Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 3, 427-431

Identity of tissue culture adapted Rift Valley fever virus (ZH501)

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ARTICLE INFO

Recieved: 06 October 2023

Accepted: 15 December 2023

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Keywords:

RVFV
ZH501
Vaccine
M segment
Gn ectodomain

ABSTRACT

Rift Valley Fever (RVF) is still a threatening zoonotic disease with periodic reemergence in several countries. Egypt is endemic with RVF and uses an inactivated vaccine for control of the disease. Routine testing of the tissue culture adapted seed virus (ZH501-TC), to assess the effect of nucleotide mutations, is essential for the purpose of vaccine production. At the present work we have analyzed partial nucleotide and deduced amino acid sequence of amplified 745 bp product of M segment Gn ectodomain and checked virulence in mice. Allocation of ZH501-TC strain at the A designated lineage with the virulent parental RVFV isolated in 1977, ZH501 Egy Sh 77 indicates its genomic stability after passaging in tissue culture cells for vaccine preparation. Here we denoted a new missense nucleotide mutation A1312G corresponding to the amino acid mutation N371S, in current study the ZH501-TC strain (MZ218760) compared to the parental virulent RVFV isolate ZH501 Egy Sh 77 (previous sequence). That mutation enhanced ß sheet formation in ZH501-TC 367AQYASAYCS375 motif which might increase virus antigenicity. In addition, we have confirmed the presence of two sites of nucleotides substitutions; C1033T and A1206C, corresponding to two amino acids changes; T287I and S336R. Our study declared the false notion of the presence of one missense nucleotide mutation; A1252G corresponding to the amino acid mutation K351R, and two other silent nucleotide substitutions; T1257A and G1258C at the strain ZH501-VSVRI. Although we found these mutations, the virulence of the ZH501-TC strain was still present as approved by mice pathogenicity test.

Introduction

Rift Valley fever (RVF) is an arthropod-born viral disease of ruminants that can be transmitted to humans causing a sever zoonosis (Bob *et al*., 2017). The severity of the disease in susceptible animals can vary from mild to severe infection characterized by fever, restlessness, anorexia, disinclination to move, abortions, and high morbidity and mortality rates in neonatal animals (Bird *et al*., 2009). As an OIE-listed disease with long inter-epizootic intervals, RVF was considered as a re-emerging disease of high importance where outbreaks occur following weather conditions favoring increase in mosquito populations. Such RVF outbreaks are usually cyclical, occurring every 10–15 years (Maluleke *et al*., 2019). RVFV has appeared in Madagascar, the Comoros and the Arabian Peninsula despite it is originally endemic to sub-Saharan Africa (Shoemaker *et al*., 2002; Balkhy and Memish, 2003). The disease is caused by the Rift Valley Fever virus (RVFV) belonging to the genus Phlebovirus in the family Bunyaviridae, with a genome consisting of segmented single stranded RNA, of either negative or ambisense polarity. Genome segments are designated as L (6,404 nucleotides [nt]), M (3,885 nt), and S (1,690 nt). Coding regions of the three segments are 5′ and 3′ flanked by panhandle structures formed by two stretches of non-coding complementary stretches (Barr and Wertz, 2005; Amarasinghe *et al*., 2018). The L and M segments are of negative polarity, while S segment has ambisense polarity, encoding the nucleoprotein (N) in antisense and the non-structural protein (NSs) in sense orientation. The L segment expresses a multifunctional protein that comprises an N-terminal endonuclease (Morin *et al*., 2010) and a large RNA-dependent RNA polymerase (RdRp) domain (Schmaljohn and Hooper, 2001). Exposed on virus envelope and are responsible for cell tropism and membrane fusion, glycoproteins GN and GC are coded by the M segment. The endo-domain of GN is critical for genome packaging into infectious virus particles (Rusu *et al*., 2012). Climate changes enhances the spread of competent mosquito vector species leading to emergence of RVF virus in new ecosystems, including Europe and the United States, and re-emergence in the endemic areas (Chevalier, 2013; Elliott, 2009; Golnar *et al*., 2014; Rolin *et al*., 2013). Since 2015, RVF has been listed as a priority emerging disease by the World Health Organization (WHO) R&D Blueprint (WHO, 2018). A major concern about RVF is the expansion of its geographical range over recent decades (Nanyingi *et al*., 2015; Hatchett and Lurie, 2019). Increased animals transportation and trading worldwide raised the risk of RVFV transmission. On 10th of October 2019, the National IHR Focal Point for Sudan notified WHO of 47 suspected cases of RVF, including two deaths in Arb'aat Area (Tong *et al*., 2019; WHO, 2019). The disease remains a threat in countries where its mosquito vector thrives. Current disease control options to reduce human risk mainly rely on controlling virus transmission in animal populations (Métras *et al*., 2020). Vaccines constitute the major pillar to control RVFV spread and protection of livestock in endemic areas like Egypt. Veterinary Serum and Vaccines Research Institute (VSVRI) in Egypt produced Binary inactivated vaccine which produced from a seed ZH501virulent virus strain originally isolated from human in Zagazig in 1977 (Meegan *et al*., 1977; Meegan, 1979) Veterinary Serum and Vaccines Research Institute (VSVRI) Egypt produces the tissue culture inactivated Rift Valley Fever vaccine (El-Nimr; 1988). Periodical check for virus virulence and genomic stability after more than a decade of the last analysis made by Atwa *et al* (2011) is required. Our present work was designed for amplification and sequence analysis of a partial fragment of the M segment of the tissue culture vaccine virus (ZH501-Tc) in comparison with the parental virulent ZH501 strain and to check its virulence in mice aiming to evaluate its

ISSN: 2090-6277/2090-6269/

efficacy for vaccine production.

Materials and methods

Ethical Approval

Institutional Animal Care and Use Committee at the Veterinary Serum and Vaccine Research Institute, acknowledges the research manuscript. It has been reviewed under our research authority and deemed compliance with bioethical standards in good faith.

Virus and cells

The tissue culture-adapted Rift Valley Fever Virus (RVFV) strain (ZH-501-TC) with a titer of 108 was kindly obtained from the Rift Valley Fever Department, VSVRI, Cairo, Egypt.

Virus titration

Titration of ZH-501-TC was carried out using the tissue culture cells and Swiss albino mice according to El-Nimr (1980) and Taha (1982), and the virus titer was expressed as TCID50/ml and MIPLD50 according to Reed and Muench (1938).

Nucleic acid extraction

PureLink™ Viral RNA/DNA Mini Kit (ThermoFisher, USA) was used to extract viral RNA from ZH501-TC infected tissue culture cells following the manufacturer instructions. Briefly, 200µl of clarified supernatant of virus-infected tissue culture was added to 25 µL proteinase K in a sterile micro-centrifuge tube before adding 200µl lysis buffer containing 5.6µg carrier RNA and incubation at 56oC for 15 minutes. To precipitate nucleic acid, 250µL of 96-100% molecular biology grade ethanol was added to the tube and mixed by vortex for 15 seconds followed by incubation for 5 minutes at room temperature. To capture nucleic acids, the lysate was added to the viral spin column and centrifuged for 1 minute at 6800 x g. Thereafter, the spin column was washed with washing buffer (WII) two times before elution of the captured nucleic acid using 50 µL RNase-free water and storage at -80°C.

Reverse transcription Polymerase Chain Reaction (RT-PCR) and nucleotide sequencing of ZH501-TC Gn ectodomain partial fragment

SuperscriptTM III One-Step RT-PCR system with PlatinumTMTaq DNA Polymerase kit (Invitrogen, USA) was used to partially amplify the Gn ectodomain at the genomic M segment of the ZH501-TC strain, as a745 bp fragment (Shoemaker *et al*., 2002). The 50 µl reaction mix composed of 2x reaction mix (25 µl), template RNA (7 µl), 10µM of each of the sense and antisense primers (Table 1), SuperscriptTM III RT/PlatinumTMTaq Mix (2 µl), RNase free distilled water to 50 µl. the reaction mix was forwarded to the following thermal cycling: [1 cycle for RT at50°C for 30 minutes; 1 cycle at94°C for 2 minutes for denaturation of reverse transcriptase enzyme and activation of PlatinumTMTaq polymerase; 40 cycles of 94°C for 15 seconds, primers annealing at 55°C for 30 seconds, and extension of amplified fragment at 68°C for 30 seconds; one cycle for final extension at 68°C for 5 minutes]. After amplification of target fragment, whole volume of amplified PCR product was electrophoresed on 1% agarose gel. DNA concentration was measured using Qubit 4 fluorometer and DNA high sensitivity (HS) quantification kit (ThermoFisher scientific, USA). For sequencing, purified amplicons were submitted to GATC Bioteck AG (Germany) for sequencing. Nucleotides and deduced amino acids sequences were aligned with RVFV M segment sequences from GenBank (Table 2) using the nucleotides Basic Local Alignment Search Tool (BLASTn). MEGA-X software package was used to assess sequence homology and to perform phylogenetic analysis.

Results

ZH501-TC virus propagation and titration

ZH-501-TC strain of RVFV propagated in Vero cell cultures showed obvious cytopathic effect (CPE) 48-72 hours post-inoculation represented by rounding of cells, particle increasing in size, disruption and necro-cytosis (Fig. 1). The virus infectitivity titer was found to be 108 TCID50 /ml.

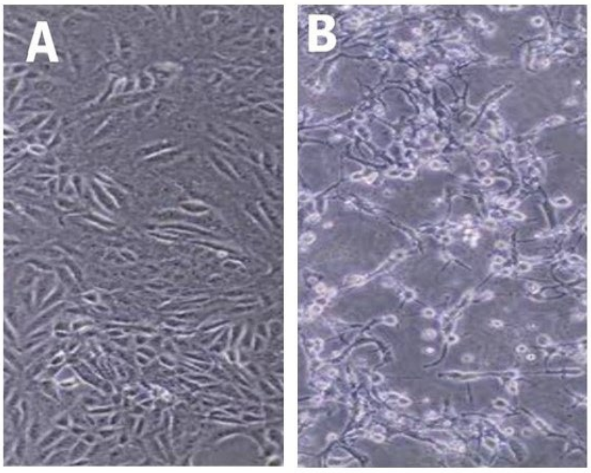

Fig. 1. Normal and RVFV (ZH-501-TC) infected Vero cells. A) Normal Vero cells monolayer sheet showing spindle cells. B) CPE of 48 hours RVFV (ZH-501-TC) infected Vero cells showing cell detachment, and cell rounding.

Mouse pathogenicity test

Mouse Intraperitoneal Lethal Dose 50 (MILD50) of ZH-501-TC RVF virus conducted in Swiss albino mouse was 108 MIPLD50 /ml. Mice started to succumb 5 days post infection with the ZH501-TC.

Amplification of partial Gn ectodomain fragment, sequencing and phylogenetic analysis

A 745 bp fragment partially covering the M segment Gn ectodomain of the TC adapted ZH501 strain was amplified as a single sharp band as appeared in gel electrophoresis (Fig. 2).

Phylogenetic analysis of ZH501-TC strain M segment Gn ectodomain partial fragment sequence and protein structure folding analysis

Deduced amino acids sequence of the obtained fragment of ZH501-TC virus was compared with sequences of twenty six ecologically and geographically different RVFV strains collected from Africa and Saudi Arabia in the period from 1944 to 2000 (Table 2 and Fig. 6). These strains

Table 1. Primers used for Identity M segment of RVFV (Shoemaker *et al*., 2002)

Primer name	5'-Primer sequence-3'	Location in RVFV genome	Product length
RVF-FORI RVF-REVE	GTCTTGCTTGAAAAGGGAAAA CCTGACCCATTAGCATG	M segment nt. 597-617 complementary to nt. 1326-1342 in M segment	745 bp

were clustered into six different lineages designated A, B, C, D, E and F, according to their full genome sequences (Fig. 3). Three nucleotide substitution (A1252G, T1257A, and G1258C) at the end of the parental vaccine strain (GU953292) sequence, were observed in comparison to the original ZH501 isolate (DQ380200) and the TC adapted virus (MZ218760) (Fig. 4) Deduced amino acids residues. Deduced amino acids residues of ZH501-TC virus (MZ218760) (A) and virulent ZH501 (DQ380200) (B) partially representing the Gn ectodomain, have been modelled by the single highest scoring to 5Y0Y RVFV GN-AU (DOI: 10.2210/pdb5Y05/pdb) template using Protein Homology/analogy Recognition (PHYR2) Engine V2.0 for protein modeling (Fig. 5).

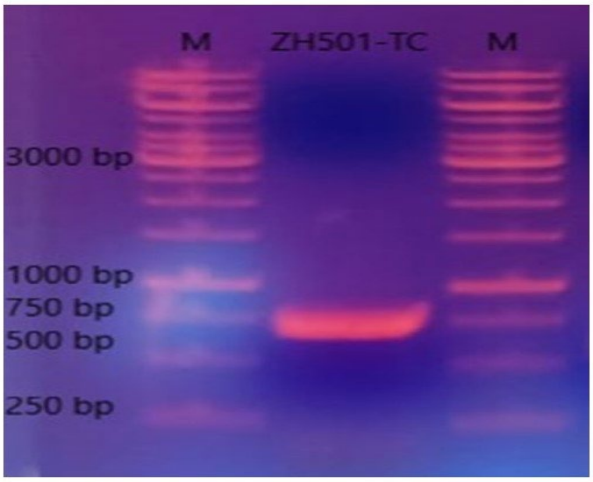

Fig. 2. Amplification of M segment fragment. RT-PCR of RVFV strain ZH-501-C using primer pair specific to RVFV M segment (table 1) revealed an amplicon of 745 bp approximate size. M lanes represent O'GeneRuler® ready to use 1 Kb DNA ladder.

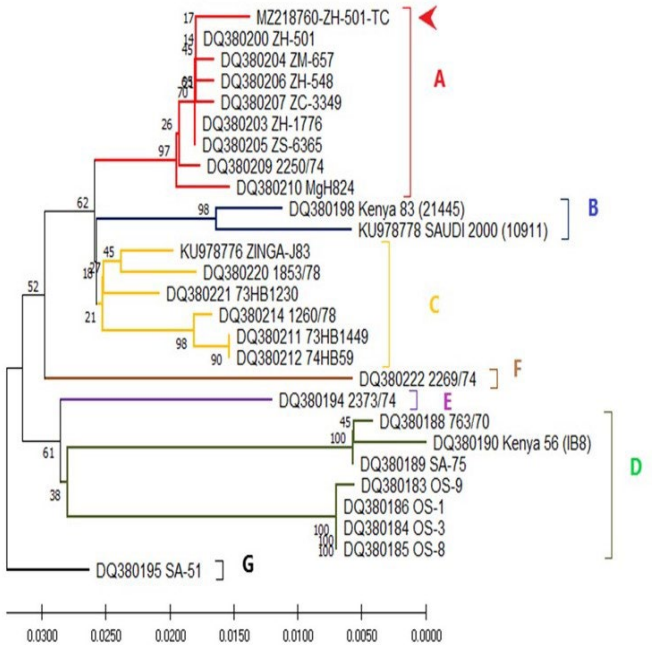

Fig. 3. Evolutionary relationships of different RVFV: The evolutionary history of RVFV-ZH501-tissue culture (TC) adapted strain was inferred using the Neighbor-Joining method based on a 714 base pair truncated fragment of M segment glycoprotein 2 (gp2). This analysis involved 26 nucleotide sequences in addition to RVFV-ZH501-TC adapted strain (denoted with red arrow). Each taxon name indicates GenBank accession numbers for the virus M segments, the strain, host, country of origin, and year of isolation were mentioned at table 2. Different RVFV lineages A, B, C, D, E, F, and G, were indicated for associated cluster. Evolutionary analyses were conducted using MEGA X software.

Discussion

Vaccines are the major control measure to protect animals from infection with RVFV and preventing transmission of infection to humans. In Egypt, inactivated RVFV vaccine is produced by Veterinary Serum and

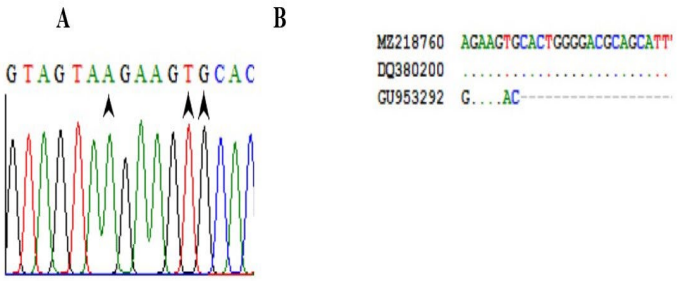

Fig. 4. Chromatogram and nucleotide sequence alignment of RVFV. (A) Sharp evenly-spaced peaks for M segment nucleotides 1246 to 1261 at RVFV-ZH501-TC sequence (MZ218760), with no base line noise. Three positions of nucleotide substitutions;A1252G, T1257A, and G1258C, at the end of the parental vaccine strain (GU953292) sequence, when compared to the original ZH501 isolate (DQ380200) and the TC adapted vaccine strain (MZ218760) (B).These three nucleotides (black arrows) appear with clear sharp peaks in the chromatogram of the tissue culture adapted strain (MZ218760) (A). Dots (.) indicate similar nucleotide and

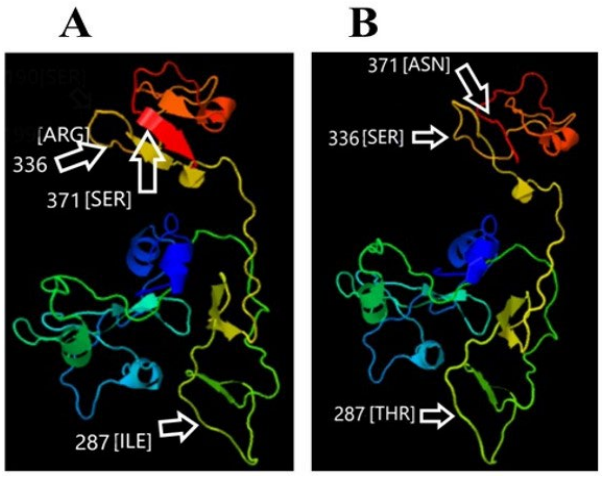

Fig. 5. Protein folding structure of sequenced M segment fragment.214 residues of ZH501-VSVRI-TC adapted virus (MZ218760) (A) and virulent ZH501 (DQ380200) (B) M segment fragment sequence, partially representing the Gn ectodomain, have been modelled with 100.0% confidence. Modelling was performed by the single highest scoring to 5Y0Y RVFV GN-AU (PDB DOI: 10.2210/pdb5Y05/pdb) template using Protein Homology/analogy Recognition (PHYR2) Engine V2.0 for protein modeling. Three amino acids missense mutations T287I, S336R and N371Sare pointed with white arrows. The missense mutation [ASN]N 371 S[SER]in the 367AQYASAYCS375 motif enhanced a ß sheet formation (red arrow) ZH501-VSVRI-TC adapted virus (MZ218760) (A) in as compared by virulent ZH501(DQ380200) (B).

Vaccines Research Institute (VSVRI), from the wild-type ZH-501 strain (El-Nimr, 1980). The vaccine is licensed for national use to vaccinate livestock in Egypt. Periodical analysis of the vaccine seed virus, following OIE guidelines for conventional vaccines production (OIE, 2018), is required to check for virus identity genetic stability. Previous studies analyzed the master seed of VSVRI vaccine virus nucleotide/amino acids sequences at different genome loci (Atwa *et al.*, 2011; Abo Hatab *et al.*, 2019). In this study, we used the same fragment of the GN ectodomain (eGN) which has been used before by Atwa *et al.* (2011) to analyze the identity and stability of the ZH501-TC adapted strain which evolved from the parental ZH501-VSVRI vaccine virus after ten years of vaccine production and adaptation in tissue culture.

Although, RVFV neutralizing antibodies are elicited against the dimeric surface glycoproteins GN and GC which are involved in virus attachment and entry and encoded by the viral M segment. The GC is imbedded in the viral envelope bilayer forming the iscosahedral scaffold and the ridges between capsomers, whereas the GN ectodomain (eGN) forms the protrusion spikes exposed to IgG antibodies (Schmaljohn *et al.*, 1989; Rusu *et al.*, 2012; Wu *et al.*, 2017). This highlights the importance of this fragment for analyzing its stability in the tissue culture adapted vaccine strain, ZH501-TC, used for vaccine production in Egypt.

A truncated 714 bases M segment fragment (nt. 612 – nt. 1325) nucleotide sequence, partially covering the eGN of the TC adapted ZH-501 strain (MZ218760), was analyzed in this study. Although the TC adapted strain showed close genetic relation to the virulent ZH501 strain in phylogenetic analysis, analysis of nucleotide sequence revealed two sites of nucleotides substitutions; C1033T and A1206C, corresponding to two amino acids changes; T287I and S336R, at the M segment of the

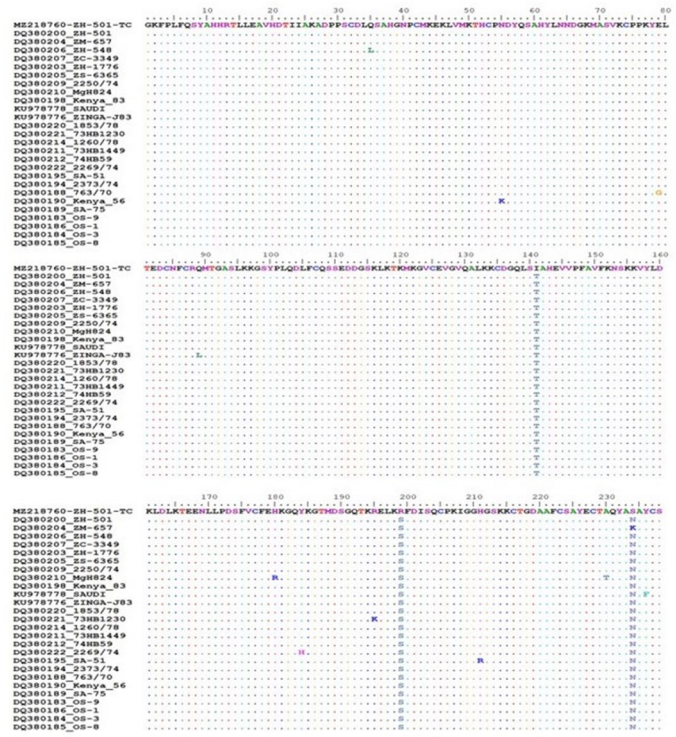

Fig. 6. Deduced amino acids alignment of amplified M segment fragment. Deduced amino acids of a truncated 714 bp amplified fragment of M segment representing partial RVFV G2 sequence, were aligned using BioEdit® software package. Identical amino acids with ZH-501-TC adapted strain are represented with dots (.) and colored characters represent mismatches.

TC adapted ZH-501 strain (MZ218760) compared to the parental virulent RVFV isolate ZH501 Egy Sh 77 (DQ380200). These mutations evolved in the parental vaccine strain ZH-501-VSVRI sequence (GU953292), as reported before by Atwa *et al.* (2011), and are stable by passaging in tissue culture for vaccine production as obtained for the TC adapted ZH-501 strain (MZ218760).

In our current study, we report a missense nucleotide substitution A1312G corresponding to the amino acid mutation N371S, in the ZH501-TC (MZ218760) compared to the parental virulent RVFV isolate ZH501 Egy Sh 77 (DQ380200) and other sequences published in the Gen Bank (Fig. 2 and Fig. 4). Unfortunately, that stretch of eGn sequence is not covered by the sequence published for the parental vaccine strain ZH-501-VSVRI (GU953292) (Atwa *et al.*, 2011) despite using the same primers set for M segment fragment amplification. We assume this missense mutation is evolved in the parental virulent RVFV isolate ZH501 Egy Sh 77 (DQ380200) due to passaging in tissue culture. This assumption comes in accordance with the results obtained by Lokugamage and Ikegami (2017), where potential risk of reversion mutation at the L-G3750A temperature-sensitive mutation after excessive viral passages in Vero culture cells was proved. Moreover, Moutailler *et al.* (2011) reported deletion of virulence factors encoded sequences at the NSs gene at the 15th serial passage onwards in BHK21 cells.

RVFV Gn 3D structure was predicted by Rusu *et al.* (2012) as a mixture of α- helical, ß-strands, and coil secondary structural elements where the Gn ectodomain (eGn) was rich in ß-strands than the transmembrane (TMD) and cytoplasmic tail domains (CTD). We have analyzed the protein folding structure of that stretch of eGn using Protein Homology/analogy Recognition (PHYR2) Engine V2.0 for protein modeling, prediction and analysis (Kelley *et al.*, 2015). It is noteworthy that the missense mutation [ASN]N371S[SER] (Fig. 4) enhanced ß sheet formation in ZH501-TC 367AQYASAYCS375 motif (Fig. 4), which might increase antigenicity of the eGn protein and hence increasing antigenicity of ZH501-TC vaccine strain.

Ikegami *et al.* (2015) identified a combination of three amino acid

Table 2. RVF viruses used in genetic analysis with ZH-501-TC strain at this study.

MZ Lineage	GenBank Accession No.	Virus strain	Isolated from	Country	Year of Isolation
	MZ218760	ZH-501-TC	Tissue culture adapted strain of ZH501 human isolate 1977	Egypt	1977 (current study)
A	DQ380200	ZH-501	Human	Egypt	1977
	DQ380204	ZM-657	Mosquito	Egypt	1978
	DQ380206	ZH-548	Human	Egypt	1977
	DQ380207	ZC-3349	Bovine	Egypt	1978
	DQ380203	ZH-1776	Human	Egypt	1978
	DQ380205	ZS-6365	Ovine	Egypt	1979
	DQ380209	2250/74	Bovine	Zimbabwe	1974
	DQ380210	MgH824	Human	Madagascar	1979
B	DQ380198	Kenya 83 (21445)	*Aedes macintoshi*	Kenya	1983
	KU978778	Saudi 2000 (10911)	Human	Saudi Arabia	2000
C	KU978776	Zinga	Human	CAR	1969
	DQ380220	1853/78	Bovine	Zimbabwe	1978
	DQ380221	73HB1230	Human	CAR	1973
	DQ380214	1260/78	Bovine	Zimbabwe	1978
	DQ380211	73HB1449	Human	CAR	1973
	DQ380212	74HB59	Human	CAR	1974
D	DQ380188	763/70	Bovine	Zimbabwe	1970
	DQ380190	Kenya 56 (IB8)	Bovine	Kenya	1956
	DQ380189	SA-75	Human	South Africa	1975
	DQ380183	OS-9	Human	Mauritania	1987
	DQ380186	OS-1	Human	Mauritania	1987
	DQ380184	OS-3	Human	Mauritania	1987
	DQ380185	OS-8	Human	Mauritania	1987
E	DQ380194	2373/74	Bovine	Zimbabwe	1974
F	DQ380222	2269/74	Bovine	Zimbabwe	1974
G	DQ380195	SA-51	Ovine	South Africa	1951

changes; two in M segment (Y259H [Gn] and R1182G [Gc]) and one in L segment (R1029K) to induce full attenuation of the wild type ZH-501 strain driving the MP12 vaccine strain. In addition, in vivo attenuation of RVFV was demonstrated in swiss albino mice through a single, P82I, amino acid mutation in the NSs protein (Borrego *et al*., 2021). At the same context, RVFV mutants lacking the M segment non-structural protein, Nsm, was strongly attenuated in mice (Kreher, *et al*., 2014).

At this study, pathogenicity test in mice was performed to investigate whether the aforementioned missense mutations and changes in the Gn protein folding affected the virulence of the tissue culture adapted ZH501-TC strain (MZ218760) in comparison to the parental virulent RVFV isolate ZH501 Egy Sh 77 (DQ380200) or not. Here we denote that the aforementioned mutations at the M segment T287I, S336R, and N371R did not affect virus virulence in Swiss albino mice, as fast progression of the disease and death of all tested mice was observed, indicating keeping of virulence of the tissue culture adapted vaccine virus strain (ZH501-TC) (MZ218760). This result comes in accordance with the high homology between ZH501-TC and the parental virulent strains isolated in 1977-1979. ZH501-TC strain was clustered with the parental ancestral virulent strains of ZH-501 isolated from Human, Bovine, Ovine and Mosquito in Egypt during 1977-1979 time periods. The allocation of ZH501-TC strain at the A designated lineage (Figure 2) indicating the genomic stability of ZH-501-TC vaccine strain and compatibility for vaccine preparation.

In addition, Atwa *et al*. (2011) mentioned other one missense nucleotide mutation; A1252G corresponding to the amino acid mutation K351R, and two other silent nucleotide substitutions; T1257A and G1258C, in ZH-501-VSVRI (GU953292) M segment; compared to the virulent parental RVFV isolated in 1977, ZH501 Egy Sh 77 (DQ380200). The TC adapted ZH-501 strain (MZ218760), did not show any nucleotide substitutions at the aforementioned three positions.In parallel, the chromatogram obtained for the tissue culture adapted vaccine seed virus strain (ZH501-TC) (MZ218760) showed clear sharp peaks for that part of the sequence (Fig. 3A). This misleading sequence reading could be explained by sequence artifacts at the end of sequence read of the truncated M segment fragment obtained by Atwa *et al*. (2011) as shown in Fig. 3B. Noteworthy, Atwa *et al*. (2011) obtained a shorter truncated sequence of 586 bases (GU953292), but we have obtained 714 bases (MZ218760) nucleotide sequence fragment of the eGn, despite using the same primer set indicating a better quality of the sequencing reaction.

Conclusion

In conclusion, reanalysis and data recall for the eGn partial nucleotide sequence of the TC adapted ZH501 seed vaccine virus confirmed the stability of the nucleotide mutations C1033T and A1206C, corresponding to two amino acids changes: T287I and S336R, by passaging in tissue culture cells. Here, we report a missense nucleotide substitution A1312G corresponding to the amino acid mutation N371S, in the ZH501-TC vaccine strain (MZ218760). This mutation enhanced ß sheet formation at the eGn antigenic motif 367AQYASAYCS375 which might increase antigenicity of the TC adapted seed virus vaccine. Despite these mutations at the eGn, the TC adapted ZH501 kept its virulence when infected mice. In addition, we resolved the incorrectness of the previously published two nucleotide mutations at the same fragment of eGn. These results confirm the compatibility of ZH501-TC virus for inactivated RVFV vaccine production.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abo Hatab, E.M., Ali, M.H., Atwa, M.H., Abul Magd, D.M., Soudy, A.F., Moussa, S.A., 2019. Propagation, purification and molecular characterization of Rift Valley Fever virus (ZH 501) for vaccine production in Egypt. J. App. Vet. Sci. 4, 13-17.

Amarasinghe, G. K., Arechiga Ceballos, N.G., Banyard, A.C., Basler, C.F., Bavari, S., Bennett, A.J., Blasdell, K.R., Briese, T., 2018. Taxonomy of the order Mononegavirales: update 2018. Arch. Virol. 163, 2283-2294.

Atwa, M.H., El-Sabagh, I.M., Amer, H.M., Saad, S., Yousif, A.A., Shalaby, M.A., 2011. ZH501-VSVRI: Is it Still the Best Choice for Vaccination Against RiftValley Fever in Egypt? J. Vaccines 2, DOI: 10.4172/2157-7560.1000121.

Balkhy, H.H., Memish, Z.A., 2003. Rift Valley fever: an uninvited zoonosis in the Arabian Peninsula. Int. J. Antimicrob. Agents 21, 153-157.

Barr, J.N., Wertz, G.W., 2005. Role of the conserved nucleotide mismatch within 3'- and 5'-terminal regions of Bunyamwera virus in signaling transcription. J Virol, 79, 3586-3594.

Bird, B.H., Ksiazek, T.G., Nichol, S.T., Maclachlan, N.J., 2009. Rift Valley fever virus. J. Am. Vet Med Assoc. 234, 883–93.

Bob, N.S., Hampate, B., Gamou F., El Khalil, I., Mamadou, Y., Diallo, Abdourahmane, S., Pape, M.S., Ousmane, F., Brahime, Mohamed L.S., Amadou, A.S., 2017. Detection of the Northeastern African Rift Valley Fever Virus Lineage during the 2015 Outbreak in Mauritania. Mauritania Open Forum Infect. Dis. 4, ofx087.

Borrego, B., Moreno, S., de la Losa, N., Weber, F., Brun, A., 2021. The Change P82L in the Rift Valley Fever Virus NSs Protein Confers Attenuation in Mice. Viruses 13.

Bridgen, A., Elliott, R.M., 1996. Rescue of a segmented negative-strand RNA virus entirely from cloned complementary DNAs. Proc. Natl. Acad. Sci. USA, 93, 15400-15404.

Chevalier, V., 2013. Relevance of Rift Valley fever to public health in the European Union. Clin. Microbiol. Infect. 19, 705-708.

Elliott, R.M., 2009. Bunyaviruses and climate change. Clin Microbiol Infect, 15, 510-517.

El-Nimr, M.M,. 1980. Studies on inactivated vaccine against RVFV. Ph.D. Thesis (Microbiology), Fac. Vet. Med. Assiut Univ. Egypt.

Golnar, A.J., Turell, M.J., LaBeaud, A.D., Kading, R.C., Hamer, G.L., 2014. Predicting the mosquito species and vertebrate species involved in the theoretical transmission of Rift Valley fever virus in the United States. PLoS Negl, Trop, Dis. 8, e3163.

Hatchett, R., Lurie, N., 2019. Outbreak response as an essential component of vaccine development. Lancet Infect. Dis. 19, e399-e403.

Ikegami, T., Hill, T.E., Smith, J.K., Zhang, L., Juelich, T.L., Gong, B., Slack, O.A., Ly, H.J., Lokugamage, N., Freiberg, A.N., 2015. Rift Valley Fever Virus MP-12 Vaccine Is Fully Attenuated by a Combination of Partial Attenuations in the S, M, and L Segments. J. Virol. 89, 7262-7276.

Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N., Sternberg, M.J., 2015. The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Protoc. 10, 845-858.

Kreher, F., Tamietti, C., Gommet, C., Guillemot, L., Ermonval, M., Failloux, A.B., Panthier, J.J., Bouloy, M., Flamand, M., 2014. The Rift Valley fever accessory proteins NSm and P78/NSm-GN are distinct determinants of virus propagation in vertebrate and invertebrate hosts. Emerg. Microbes Infect. 3, e71.

Lokugamage, N., Ikegami, T., 2017. Genetic stability of Rift Valley fever virus MP-12 vaccine during serial passages in culture cells. NPJ. Vaccines 2.

Maluleke, M.R., Phosiwa, M., van Schalkwyk, A., Michuki, G., Lubisi, B.A., Kegakilwe, P.S., Kemp, S.J., Majiwa, P.A.O., 2019. A comparative genome analysis of Rift Valley Fever virus isolates from foci of the disease outbreak in South Africa in 2008-2010. PLoS Negl. Trop. Dis. 13, e0006576.

Meegan J.M., Hoogstraal H., Moussa M.I., 1977. An epizootic of Rift Valley inEgypt in 1977. Vet. Rec. 105, 124-125.

Meegan J.M., 1979. The Rift Valley fever epizootic in Egypt 1977-78. 1. Description of the epizootic and virologic studies. Trans. Soc. Trop. Med. Hyg. 73, 618-623.

Metras, R., Edmunds, W.J., Youssouffi, C., Dommergues, L., Fournie, G., Camacho, A., Funk, S., Cardinale, E., Le Godais, G., Combo, S., Filleul, L., Youssouf, H., Subiros, M., 2020. Estimation of Rift Valley fever virus spillover to humans during the Mayotte 2018-2019 epidemic. Proc. Natl. Acad. Sci. USA 117, 24567-24574.

Morin, B., Coutard, B., Lelke, M., Ferron, F., Kerber, R., Jamal, S., Frangeul, A., Baronti, C., Charrel, R., de Lamballerie, X., Vonrhein, C., Lescar, J., Bricogne, G., Gunther, S., Canard, B., 2010. The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription. PLoS Pathog. 6, e1001038.

Moutailler, S., Roche, B., Thiberge, J.M., Caro, V.R., Rougeon, F., Failloux, A.B., 2011. Host Alternation Is necessary to Maintain the GenomeStability of Rift Valley Fever Virus. Plos Neg. Trop. Dis. 5, e1156.

Nanyingi, M.O., Munyua, P., Kiama, S.G., Muchemi, G.M., Thumbi, S.M., Bitek, A.O., Bett, B., Muriithi, R.M., Njenga, M.K., 2015. A systematic review of Rift Valley Fever epidemiology 1931-2014. Infect. Ecol. Epidemiol. 5, 28024.

OIE Terrestrial Manual, 2018. Chapter 3.1.18. – (Rift Valley fever (infection with Rift Valley fever virus). pp. 613-633.

Reed L, Muench D., 1938. A simple method of estimating fifty percent endpoints Am. J. Hyg., 27, 493-497.

Rolin, A.I., Berrang-Ford, L., Kulkarni, M.A., 2013. The risk of Rift Valley fever virus introduction and establishment in the United States and European Union. Emerg. Microbes Infect. 2, e81.

Rusu, M., Bonneau, R., Holbrook, M.R., Watowich, S.J., Birmanns, S., Wriggers, W., Freiberg, A.N., 2012. An assembly model of rift valley Fever virus. Front. Microbiol. 3, 254.

Schmaljohn, C.S., Parker, M.D., Ennis, W.H., Dalrymple, J.M., Collett, M.S., Suzich, J.A., Schmaljohn, A.L., 1989. Baculovirus expression of the M genome segment of Rift Valley fever virus and examination of antigenic and immunogenic properties of the expressed proteins. Virology 170, 184-192.

Schmaljohn, C., Hooper, J.W., 2001. Bunyaviridae: the viruses and their replication. In: Fields, B.N., Knipe, D.M. (Eds.), Fields Virology, 2.2 vols. Lippincott Williams & Wilkins, Philadelphia.

Shoemaker, T., Boulianne, C., Vincent, M.J., Pezzanite, L., Al-Qahtani M.M., 2002. Genetic analysis of viruses associated with emergence of Rift Valley Fever in Saudi Arabia and Yemen, 2000-2001. Emerg. Infect. Dis. 8, 1415-1420.

Taha, M.M., 1982. Studies on inactivated vaccine against RVFV. Ph D Thesis (Microbiology), Fac. Vet. Med. Cairo Univ. Egypt.

Tong, C., Javelle, E., Grard, G., Dia, A., Lacrosse, C., Fourie, T., Gravier, P., Watier-Grillot, S., Lancelot, R., Letourneur, F., Comby, F., Grau, M., Cassou, L., Meynard, J. B., Briolant, S., Leparc-Goffart, I., Pommier de Santi, V., 2019. Tracking Rift Valley fever: From Mali to Europe and other countries, 2016. Euro. Surveill. 24.

WHO, 2018. Rift Valley Fever. https://apps.who.int/iris/bitstream/handle/10665/273028/OEW27-300606072018.pdf)

WHO, 2019.Rift Valley Fever. https://www.who.int/emergencies/disease-outbreak-news/item/2019-DON205)

Wu, Y., Zhu, Y., Gao, F., Jiao, Y., Oladejo, B.O., Chai, Y., Bi, Y., Lu, S., Dong, M., Zhang, C., 2017. Structures of phlebovirus glycoprotein Gn and identification of a neutralizing antibody epitope. Proc. Natl. Acad. Sci. USA. 114, E7564–E7573.