be carried out to assess the relationship between vector bionomics and improved health care facilities for the farming community.

4) Control of livestock transport and cattle products: It is vital that cattle trades with neighboring countries are reviewed and strict guidelines should be adhered to in order to control the transportation of infected cattle.

5) Mitigation strategies: Healthcare organizations should focus on key activities, such as early detection of the virus as well as timely and accurate verification of the presence or absence of the virus using diagnostic methods in the field, laboratories, and healthcare settings. A comprehensive and rapid response to care for infected patients and reduced exposure of the wider population to contaminated food will significantly reduce infections in humans and animals.

CONCLUSION

There is an urgent need for identifying gaps and competencies across the region that may enhance the prevention and response capabilities of AHFV infection. In the area of AHFV research, these gaps emphasize the need for improved tools and facilities for detection and verification as well as the need to speed up development and production of interventions and therapeutics through translational research. In the animal trade and food production, cooperation between governments and industry is critical for preventing contaminated food products from reaching consumers. Therefore, accepted infection control practices and isolation precautions should be implemented and strictly enforced. History has shown that pathogens can and do exploit gaps in these efforts.

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