Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(S1-2): 22-30. **OPEN ACCESS**

Isolation of Bovine Ephemeral fever virus and the potential transmission in Egypt and Middle East

Mohamed Nayel[1], Akram Salama[1], Walid Mousa[1], Amal Mahmoud[2], Mohammed Abo Elkhair[3], Ahmed Zaghawa[1], Mohammad S. Al-Aboody[4], Mosaab A. Omar[5,6] and Ahmed Elsify[1,*]

[1]Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, **Egypt.**
[2]Genetic Engineering & Biotechnology Research Institute, University of Sadat City, **Egypt.**
[3]Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, **Egypt.**
[4]Department of Biology, College of Science in Zulfi, Majmaah University, Majmaah 11952, **Kingdom of Saudi Arabia.**
[5] Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Qassim, 51452, **Kingdom of Saudi Arabia.**
[6] Department of parasitology, Faculty of Veterinary Medicine, South-Valley University, 83523 Qena, **Egypt**.

***Correspondence: ahmedelsify@gmail.com** **Received: 14 Nov. 2019, Revised: 19 Dec. 2019 Accepted: 20 Dec. 2019 e-Published: 26 Dec. 2019, Reviewed by: Prof. Dr. Adel Khadr, Prof. Dr. Magdy ElGabry**

Bovine ephemeral fever virus (BEFV) is an economically important arbovirus of cattle. The disease occurs seasonally in Egypt. Several observations suggest there may be a connection between the outbreaks which have occurred simultaneously in Middle East countries. This study aimed to explore BEFV transmission link between Egypt and other Middle East countries. The viral agent was successfully isolated from preserved leucocytic fractions of feverish cattle obtained during 2000, 2001 and 2004 Egyptian outbreaks through serial passages in brain of suckling mice and cell culture. Isolation of BEFV was confirmed by RT-PCR. Phylogenetic analysis based on the surface glycoprotein (G) gene including this study sequences and 97 BEFV isolates from Middle East, Australia and East Asia revealed that BEFVs detected in Egypt during 2000, 2001 and 2004 were almost identical and genetically close to the Japanese vaccinal strain (YHL isolate). All the Egyptian isolates clustered phylogenetically within the Middle East clade except the 2005 isolates, which clustered, with the China/East Asia clade. The Egyptian BEFV 2000, 2001 and 2004 isolates were genetically distance from Israeli isolates within the same periods. Even the Egyptian 2012 and 2017 isolates are not so close genetically to Israeli and Turkish isolates. These results suggest that the link between BEFV outbreaks that occurred in Middle East countries at approximately the same periods cannot be attributed to winds and animal transport only but also, environmental factors such as hot and humid weather that favored the abundancy of the vectors in the region during the same time.

Keywords: Bovine ephemeral fever; Isolation; RT-PCR; Phylogeny; Middle East

INTRODUCTION

Bovine ephemeral fever (BEFV) is a non-contagious, arthropod-borne disease of cattle and water buffaloes transmitted by hematophagous arthropod vectors (Culicoides biting midges and mosquitoes) (St George, 1988; Walker, 2005). BEFV is the type species of the genus Ephemero virus, Rhabdoviridae family. BEFV is bullet shaped possesses a single stranded, negative-sense RNA genome (14,900 nucleotides) consists

of five structural protein genes; (N), a polymerase-associated protein (P), a matrix protein (M), a large RNA-dependent RNA polymerase (L) and a glycoprotein (G) spanning the viral envelope (Walker et al., 2012). The G gene encodes glycoprotein G which is responsible for cell attachment and entry, It is also a type-specific neutralizing antigen and induces protective immunity in cattle (Uren et al., 1994; Hertig et al., 1996; Johal et al., 2008).

BEFV is common in tropical, subtropical and temperate regions of Africa, Asia, Australia and the Middle-East with considerable economic impact due to reduced milk production at dairy farms and loss of conditioning of beef cattle (Walker et al., 2012).

In Egypt, BEF was known as “dengue fever” of cattle in the late 19th century but the first detailed report was of an epizootic in 1909 that commenced at Aswan, travelled down the Nile Valley to Cairo and spread across the Delta to the coast (Ragbagliati, 1924). Subsequent outbreaks affecting hundreds of cattle occurred in 1915 and 1919–1920. More recently, the disease was reported during the summers of 1990–1991, 2000–2001, 2004–2005, 2009-2010 and 2012 (Davies et al., 1992; Zaher and Ahmed, 2011; Kasem et al., 2014).

As previously reported, BEFV sequences from the world are clustered into three distinct lineages: East Asia, Australia and the Middle East (Kato et al., 2009; Aziz-Boaron et al., 2012; Walker et al., 2012; Zheng and Qiu, 2012a). This geographic distinction explained to be resulted from circulation of the virus in a vast distance by vectors and winds in Australia, East Asia and Middle East (Murray, 1970; Ogawa, 1992; Shirakawa et al., 1994; Finlaison et al., 2010).

The aim of this study was isolation of the BEFV and comparative phylogenetic analysis of BEFV isolates to reveal the BEFV geodynamics in Egypt and Middle East.

MATERIALS AND METHODS

Samples:

Heparinized blood samples were collected from clinically suspected feverish cattle for separation of buffy coat as described previously (van der Westhuizen, 1967). Briefly, the blood samples were centrifuged at 2000 ×g for 5 min. Buffy coats were aspirated, washed with phosphate-buffered saline (PBS), re-suspended in PBS and stored at-70°C for virus isolation

Virus Isolation:

Isolation of BEFV was carried out in the brains of suckling mice and baby hamster kidney (BHK-21) cells as described previously (van der Westhuizen, 1967). Briefly, the buffy coats of the feverish cattle were frozen and thawed 3 times and 10 µl was inoculated intracerebrally into suckling mice (1-3 day age). The brain of inoculated baby mice were extracted 7 days post inoculation and were grinded with PBS, the homogenates were frozen and thawed 3 times, centrifuged at 2000 × g for 5 min and the supernatant was inoculated into suckling mice’s brains again until the 4th passages when nervous signs were observed. Confluent BHK-21 cells monolayers in 25 cm^2 flask were inoculated with 400 µl of homogenate from the 4th passages of infected suckling mice with clear nervous manifestations and incubated at 37 °C for 1 h, then cell maintenance medium containing: DMEM (Gibco), Glucose, Calf serum (5%), NaHCO3, Penecillin and Streptomycin, was added into the monolayers. The cultures were maintained at 37 °C for 8 days and observed every 24 h intervals. The flasks were frozen-thawed 3 times, they were centrifuged and the supernatant were inoculated into new monolayers. The virus underwent 4 passages until cytopathogenic effects (CPE) were observed.

RNA extraction and PCR amplification:

The existence of BEFV was confirmed by reverse transcription polymerase chain reaction (RT-PCR) as described previously (Zaghloul et al., 2012). Viral RNA was extracted from whole blood, baby mice brain tissue and cell culture by means of one step-RNA reagent (Bio Basic Inc, Canada) according to the manufacturer’s instructions. RNA reverse transcription was performed using a forward primer BEFV F 5' CATTATGGGATAGGATCC 3' and a reverse primer BEFV R 5' TACAACAGCAGATAAAAC 3' obtained from the virion transmembrane glycoprotein G gene (3058-4929 nt) of the BEFV isolate BB7721 accession number (AF234533). The RT-PCR was carried out using QIAGEN One-Step RT-PCR Kit. The RT-PCR thermocycling were 30 min at 50°C, 2 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 45°C, 1 min at 72°C and 10 at 72°C. The amplified RT-PCR products were visualized in 1% agarose gel.

Sequence and phylogenetic analysis:

The PCR products were purified and sequenced using an AB1 PRISM 3100 genetic

analyzer (Applied Biosystems, USA, gene link DNA Sequencing service, New York, USA). The obtained sequences were edited and aligned with 97 other sequences retrieved from the GenBank using ClustalW algorithms available in the Molecular Evolutionary Genetic Analysis (MEGA version X) software, Phylogenetic tree was built by Maximum Parsimony method in MEGA version X (Kumar et al., 2018). The evolutionary distances were computed using the Jukes-Cantor method. One thousand bootstrap replicates were conducted to assess statistical support for the tree topology.

RESULTS

Virus isolation

The clinical manifestation of suckling mice after intracerebral inoculation with leucocytic fraction from cattle clinically affected with bovine ephemeral fever virus appeared gradually, beginning from arched back, circling, paresis, paralysis and finally death. The severity of clinical signs is different according to the level of passage. Additionally, the BHK-21 monolayer showed cells rounding, chromatin condensation, aggregation, lysis, and detachment fourth day after inoculation.

Reverse transcription polymerase chain reaction (RT-PCR)

The designed primers amplified specific DNA fragments of 438 bp (4201-4638) of the virion transmembrane glycoprotein G gene from BHK-21 cells and suckling mice brains inoculated with the BEFV (Fig. 1).

Phylogenetic analysis of the partial G gene sequences

The three sequences; Monufiya 2001, Alexandria 2000 and Demitta 2004 were almost identical (99.68% – 100%) using Clustal W. Additionally, alignment of this study BEFV sequences with 97 sequences from the world revealed that the BEFV isolates in the present case, were found to be most closely related to a Japanese vaccine strain, YHL (99%). Also, it were closely related (97%) to several other Japanese isolates from 1988 and 1989, as well as three isolates in Taiwan from 1984, 1996 and 1999 and the 1976 isolate JB76H from China. For the Middle East isolates, this study BEFV sequences were almost 96% identical with the Egyptian isolates EGY12 which was isolated in 2012 and strain Egy which was isolated in 2017. Also, they were almost 95% related to the 2012 Turkish isolates, 94% related to the Iranian 2012/2013 isolates, 93% related to the Israeli isolates in 2000, 2001, 2004 and 2010 isolates and 92% related to 2008 and Ad12 which was isolated in 2017 Turkish isolates Tab. 1.

In the present study, All the Egyptian isolates clustered phylogenetically together except the 2005 isolates, which clustered, with the China/East Asia isolates (Fig. 2.).

Israeli isolates clustered phylogenetically together. Turkish isolates were divided into two groups; Turkish isolates 2012/TR/CU15, 2012/TR/CU16 and 2012/TR/CP3 together with isolates IR-2012 and IR-2013 from Iran were clustered within the China/East Asia isolates, while Turkish isolates 2012/TR/ADYMN, 2008/TR/CP77 and 2008/TR/CP62 clustered more closer to the Israeli isolates (Fig. 3.).

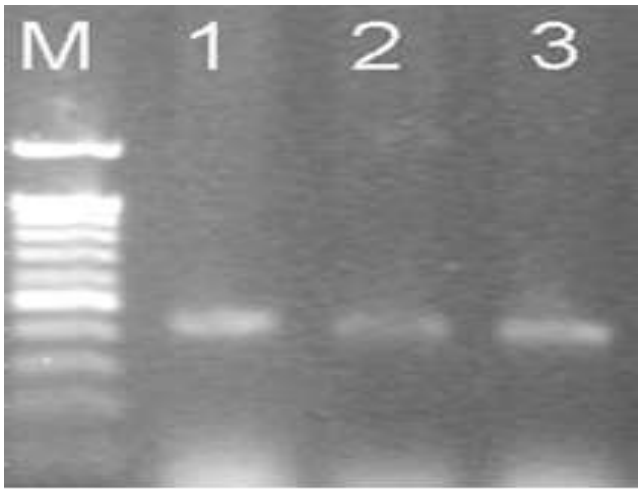

Figure 1: RT-PCR detection of BEFV. M is 1kb ladder. Lanes 1, 2 and 3 are BEFV isolates.

Table 1: Identity % of Monufiya 2001 isolates and other Bovine ephemeral fever virus (BEFV) sequences.

Accession number	Strain	Year	Country	Identity %
AB462028	YHL	1966	Japan	99
AB462029	Hirado-6	1988	Japan	97
AB462030	Hirado-9	1988	Japan	97
AB462031	Amakusa-1	1988	Japan	97
AB462033	Azuma	1988	Japan	97
AB462035	ON-BEF-88-3	1988	Japan	97
AB462036	ON-BEF-88-4	1988	Japan	97
AB462037	ON-BEF-89-1	1989	Japan	97
AB462038	ON-BEF-89-2	1989	Japan	97
AB462039	ON-BEF-89-3	1989	Japan	97
AF208840	Tn88128	1999	Taiwan	97
KJ605424	1996-TC-Taiwan	1996	Taiwan	97
AY935239	1984/TW/TN1	1984	Taiwan	97
AY935240	1996/TW/TN1	1996	Taiwan	97
JQ728557	JB76H	1976	China	97
KJ729108	EGY12	2012	Egypt	96
MH237603	Egy	2017	Egypt	96
KC470312	2012/TR/CU15	2012	Turkey	95
KC470313	2012/TR/CU16	2012	Turkey	95
KC470310	2012/TR/CP3	2012	Turkey	95
MF491475	IR-2012	2012	Iran	94
MF491476	IR-2013	2013	Iran	94
JN833630	ISR00	2000	Israel	93
JN833631	ISR01	2001	Israel	93
JN833632	ISR04	2004	Israel	93
JN833633	ISR10/1	2010	Israel	93
JN833634	ISR10/2	2010	Israel	93
JN833635	ISR10/3	2010	Israel	93
GQ229451	2008/TR/CP62	2008	Turkey	92
GQ229452	2008/TR/CP77	2008	Turkey	92
KY012742	BEFV/Ad12/TUR	2012	Turkey	92

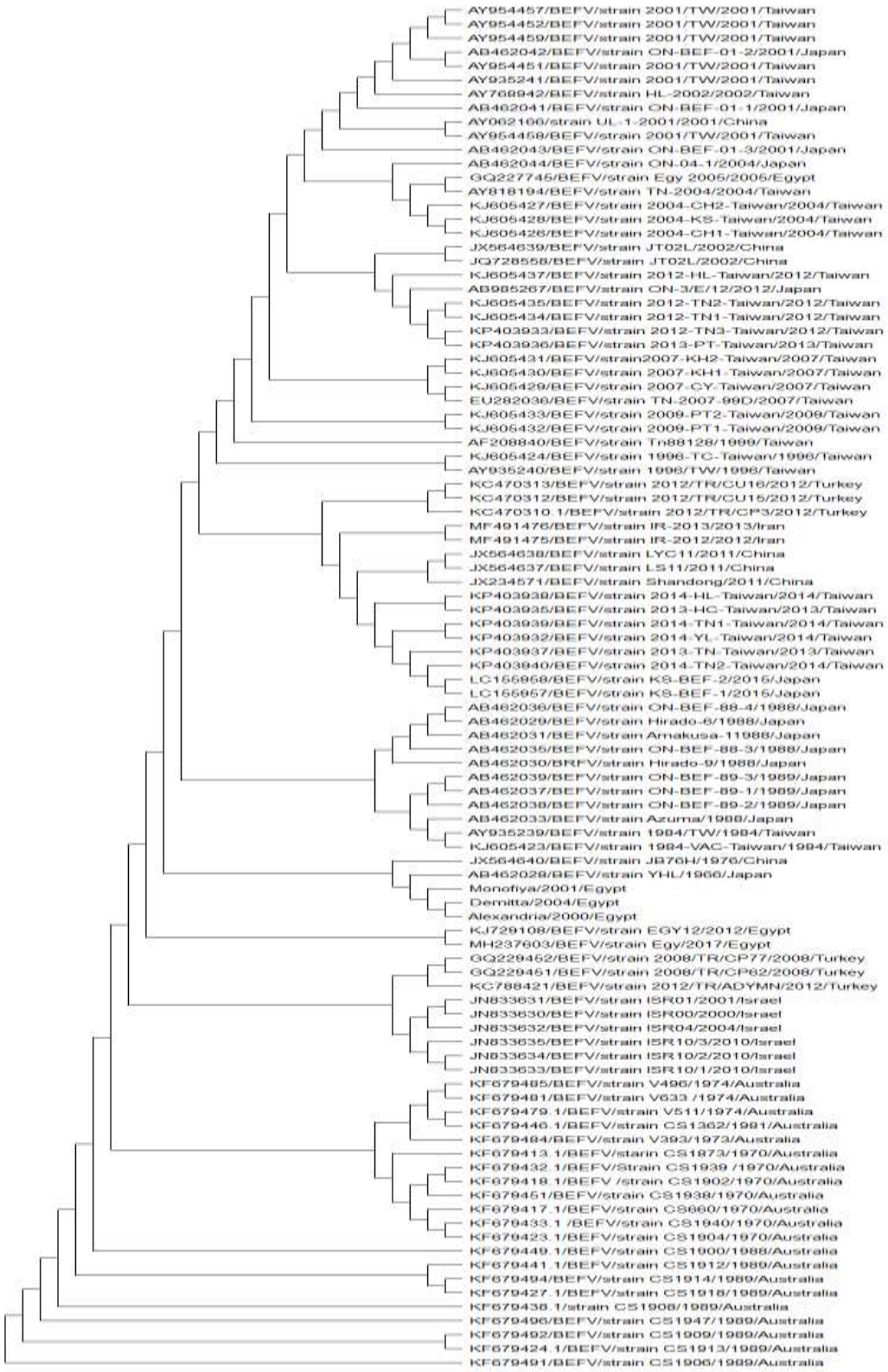

Figure 2; A Maximum Parsimony Phylogenetic tree of the BEFV partial glycoprotein G sequence of the Egyptian isolates and isolates from other parts of the world.

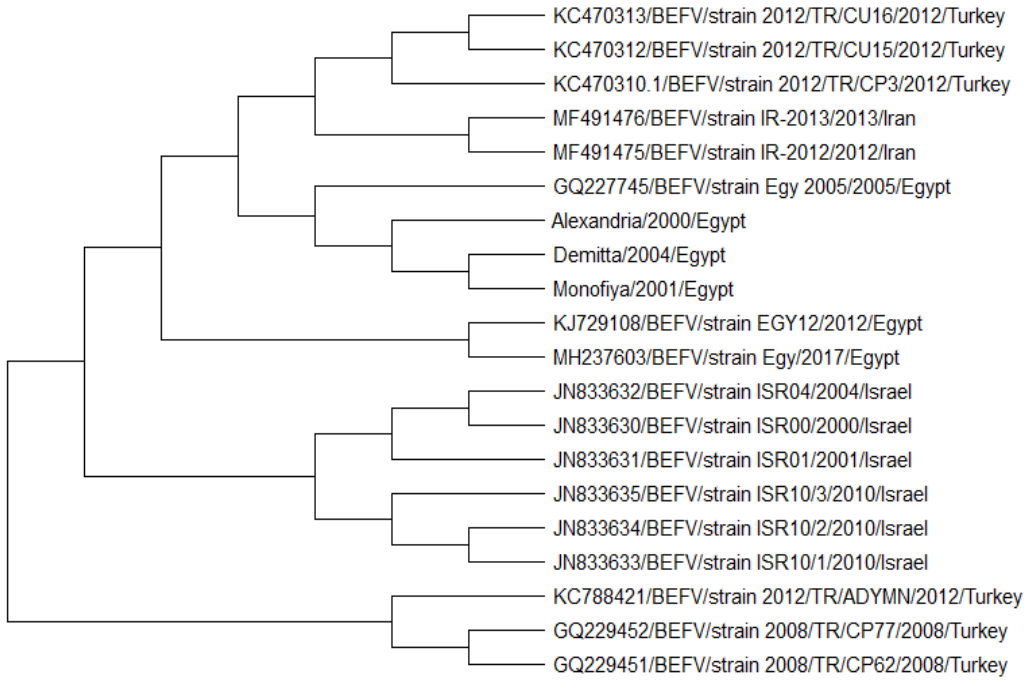

Figure 3; A Maximum Parsimony Phylogenetic tree of the BEFV partial glycoprotein G sequence of the Egyptian isolates and isolates from The Middle East.

DISCUSSION

BEF is one of the economically important cattle diseases in Egypt. Over the past 30 years, the incidence of BEF epizootics has increased in Egypt and other Middle East countries (Walker and Klement, 2015). Numerous observations suggest a connection between the epizootics which have occurred concurrently in Egypt, Israel and Saudi Arabia in 1990, in Egypt, Israel and Turkey in 1999–2000, in Egypt and Israel in 2004, and in Turkey and Israel in 2008 (Walker and Klement, 2015). The current study was designed to explore the possible methods of circulation of BEFV in Middle East and relation between isolates from different Middle East countries.

Clinical observation-based diagnosis of BEF is somewhat difficult and problematical. Therefore, confirmatory diagnosis is necessary to implement serious and accurate prophylactic action, Assessment of the disease extent and trustful epidemiological surveys (Bakhshesh and Abdollahi, 2015).

BEFV was successfully isolated from peripheral blood mononuclear cells obtained during 2000, 2001 and 2004 outbreaks in Egypt. The clinical appearance of the inoculated mice and CPE in cell culture were typical of BEF previously recorded in various reports (van der Westhuizen, 1967; Bakhshesh and Abdollahi, 2015; Zaghawa, 2017).

The conventional RT-PCR as a sensitive, specific, rapid test for detection of BEFV in clinical samples and important useful tool for testing RNA samples extracted from peripheral blood mononuclear cells (Hsieh et al., 2006; Niwa et al., 2015) was used to confirm existence of BEFV in field samples and inoculated brain and tissue culture. Our RT-PCR results support others in the belief that RT-PCR is a superior test for the provision of rapid and specific data for BEFV detection (Zaghawa, 2017).

The adaptation of BEFV to BHK-21 cells and suckling mice had no important influence on the nucleotide and amino acids sequences of the glycoprotein G (Zheng and Qiu, 2012b). Partial G protein sequences of 2000, 2001 and 2004 Egyptian BEFV isolates confirmed that these isolates were BEFV and closely related to YHL, a Japanese vaccinal strain representing the deepest China/East Asia clade ancestral node (Walker and Klement, 2015). This close relation with the Japanese sequence is difficult to interpret in this study; we could not obtain BEFV samples before

the year 2000 for further investigations.

BEFV sequences from Egypt including this study isolates together with a 2012 isolate and a 2017 isolate clustered phylogenetically together except the 2005 isolate. Egy 2005 BEFV isolate was closely related to TN-2004 isolate from Taiwan. It was proposed that the virus could be transmitted from China to the Middle East through cattle trading (Aziz-Boaron et al., 2012).

The phylogeny using the partial glycoprotein G sequences have revealed that all Egyptian BEFV strains isolated since 2000 have at least two ancestors indicating that, the virus has entered Egypt on different occasions.

In the present study, the Middle East BEFV isolates from Egypt clustered phylogenetically together, Israeli isolates clustered phylogenetically together, while Turkish isolates were clustered in two different clades. Clustering of the Turkish isolates 2012/TR/CU15, 2012/TR/CP3 and 2012/TR/CU16 together with isolates IR-2012 and IR-2013 from Iran within the China/East Asia isolates coincided with previous report of (Bakhshesh and Abdollahi, 2015). The close genetic relationship of Turkish isolates 2012/TR/ADYMN, 2008/TR/CP77 and 2008/TR/CP62 with Israeli isolates coincided with previous report of (Aziz-Boaron et al., 2012).

The phylogenetic analysis suggests that Middle East BEFV is evolving from different clades and Refers to an epidemiological distance between viruses that circulate throughout the region. The high similarity between the 2008 Turkish and Israeli isolates (Aziz-Boaron et al., 2012). In addition to the high similarity of the 2012 Iranian and Turkish isolates (Bakhshesh and Abdollahi, 2015) may point to that the BEFV has been circulating in the area for a long period and that there is transmission and occasional re-introduction of the virus between Middle East countries.

The transmission of BEFV between the Middle East countries has been suggested to occur by both animal transport and winds; this theory was supported by close genetic relationship between some Middle East isolates and East Asian isolates, confirmed animal transport from China to Middle East and separation of sequences from Turkey in 2012 into different lineages. (Aziz-Boaron et al., 2012; Bakhshesh and Abdollahi, 2015). However, this hypothesis of BEFV circulation in the Middle East by both winds and animal transport cannot explain the genetic dissimilarity reported in this study between, Egyptian and Israeli BEFV isolates during synchronous outbreaks. The possible explanation could be the similar hot humid environmental condition that favored the abundancy of the vectors in both country during the same time, which should be considered beside both winds and animal transport to explain geodynamics of BEF in the Middle East.

CONCLUSION

In this study, scientific technique for BEFV isolation and reliable diagnosis using molecular approach for detection of BEFV in Egypt were described. Comparative phylogenetic analysis of BEFV isolates certainly improves our knowledge about the geodynamics of BEFV in the Middle East and the world. Further studies are required to draw the transmission routes for BEFV in the Middle East and the world.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Authors would like to thank the staff members of the animal infectious diseases laboratory, Faculty of Veterinary Medicine, University of Sadat City for their collaboration.

AUTHOR CONTRIBUTIONS

M.N., A.S. and A.M. carried out the experiment. M.N. wrote the manuscript with support from W.M and M.A. O.A., M.O. and A. E. investigate the findings of this work. M.N. and A.Z. conceived the original idea.

REFERENCES

Aziz-Boaron, O, Klausner, Z, Hasoksuz, M, Shenkar, J, Gafni, O, Gelman, B, David, D, Klement, E, 2012. Circulation of bovine ephemeral fever in the Middle East--strong

evidence for transmission by winds and animal transport. Veterinary microbiology 158: 300-307.

Bakhshesh, M, Abdollahi, D, 2015. Bovine Ephemeral Fever in Iran: Diagnosis, Isolation and Molecular Characterization. Journal of arthropod-borne diseases 9: 195-203.

Davies, F, Moussa, A, Barsoum, G, 1992. The 1990–1991 epidemic of ephemeral fever in Egypt and the potential for spread to the Mediterranean region, Proceedings of the 1st International Symposium on Bovine Ephemeral Fever and Related Rhabdoviruses, Beijing, pp. 54-56.

Finlaison, DS, Read, AJ, Kirkland, PD, 2010. An epizootic of bovine ephemeral fever in New South Wales in 2008 associated with long-distance dispersal of vectors. Australian veterinary journal 88: 301-306.

Hertig, C, Pye, AD, Hyatt, AD, Davis, SS, McWilliam, SM, Heine, HG, Walker, PJ, Boyle, DB, 1996. Vaccinia virus-expressed bovine ephemeral fever virus G but not G(NS) glycoprotein induces neutralizing antibodies and protects against experimental infection. The Journal of general virology 77 (Pt 4): 631-640.

Hsieh, YC, Wang, SY, Lee, YF, Chen, SH, Mak, PO, Chu, CY, 2006. DNA sequence analysis of glycoprotein G gene of bovine ephemeral fever virus and development of a double oil emulsion vaccine against bovine ephemeral fever. The Journal of veterinary medical science 68: 543-548.

Johal, J, Gresty, K, Kongsuwan, K, Walker, PJ, 2008. Antigenic characterization of bovine ephemeral fever rhabdovirus G and GNS glycoproteins expressed from recombinant baculoviruses. Archives of virology 153: 1657-1665.

Kasem, S, Dawod, A, Khodier, M, Kotb, A, Abouelyazeed, M, Abdel kareem, S, Ibrahim, B, 2014. Isolation and Characterization of Bovine Ephemeral Fever Virus in Delta Provinces, Egypt 2012. Global Veterinaria 13: 972-976.

Kato, T, Aizawa, M, Takayoshi, K, Kokuba, T, Yanase, T, Shirafuji, H, Tsuda, T, Yamakawa, M, 2009. Phylogenetic relationships of the G gene sequence of bovine ephemeral fever virus isolated in Japan, Taiwan and Australia. Veterinary microbiology 137: 217-223.

Kumar, S, Stecher, G, Li, M, Knyaz, C, Tamura, K, 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35: 1547-1549.

Murray, MD, 1970. The spread of ephemeral fever of cattle during the 1967-68 epizootic in Australia. Australian veterinary journal 46: 77-82.

Niwa, T, Shirafuji, H, Ikemiyagi, K, Nitta, Y, Suzuki, M, Kato, T, Yanase, T, 2015. Occurrence of bovine ephemeral fever in Okinawa Prefecture, Japan, in 2012 and development of a reverse-transcription polymerase chain reaction assay to detect bovine ephemeral fever virus gene. The Journal of veterinary medical science 77: 455-460.

Ogawa, T, 1992. Epidemiological investigation of bovine ephemeral fever outbreaks in Kyusyu Island in Japan during the fall of 1988. Preventive veterinary medicine 14: 69-76.

Ragbagliati, D, 1924. Three day's fever or stiff sickness in cattle. The Veterinary record 4: 503-505.

Shirakawa, H, Ishibashi, K, Ogawa, T, 1994. A comparison of the epidemiology of bovine ephemeral fever in South Korea and south-western Japan. Australian veterinary journal 71: 50-52.

St George, T, 1988. Bovine ephemeral fever: a review. Tropical animal health and production 20: 194-202.

Uren, MF, Walker, PJ, Zakrzewski, H, St George, TD, Byrne, KA, 1994. Effective vaccination of cattle using the virion G protein of bovine ephemeral fever virus as an antigen. Vaccine 12: 845-850.

van der Westhuizen, B, 1967. Studies on bovine ephemeral fever. I. Isolation and preliminary characterization of a virus from natural and experimentally produced cases of bovine ephemeral fever. The Onderstepoort journal of veterinary research 34: 29-40.

Walker, P, 2005. Bovine ephemeral fever in Australia and the world, The World of Rhabdoviruses. Springer, pp. 57-80.

Walker, PJ, Blasdell, KR, Joubert, DA, Dietzgen, R, Kuzmin, I, 2012. Ephemeroviruses: arthropod-borne rhabdoviruses of ruminants, with large and complex genomes. Rhabdoviruses: Molecular Taxonomy, Evolution, Genomics, Ecology, Host-Vector Interactions, Cytopathology and Control: 59-88.

Walker, PJ, Klement, E, 2015. Epidemiology and control of bovine ephemeral fever. Veterinary research 46: 124-134.

Zaghawa, A, Housawi, F., Al-Naeem, A., Elsify, A., & Hegazy, Y. , 2017. Bovine ephemeral fever epidemics in Kingdom Saudi Arabia: clinical, epidemiological and molecular investigation. The Journal of Infection in Developing Countries 11: 854-860.

Zaghloul, AH, Amal Mahmoud, Hassan, HY, Hemeida, AA, Nayel, MA, Zaghawa, A, 2012. Establishment of Dot-blot Hybridization for Diagnosis of Bovine Ephemeral Fever Virus in Egypt. International Journal of Virology 8: 271-278.

Zaher, KS, Ahmed, WM, 2011. Investigations on bovine ephemeral fever virus in Egyprian cows and buffaloes with emphasis on isolation and identification of a field strain. Glob Vet 6: 447-452.

Zheng, F, Qiu, C, 2012a. Phylogenetic relationships of the glycoprotein gene of bovine ephemeral fever virus isolated from mainland China, Taiwan, Japan, Turkey, Israel and Australia. Virology journal 9: 268.

Zheng, F, Qiu, C, 2012b. Phylogenetic relationships of the glycoprotein gene of bovine ephemeral fever virus isolated from mainland China, Taiwan, Japan, Turkey, Israel and Australia. Virology journal 9: 268-268.