Infection, Genetics and Evolution 70 (2019) 80–83

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Genetic diversity of Alkhurma hemorrhagic fever virus in Western Asia

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ARTICLE INFO

Keywords:
Alkhurma hemorrhagic fever virus
Kyasanur forest disease virus
Genetic diversity
Phylogenetic analysis
Molecular epidemiology

ABSTRACT

Alkhurma hemorrhagic fever, caused by Alkhurma hemorrhagic fever virus (ALKV), is an arboviral infection which is further expanding in tropical and subtropical regions of the Western Asia. A number of Alkhurma hemorrhagic fever virus (ALKV) strains have been isolated from clinical cases representing Saudi Arabia and Egypt; however, the phylogenetic relationship of these particular isolates to those reported previously elsewhere in the world remains elusive. Based on the analysis of the envelope (E), and non-structural gene (NS3 and NS5), the phylogenetic and PASC analysis revealed the circulation of three sub-lineages (I-III) suggesting a continuous evolution. Also, the comparative genome analysis revealed the envelope gene to be a reliable genetic marker to elucidate the molecular epidemiology and genetic diversity of discrete strains of ALKV.

1. Introduction

The emergence and/or re-emergence of an infectious disease, such as Alkhurma hemorrhagic fever virus (ALKV), has a significant impact on animal and human health. (Saudi Gazette report, 2018; http://www.saudigazette.com.sa/). The virus belongs to the genus *Flavivirus* within the family *Flaviviridae*. Comprised of a single positive sense RNA, the genome ranges from 10,685 nt to 10,749 nt that encode a single open reading frame (ORF), termed a polyprotein. Subsequent to co- and post-translational modifications, the ORF is cleaved into three structural [capsid/core (C), pre-membrane (PrM) and envelope (E)] and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Charrel et al., 2001). Since the first report of human infection in the Alkhurma city of Jeddah District in 1994 (Zaki, 1997), a number of clinical cases have now been reported from the Makkah, Najran and Jazan Districts of Saudi Arabia (Madani, 2005; Alzahrani et al., 2010). A few clinical cases and subsequent isolation of ALKV has also been demonstrated from different hosts in Egypt and Djibouti (Charrel et al., 2007; Carletti et al., 2010; Musso et al., 2015; Horton et al., 2016).

The isolation of ALKV from ticks (*Ornithodoros, Amblyomma and Hyalomma* spp), in addition to humans, further highlights the potential of the virus to evolve, adapt and emerge in susceptible populations (Charrel et al., 2005; Carletti et al., 2010; Horton et al., 2016) with subsequent zoonotic implications. Such emergence of novel variants implies that genomic variations are under constant pressure over a period of time (Charrel et al., 2005). Therefore, in order to assess evolutionary dynamics and better elucidate ALKVs classification system worldwide, a comparative phylogenomic analysis of sequences publicly available to date is necessary to propose and develop the landscape of molecular epidemiology and genetic diversity of ALKVs.

2. Materials and methods

By December 2018, a total of 67 ALKV nucleotide sequences were reported in the public NCBI database (http://www.ncbi.nlm.nih.gov/). These included the complete genome (n = 20), partial sequences of polyprotein (n = 11), envelope (n = 14), NS3 (n = 11) and NS5 (n = 11). The complete genome and individual gene sequences were aligned according to ClustalW methods in BioEdit® version 5.0.6 (Hall, 1999). To describe the topology of phylogenetic trees, analysis of the best fit substitution model was performed in MEGA® version 6.0 software and the goodness of fit of individual models was assessed by two methods: Bayesian Information Criterion (BIC) and corrected Akaine Information Criterion (AICc) (Tamura et al., 2013). Time calibrated phylogenetic analysis was performed to reveal evolutionary dynamics using two different models: the General Time Reversible model (GTR) with discrete gamma distribution (+G) and the Real-Time-Maximum Likelihood model for invariant sites (+I) in sequences. The nucleotide homology of complete genomes, envelope, NS3 and NS5 genes of available isolates was estimated by Pairwise Sequence Comparisons (PASC) analysis in MEGA (Tamura et al., 2013). Moreover, an epidemiological link for virus transmission route was also explored while reviewing published reports related to ALKV infection and disease epidemiology.

3. Results

The reports related to infection and disease epidemiology revealed

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https://doi.org/10.1016/j.meegid.2019.02.012
Received 27 October 2018; Received in revised form 31 January 2019; Accepted 15 February 2019
Available online 16 February 2019

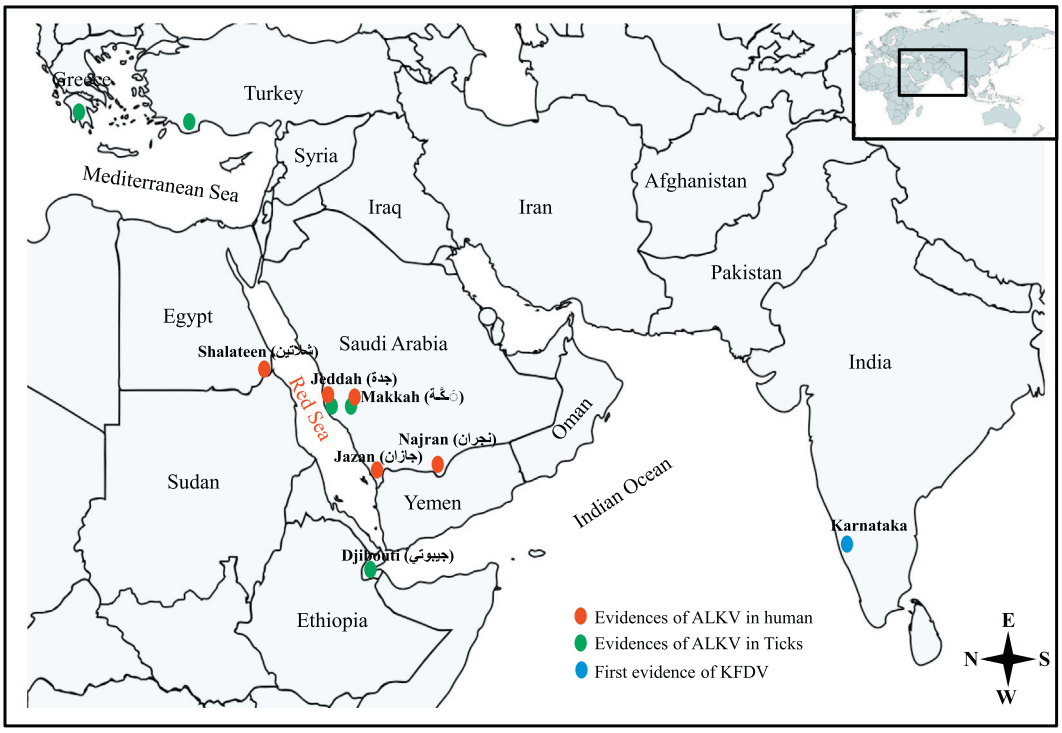

Fig. 1. Geographical distribution of ALKV strains in different hosts of Saudi Arabia and other regions around the Red Sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

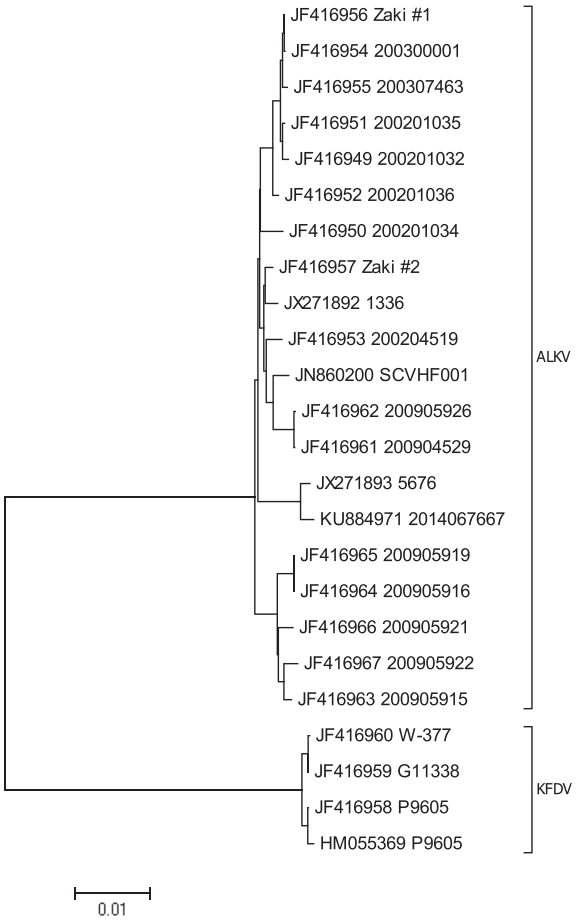

Fig. 2. Phylogenetic analysis of complete genome sequences of ALKVs and KFDVs reported to date.

that both ticks and migratory birds are crucial hosts that play a significant role in virus dissemination in inter-linked regions/countries of endemic disease around the Red Sea (Fig. 1). With a 7.6–8.1% nucleotide divergence, the complete genome based phylogeny of ALKVs and Kyasanur Forest disease virus (KFDVs) clustered the isolates into two distinct clades named Alkhurma lineage and India originated KFDVs lineage (Fig. 2). Phylogenetic analysis of ALKV strains suggested three distinct clades. Clade-I, designated as sub-lineage I, comprised of strains isolated from human beings during 1994–2003 and 2009–2014 in Saudi Arabia. Tick-originated strains isolated from Saudi Arabia in 2009 clustered in clade-II or sub-lineage II. Likewise, the strains isolated from Egypt during 2010 clustered in a distinct clade-III or sub-lineage III (Fig. 3A). Based on the envelope gene, a discrete phylogenetic pattern of isolate clustering was observed according to their hosts and geography (Fig. 3A). On the other hand, phylogenic clustering was not as discrete as required in such molecular epidemiological studies for NS3 and NS5 genes (Fig. 3B and C). The PASC analysis of the envelope gene revealed 0.75–1.31% nucleotide divergence between sub-lineages I and II, 1.31–2.3% between sub-lineages I and III, and 1.5–1.9% between sub-lineages II and III (Table S1). A 0.71–1.3% and 0.7–1.5% nucleotide divergence was observed between sub-lineages I and II for NS3 and NS5 gene-based PASC analysis (Tables S2 and S3).

4. Discussion

Owing to the paucity of genome sequencing data for these very few isolates (Charrel et al., 2001; Madani et al., 2014), the phylogenomic analysis of ALKVs remains elusive. The current study elucidates phylogeny and genetic diversity of ALKV using the complete genome and individual genes and analyzes epidemiological patterns derived from published reports (Charrel et al., 2001, 2005, 2007; Madani, 2005; Madani et al., 2011, 2012, 2014; Alzahrani et al., 2010; Carletti et al., 2010; Musso et al., 2015; Horton et al., 2016; Hoffman et al., 2018). In contrast to previous observations (Charrel et al., 2005), the study analysis revealed rich genetic diversity among ALKVs isolated from different hosts and geographical regions. This is quite understandable

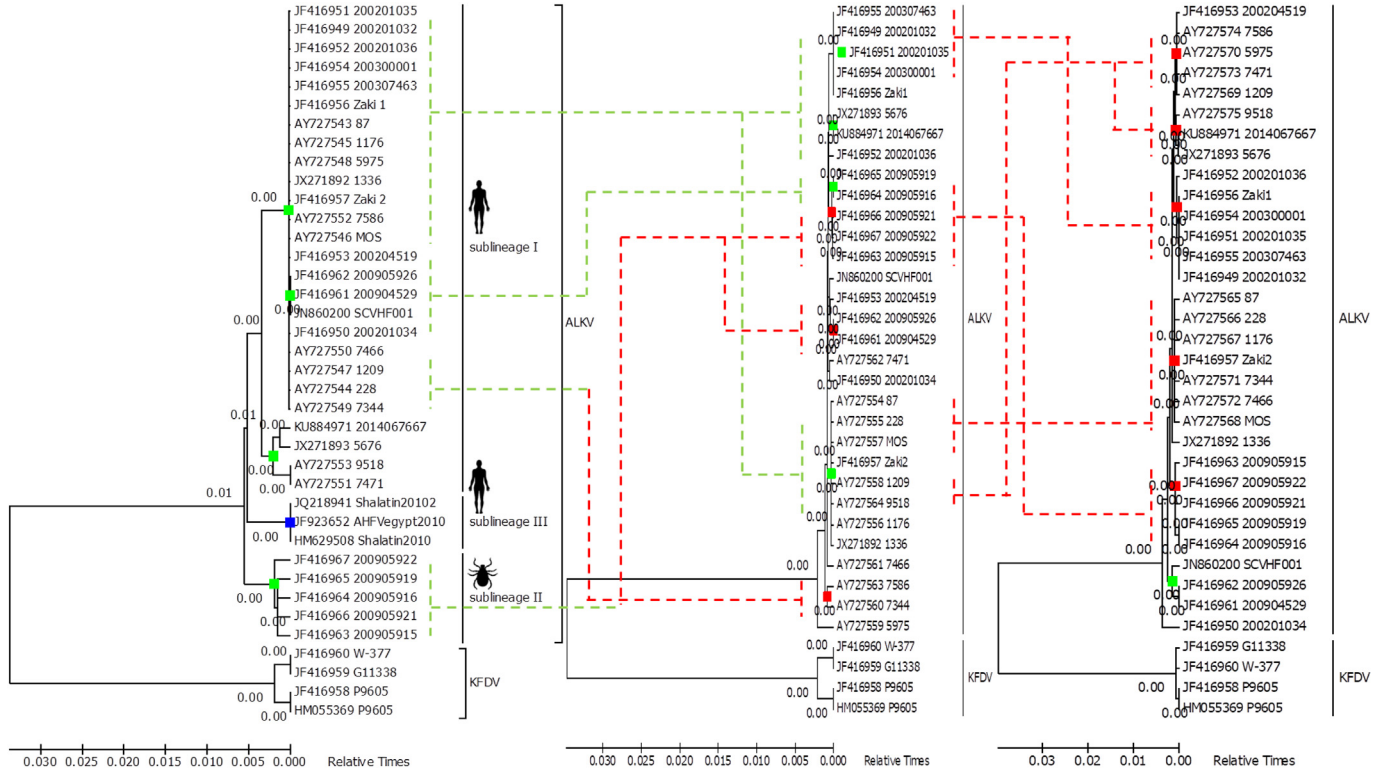

Fig. 3. Time calibrated molecular phylogenetic analysis of ALKVs based on envelope (A), NS3 (B) and NS5 (C) genes was constructed in MEGA6. The apt clustering is shown with a green colour box at node of cluster whereas the inapt or possibility of occurrence of recombination in clustering pattern is shown as red colour at node of cluster. The Blue colour box indicates the clustering of strains reported only from Egypt. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

given that previous analyses by Charrel et al. (2005) were limited to strains isolated between 1994 and 1999, while a significant number of isolates originating from ticks in Saudi Arabia and hosts in Egypt were reported during 2009–10.

The envelope gene was found to be the most suitable genetic marker for comparative phylogenetic analysis that provided a discrete clustering pattern based on host and geography. This finding is of particular importance because a large number of ALKV envelope gene sequences are available in the public NCBI database. Also located in the envelope gene are the antigenic domains, where a genetic variation may result in the emergence of novel antigenic variants (Roehrig, 2003). Furthermore, the finding that the envelope gene is a reliable genetic marker is important for our understanding of the genetic diversity of other members of the genus *Flavivirus* (Mir et al., 2017; Hashem et al., 2018). Since a genetic marker appropriate for investigating the evolutionary dynamics of prevailing ALKVs isolates has not been identified to date, this study's analysis using both the complete genome and the envelope gene will provide a baseline criteria for the classification of ALKV strains reported so-far and those that will be reported in the future.

Since there exist 89–92% sequence homology between ALKV and KFDV, the ALKVs are considered a prototype strain/variant of the serological and antigenic variant genotype of KFDV. This is important because both viruses share similar clinical presentation, susceptible hosts, and pattern of transmission via ticks as vectors. Therefore, ALKV is now classified as a species-type of KFDV (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=33743). KFDV was first isolated from Karnataka, India near the Indian Ocean. It has been speculated that the movements of ticks may help to disseminate the virus to nearby regions (Mehla et al., 2009). Complete genome-based phylogenetic analysis clustered the KFDV strains into an Indian clade while the ALKV strains grouped together into an Alkhurma clade, which is in agreement with the observations made previously (Palanisamy et al., 2018).

The presence of ALKV has been detected in ticks (*Hyalomma rufipes*) infesting migratory birds including the Eastern woodchat shrike (*Lanius senator niloticus*), the Sedge warbler (*Acrocephalus schoenobaenus*), the Western yellow wagtail (*Motacilla flava*) and the Common redstart (*Phoenicurus phoenicurus*) in Greece and Turkey using real time polymerase chain reaction (PCR) (Hoffman et al., 2018), as well as in ticks (*Ornithodoros savignyi*) and camels in Egypt and Saudi Arabia (Charrel et al., 2007) and ticks (*Amblyomma lepidum*) and cattle in Djibouti using reverse transcriptase PCR (Horton et al., 2016). Though further analysis is needed for ALKVs isolated from ticks infesting multiple species, the maximum nucleotide homology (99.7%) between ALKV strains isolated from sand tampans (*Ornithodoros savignyi*) and humans highlights their role in cross-species dissemination and subsequent zoonotic implications (Dodd et al., 2011). This is important because tick-associated human infection has previously been observed in Egypt and Saudi Arabia (Charrel et al., 2007; Musso et al., 2015). Taken together, owing to a seasonal abundance of ticks over large geographical regions in humid climates, there needs to be constant surveillance of multiple hosts, including ticks, to better elucidate the underlying mechanism responsible for the transmission of the virus between, as well as within, animals and humans, particularly in settings of endemic disease.

5. Conclusions

ALKV is a prototype species of KFDV that is further expanding its geographical boundaries and susceptible host species. The envelope gene could be employed as a genetic marker to better elucidate molecular epidemiology and the subsequent evolutionary dynamics of prevailing strains. Future studies involving ticks infesting migratory birds and multiple susceptible hosts are necessary to further ascertain epidemiological links between disease incidences occurring either simultaneously or non-simultaneously from different geographical regions.

Conflict of interest

None to declare.

Acknowledgement

The author thanks Dr. Juliette Rando (University of Pennsylvania, USA) and Dr. Muhammad Zubair Shabbir (University of Veterinary and Animal Sciences, Pakistan) and anonymous reviewers for critical edits in improving the manuscript contents and language.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.02.012.

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