Transboundary and Emerging Diseases

SHORT COMMUNICATION

Phylogeographic Reconstructions of a Rift Valley Fever Virus Strain Reveals Transboundary Animal Movements from Eastern Continental Africa to the Union of the Comoros

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Summary

Major explosive outbreaks of Rift Valley fever (RVF), an arthropod borne zoonotic disease, occur in humans and animals with significant mortality and economic impact across continental Africa and the Indian Ocean region (Madagascar, the Comoros archipelago). Recently, sporadic human cases have been reported in Mayotte and Grande Comore, two islands belonging to the Comoros archipelago. To identify the hypothetical source of virus introduction in an inter-epidemic or a post-epidemic period, a longitudinal survey of livestock was set up in Comorian ruminant populations, known to be susceptible hosts. The phylogeographic genomic analysis has shown that RVF virus (RVFV) detected in a zebu collected in Anjouan in August 2011 seems to be related to the last known epidemic of RVF which occurred in East Africa and Madagascar (2007–2009). This result highlights the fact that RVFV is maintained within local livestock populations and transboundary animal movements from eastern continental Africa to Indian Ocean islands likely result in RVFV crossover.

Introduction

Rift Valley fever (RVF) epidemics occur in various areas of sub-Saharan Africa, spreading northward to Egypt and eastward across the Red Sea into Saudi Arabia and Yemen, causing significant mortality and economic impact (Easterday, 1965; Madani et al., 2003; Bird et al., 2009). Climatic changes like episodes of heavy rainfall in eastern and southern Africa as well as intensified livestock trade are known to favour the re-emergence of arboviruses such as RVF virus (RVFV).

The RVFV genome is composed of three negative-sense single-stranded RNA segments with a total combined length of approximately 11.9 kb (Nichol, 2001). Phylogeographic reconstructions of the RVFV genome have unveiled a complex pattern of viral movements across long distances in the African continent (Soumaré et al., 2012). Within the Indian Ocean region, a large outbreak of RVF originating from mainland Africa hits Madagascar during the rainy seasons of 2008 and 2009, causing 59 confirmed human cases including seven deaths and infecting a great number of livestock (Andriamandimby et al., 2010; Carroll et al., 2011). The Comoros archipelago, consisting of the Union of the Comoros (Grande Comore, Moheli and Anjouan) and Mayotte, is located in the south-western Indian Ocean at the northern end of the Mozambique Channel and is considered as the main gate for uncontrolled animal and animal-associated pathogen movements to the Indian Ocean islands from neighbouring African countries (Fig. 1) (Cêtre-Sossah et al., 2012a; Tortosa et al., 2012).

In Mayotte in 2007–2008, two human isolates were shown to be genetically similar to those observed in Kenya

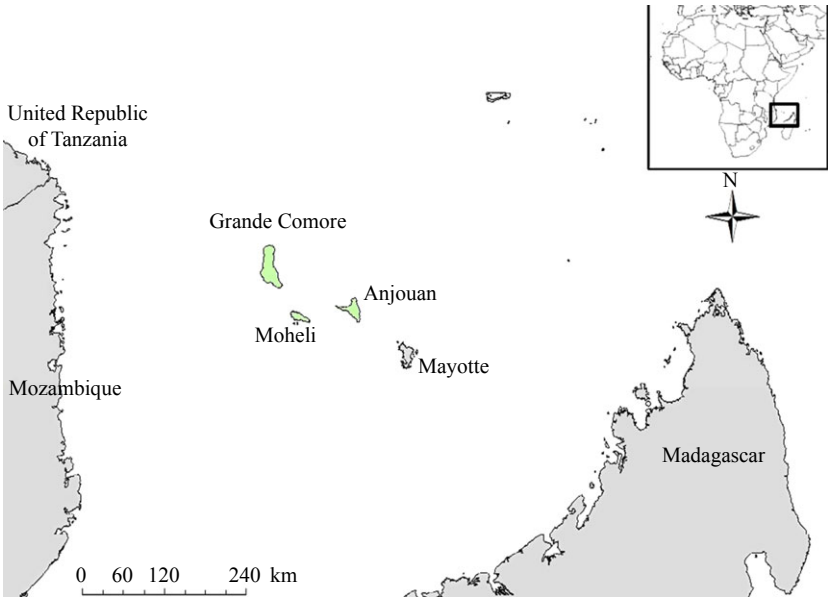

Fig. 1. Geographical location of the Comoros archipelago.

in 2006–2007 (BEH 2009; Sissoko et al., 2009; Cêtre-Sossah et al., 2012b), and RVFV has been circulating in Mayotte livestock populations since at least 2004 (Cêtre-Sossah et al., 2012a). In the Union of the Comoros in 2011, seroprevalence surveys of livestock demonstrated exposure to RVFV of unknown origin in 32.8% of tested animals (Roger et al., 2011). Several sporadic human cases were also reported in the Union of the Comoros between 2011 and 2012 (BHI 2011, 2012), implying an ongoing, silent circulation of RVFV in cattle displaying no clinical symptoms (Maquart et al., 2014). Non-classical transmission of RVFV has also been observed in Madagascar (Nicolas et al., 2014). Here, a longitudinal survey of Comorian ruminant populations was designed to clarify the status of RVFV circulation in the Comoros archipelago. We present a partial M segment analysis for a RVFV-positive zebu that was sampled on Anjouan in August 2011. A better understanding of RVFV phylogeography is of great interest to the region, and understanding how trade exchanges influence viral circulation is essential to limit the socio-economic burden of RVF.

Materials and Methods

A total of 191 ruminants (88 cattle and 103 goats) – 135 animals in Grande Comore, 27 in Moheli and 29 on Anjouan – were included in the longitudinal survey conducted from April 2010 to August 2011. Blood samples were taken monthly from April to August 2010 and every 4 months from August 2010 to August 2011. Twenty distinct animals tested positive for RVFV by the newly developed RT-PCR detection assay described in Maquart et al., (2014). The presence of RVFV was then confirmed by sequencing of these 90 bp long PCR products. Briefly, viral RNA was extracted and eluted in a volume of 50 μl using the EZ1 robot and EZ1® Virus Mini Kit v2.0 (Qiagen, Courtaboeuf, France). Six microlitres of total RNA was reverse-transcribed (RT) and amplified using the two-step GoScript-RT and GoTaq qPCR SYBR Green Mastermix 2X (Promega, Madison, WI, USA) according to the manufacturer instructions. Regarding the sequencing of the whole M segment, the method used two steps of amplification. A first round PCR was performed using the M segment primers described in Bird et al., (2007). The second round PCR was performed using 0.2 μM of each of the following primer sets: RVFV-M-2152F: 5′-CCAAGTGTAGACTGTCTGGCA CAG-3′ and RVFV-M-2766R: 5′-CAATTGCATACCCT TTGCCTGGGC-3′ or RVFV-M-2766F: 5′-GCCCAGGCA AAGGGTATGCAATTG-3′ and RVFV-M-3078R: 5′- GAG ACTACAAACTATAAAAACTCCTC -3′. Amplification reactions were performed in a final volume of 50 μl consisting of 25 μl GoTaq mastermix 2X (Promega) and 3 μl of the primary PCR product in a ABI 7300 thermocycler (Applied Biosystems, Saint Aubin, France) using the following temperature cycles: 95°C for 15 min; 45 cycles of (95°C for 5 s, 56°C for 30 s) and 72°C for 90 s. PCR products were sequenced by Beckman's Coulter Genomics, UK. Viral isolations were attempted without any success. To compare the genetic relatedness of the partial virus genome, phylogenetic analyses were performed against a panel of 98 RVFV sequences, identified between 1944 and 2011. Before phylogenetic inference, datasets and multiple sequence alignments were thoroughly examined to eliminate misalignments and ensure correct framing of coding sequences.

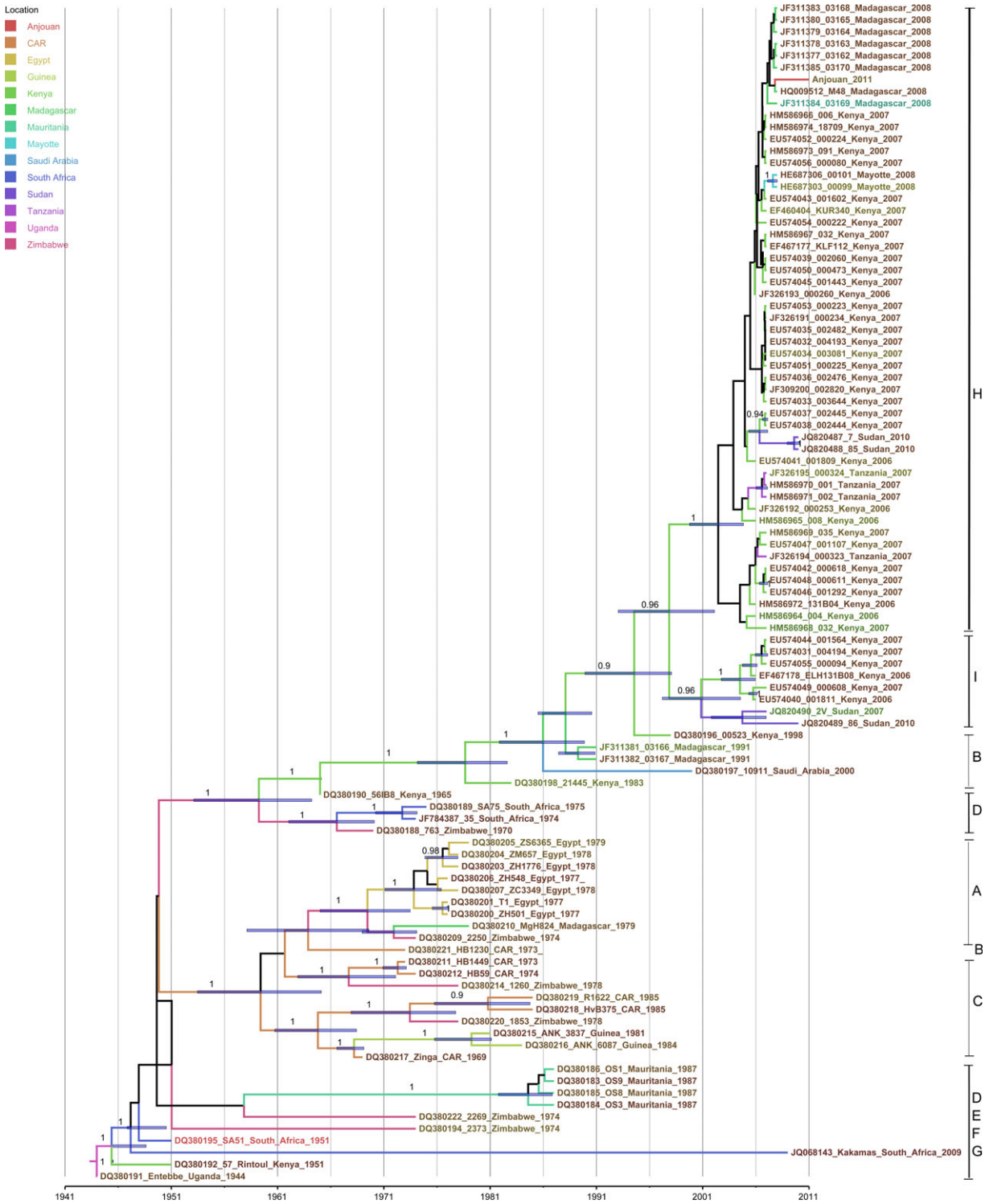

Fig. 2. Ninety-nine partial 927 bp M segment including the CVII14 Anjouan 2011 were analysed by the Bayesian statistical program (BEAST), a GTR+Γ nucleotide rate substitution model, a relaxed molecular clock and sampling every 1.000 states (60 million iterations). Trees and support values from maximum-likelihood techniques and BEAST analyses were similar. The estimated TMRCA of the tree nodes was calculated by Bayesian analysis, and the posterior Bayesian probability with a value greater than 0.90 is reported for each node. Each taxon name indicates the strain, country of origin and collecting date. Clade nomenclature was taken from Bird et al., (2007). Black branches of the groups H and I illustrate the association between the different locations and strain circulations. Group B represents strains which were defined as group B by Bird et al., (2007).

Sequence alignments were performed in Mega 6 using the Clustal W multiple alignment algorithms (Tamura et al., 2013). The best-fit evolutionary model determined using jModelTest version 0.1 (Posada, 2008) was the General Time Reversible with a gamma-distribution model of among site rate heterogeneity (GTR+Γ). The evolutionary history was inferred using the Bayesian MCMC method available in the BEAST v1.8 package (Drummond et al., 2012). We incorporated a relaxed (uncorrelated lognormal) molecular clock (60 million of iterations), with an extended Bayesian Skyline tree prior. Two independent runs were undertaken each sampling every 1000 generations until effective sample size (ESS) values greater than 200 were achieved for all parameters. Statistical analysis was performed with Tracer v1.5 (Rambaut, 2003).

Results and Discussion

Of the 20 RVFV-positive samples, a highly conserved partial M sequence of 927 bp corresponding to Genbank referenced strains positions 2152–3078 obtained from a zebu sample collected on Anjouan in August 2011 identified as Anjouan 2011 (CVII-14) is embedded within the largest East African clade. This clade includes strains isolated over the two last epidemics in Kenya from 2006 to 2007 (Bird et al., 2007) and Madagascar from 2008 to 2009 (Andriamandimby et al., 2010), as well as previous isolates from Mayotte in 2008 (Cêtre-Sossah et al., 2012b) (Fig. 2). Molecular evolutionary rates (nucleotide substitutions per site per year) were estimated by Bayesian analysis of sequence differences among the partial M segment of the 99 RVF viral strains. The mean estimated rate of mutation was 3.26×10^{-4} nt substitutions per year per site with 95% high posterior distribution (HPD) values of 1.72×10^{-4}, 4.24×10^{-4}. The mean estimate of the most recent common ancestor (TMRCA) for the tree root was approximately 1913, in accordance with the first description of a clinical disease similar to RVF reported in 1911 in the vicinity of Lake Naivasha in Kenya (Montgomery, 1912; Stordy, 1913). The mean TMRCAs for phylogenetic groups H and I were, respectively, 1944 (HPD 95%, $\pm 1.16 \times 10^{-4}$) and 1955 (HPD $\pm$ 4 years). Difficulty was experienced in obtaining genetic sequence data and viral isolations were unsuccessful, potentially indicating a low viral load in the infected animal sampled in a post-epidemic or an inter-epidemic period or a partially deleted virus.

Due to the associated livestock trade routes, recent detection of RVFV in Madagascar and Mayotte could suggest its geographical spread across the south-west Indian Ocean region. Here, we have reported evidence for the circulation of a likely East African strain of RVFV on the island of Anjouan. Specific RVF antibody acquisition (Maquart et al., 2014) and genetic data pertaining to RVFV suggest recent livestock-associated disease importation to Anjouan, most likely following the epidemics in Kenya–Tanzania (2006–2007) and in Madagascar (2008–2009). Furthermore, all eastern African lineages of RVFV appear to originate from the outbreak in Kenya (2006–2007). In the context of frequent trades, regional environmental conditions and vector activities (diversity, abundance) are seemingly sufficient to maintain RVFV active circulation in the inter-epidemic period.

Intense human and animal traffic has existed for centuries between Madagascar, the Comoros archipelago and the countries of the East African coast. Commercial trade has resulted in the importation of many diseases, such as blackleg in 1970 and 1995, the contagious ecthyma in 1999 and East Coast fever in 2003 and 2004 (De Deken et al., 2007; Yssouf et al., 2011).

Amongst other risk factors, the intensive trade between East Africa, Madagascar and the Comoros archipelago in combination with regional low-level RVFV circulation and the presence of Aedes vector species put the Comoros archipelago at high risk of RVF epidemic emergence (Roger et al., 2014). Therefore, the surveillance improvement of RVFV circulation is essential to better anticipate the risk of a new RVF outbreak and to eventually lower the social and economic burden that disease outbreaks can have on this developing region of the world.

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