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## Genetic Characterization of Rift Valley Fever Virus in Insectivorous Bats, Egypt

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### Abstract

**Background:** The endemic character of Rift Valley fever (RVF) disease points toward an interepidemic reservoir. Although not yet identified, bats and rodents may be implicated in RVF virus (RVFV) epidemiology. In this study, we investigated the putative role of Egyptian frugivorous and insectivorous bats in RVFV epidemiology in Egypt.

**Methods:** From 2019 to 2021, 200 bats of two different species from six Egyptian governorates were tested for phleboviruses using real-time RT-PCR (rRT-PCR) and sequence analysis.

**Results:** Screening through rRT-PCR showed evidence of the RVFV genome only in insectivorous bats. Partial sequence and phylogenetic analysis based on S and M genome segments showed that these viruses are genetically similar to those circulating (clade A) in livestock and humans during previously reported RVFV outbreaks in 1977/78 and 2003 in Egypt.

**Conclusions:** Our molecular data suggest that the bat *Pipistrellus deserti* could play a role in RVFV ecology in Egypt.

**Keywords:** *Pipistrellus deserti* bat, Egypt, interepidemic periods, Rift Valley fever virus, sequencing

### Introduction

THE EMERGENCE OF the Rift Valley fever virus (RVFV) in new territories, as reported during outbreaks in Egypt (1977), Western Africa (1988), and the Arabian Peninsula (2000), or re-emergence after long periods of silence, as observed in Kenya and South Africa in 2018, has highlighted the importance of RVFV surveillance and monitoring (Jansen van Vuren et al. 2019). The first Egyptian RVFV epidemic occurred in 1977/78 in five governorates of the Nile Valley (Sharqia, Aswan, Qalyubia, Giza, and Asyut), resulting in a large number of human infections and massive economic losses in livestock.

After this outbreak, further outbreaks recurred at irregular intervals (1993, 1994, 1997, and 2003) (Kamal 2011). The source of the diverse outbreaks has been extensively discussed, but the maintenance of the virus during interepidemic periods (IEPs) is still not fully understood (Meegan 1979).

Mosquitoes are considered the most important factor for long-term RVFV maintenance; however, transovarial transmission (TOT) has been reported only in a single species of mosquitoes in Kenya (Linthicum et al. 1985). Furthermore, RVFV circulates unnoticed in wildlife mammals for many generations before being noticed during unexpected spillover events (Olive et al. 2012).

Therefore, the contribution of wildlife mammals to RVFV persistence remains an important question (Bird and McElroy 2016). Egypt is continental Africa's most densely populated country, with only a few bat species. Insectivorous (*Pipistrellus deserti*) and frugivorous (*Rousettus aegyptiacus*) bats are found mostly in the aridest areas of the Sahara as well as abandoned structures and fruit gardens in proximity to humans (Benda et al. 2008). This study was conducted to investigate whether the bat population in the main Egyptian governorates harbors the RVFV genome, thus, contributing to a better understanding of the ecology of the virus. We used

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molecular detection, sequencing, and phylogenetic analyses to identify RNA sequences from the RVFV in Egyptian frugivorous and insectivorous bats.

## Materials and Methods

This study and sampling protocol were approved by the Institutional (Faculty of Veterinary Medicine, Cairo University), Animal Care and Use Committee (approval number CU28/04/2021/274).

Bats were collected from six different Egyptian governorates (Sharqia, Giza, South Sinai, Qena, Sohag, and Asyut) from December 2019 to May 2021. RVFV was present in three of the governorates (Sharqia, Qalyubia, and Giza) during the last outbreak in 2003. The collected bats were morphologically classified as frugivorous ( $n=160$ ) and insectivorous ( $n=40$ ). Eight-hundred samples, including the liver, brain, spleen, and salivary glands, were harvested separately from all individuals (four organs from each individual,  $4 \times 200$ ), inactivated in RNAlater™ Stabilization Solution (Thermo Fisher Scientific, #AM7021), and stored at  $-80^{\circ}\text{C}$  until use.

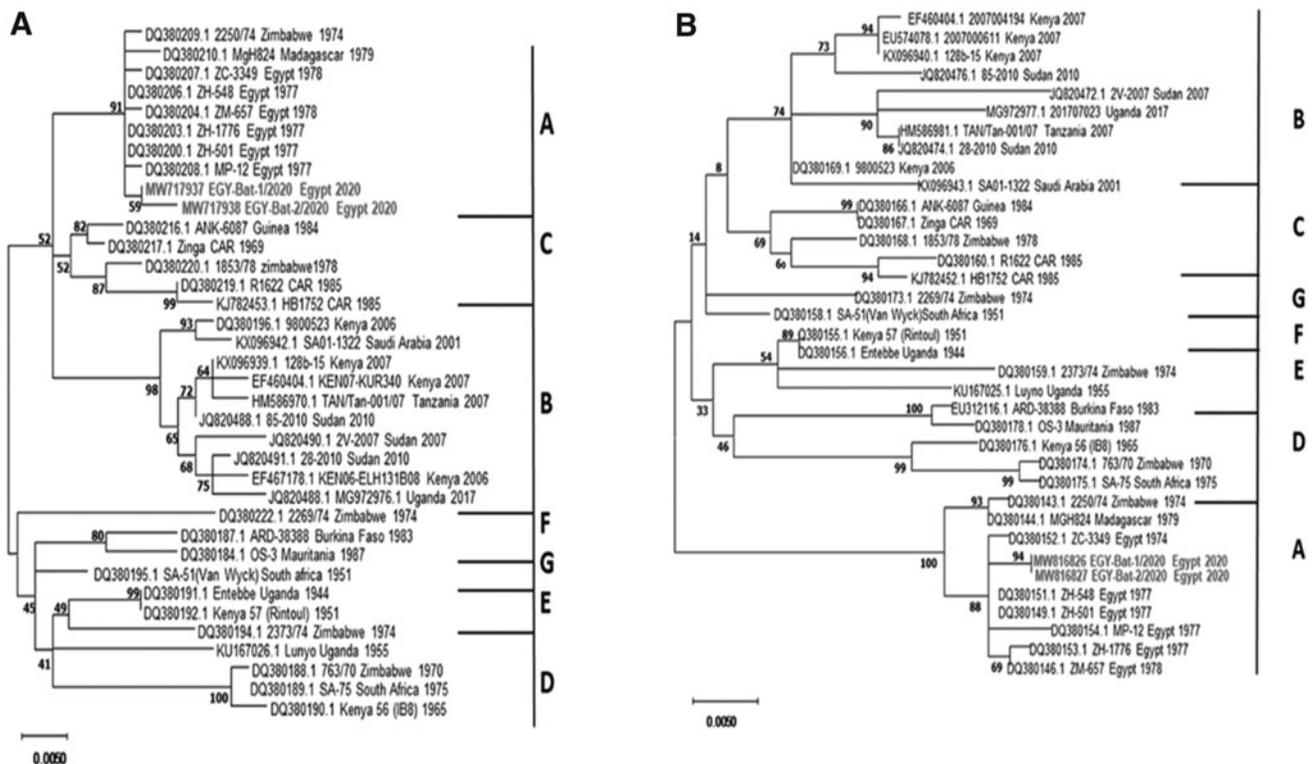
Viral RNA was extracted directly from the tissues of all individual bats using the RNeasy Mini Extraction kit (Qiagen, #74104), according to the manufacturer's instructions. RVFV screening was performed using TaqMan real-time RT-PCR (rRT-PCR) based on the highly conserved glycoprotein (Gc) gene, following the protocol recommended by the World Organization for Animal Health (OIE 2018). Sequencing was performed targeting partial nonstructural (Ns) and glycoprotein (Gn) genes, amplifying fragments of 696

and 552 bp, respectively, as previously described (Ikegami 2012). Sequences of the RVFV detected in this study were deposited in the GenBank under accession numbers (MW816826, MW816827, MW717937, and MW717938). RVFV isolation was not performed.

## Results and Discussion

Two out of 20 liver samples of insectivorous bats (family Vespertilionidae; genus *Pipistrellus*) obtained from the Asyut governorate during December 2019 to May 2020 were found positive for RVFV using rRT-PCR ( $C_t=22$ ), whereas the other 20 samples collected from South Sinai were negative. All captured frugivorous bats (family Pteropodidae; genus *Rousettus*) collected from the Egyptian governorates; Asyut ( $n=40$ ), Sohag ( $n=20$ ), Giza ( $n=40$ ), Qena ( $n=40$ ), and Sharqia ( $n=20$ ) from September 2020 to May 2021 were negative, indicating no evidence of infection during the collection period. The negative results of most samples could be attributed to several factors, such as the small sample size of insectivorous bats.

Our results are consistent with previously suggested conclusions that bats and wildlife mammals could be involved in IEPs of RVFV (Beechler et al. 2015, Olive et al. 2012). These conclusions are supported by the results of Boiro, who isolated RVFV from *Micropteropus pusillus* and *Hipposideros abae* bats (Boiro et al. 1987), as well as those of Oelofsen and Van Der Ryst who detected RVFV nucleic acids in the tissues of Guinean insectivorous bats (Oelofsen and Van der Ryst 1999). Furthermore, Mourya et al. (2014) reported the



**FIG. 1.** A higher resolution maximum likelihood phylogenetic trees based on partial Gn (A) and (B) NSs genes of the newly identified RVFV in Egyptian *Pipistrellus deserti* bat in 2020 showed that the clustering pattern of these studied strains with Egyptian RVFV clade (A). The year of isolation and geographical origin of the virus sequences are included in the tree. The scale bar represents branch lengths measured in the number of substitutions.

isolation of phleboviruses in *Rousettus* bats in 2014, whereas Muller et al. (2016) detected Bunya-like viruses in African bats in 2016.

Based on phylogenetic analyses of full-length genome sequences, RVFV strains that have been isolated from endemic countries can be classified into seven genetic lineages: A (e.g., ZH501), B (e.g., Kenya 199800523, Saudi Arabia 200010911), C (e.g., Zinga), lineage D (e.g., OS1, OS7, SA75), E (e.g., Entebbe), F (e.g., Zimbabwe 2269/74), and G (e.g., SA51) (Bird et al. 2007).

Notably, sequence analysis of the obtained RVFV genome, in comparison with RVFV reference strains, revealed 98.3% identity with the sequences previously obtained from humans, sheep, and cattle RVFV strains (ZH-501-177, ZH-501, ZC-3349, ZM-657, and 2250/74) that circulated in Egypt during the outbreaks in 1977 and 2003. Phylogenetic trees of the partial Gn (Fig. 1A) and Ns (Fig. 1B) sequences obtained in this study clustered with the Egyptian clade (A) strains, the strain from Madagascar (1979) and Zimbabwe (1974), according to classification by (Bird et al. 2007). This finding suggests that *P. deserti* may play a role in the maintenance, transmission, and evolution of RVFV in humans and domestic animal populations, specifically when unfavorable conditions for virus spread among its natural hosts are halted.

There are many factors that provide proper ecoepidemiological conditions for RVFV adaptation to wild ecosystems, including the sporadic detection of RVFV in different Egyptian governorates and the presence of multiple vectors such as *Culex pipiens*, *Cx. antennatus*, *Cx. perexiguus*, and *Aedes caspius* in close contact with domestic animals, wild mammals, and humans (Kamal 2011, Kenawy et al. 2018).

Sulkin et al. (1965) suggested that bats are ecologically related to arthropods, specifically insectivorous bats that consume 50–100% of their body weight in insects in 24 h (Kunz et al. 2011). Thus, insectivorous bats are exposed to diverse arthropod viruses through ingestion (Kuno and Chang 2005). Consequently, a wide range of insect-origin viruses have been discovered in the guano of bats (Reuter et al. 2014). These viruses have found their way to internal tissues through passive transport from the gastrointestinal tract; however, it remains speculative whether viral nucleic acids or viral particles can pass through the gut wall or replicate inside bat tissues (Sulkin et al. 1965, Szentivanyi et al. 2019).

## Conclusions

A longitudinal study on bats is needed to confirm our hypothesis that bats may be implicated in RVF transmission in Egypt and to determine whether RVFV detected is the result of accidental infection of bats, spillover of the virus from vertebrates/wild mammals in the epidemic areas, and/or an adaptation of RVFV to this ecological niche. This study only highlights the possibility of the implication of bats in the ecology of RVFV and that bats may act as one of the components of the reservoir system of this virus. Notably, this is the first case of RVFV genome detection in *P. deserti* bats in Egypt.

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## Authors' Contributions

O.S.S. conducted the laboratory work and drafted the article. A.H.E. supervised the laboratory work, analyzed the results, interpreted data, and revised the article. M.R.G. generated the sequence data and S.A.G.E. collected the samples. H.A.H.A. developed the concept, designed the study, analyzed the results, and critically reviewed the article.

## Author Disclosure Statement

No competing financial interests exist.

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