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Short communication

New genetic mechanism, origin and population dynamic of bovine ephemeral fever virus

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ABSTRACT

Bovine ephemeral fever virus (BEFV) is a typical species of the genus*Ephemerovirus* in the family Rhabdoviridae. Today, prevailing BEFV can be divided into three phylogeographic lineages, East Asia, Mideast, and Australia. In this study, we provide evidence that the whole East Asia lineage originates from a homologous recombination (HR) between the Mideast and Australia lineages that probably occurred in the 1940s. To our knowledge, HR has not been proposed before as the genetic mechanism of BEFV. According to the HR event and Bayesian estimation, the three BEFV lineages might originate from Africa, and may have spread to Asia and Australia through the Mideast. In addition, the population of the virus may have augmented significantly in the 2000s, suggesting that the risk for outbreaks of BEFV may be high at present.

1. Introduction

Bovine ephemeral fever (BEF) is a disabling viral disease of cattle and water buffalos that occurs seasonally in tropical, subtropical, and temperate regions of Africa, Asia, and Australia (Walker, 2005). Its causative agent, the BEF virus (BEFV), is a typical member of the genus *Ephemerovirus* in the family Rhabdoviridae.

The virus has a single-stranded negative RNA genome of approximate 15,000 nt in length (Walker, 2005). Its G protein contains type-specific and neutralizing antigenic sites (Cybinski et al., 1990) and vaccination with this protein can protect cattle against infection (Hertig et al., 1996; Uren et al., 1994). With reference to the gene encoding the G protein, BEFVs currently known to prevail in any part of the world can be divided into three phylogeographic lineages. Those have been named East Asian, Middle Eastern, and Australian due to the areas where the viruses have been isolated (Trinidad et al., 2014). Even though a recent study suggested that G gene diversity can be driven by adaptive evolution (Trinidad et al., 2014), the genetic mechanism that has given origin to the creation of different lineages of the virus is still not fully known.

BEF is believed to be an ancient disease that has been endemic in large parts of Africa and South Asia since antiquity (St. George, 1988). Nevertheless, BEF outbreaks have not been documented until 1868 in Africa (Walker, 2005). Regarding the Mideast, in Palestine the earliest descriptions of BEF date in 1931 (Yeruham et al., 2010). Descriptions from East Asia are younger and date from 1934 in the case of China (Bai, 1992). BEFV entered Australia through the Indonesian archipelago approximately in 1936 (Walker, 2005).

In this study, we elucidate that the East Asia BEFV originates from a homologous recombination (HR) event between the Mideast and Australia lineages. We also analyzed the origin and the size of the effective BEFV population of the three prevailing lineages.

2. Materials and methods

2.1. Virus isolation and sequencing

In September 2011, an outbreak of BEF has been reported at a farm in the Shandong Province, China. Subsequently, a BEFV (Shandong/China/2011) was isolated from the blood of a cow showing symptoms of BEF. After purification by limiting dilution, the virus was passed three times through BHK cells. Its G gene was then amplified employing reverse transcription polymerase chain

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reaction (RT-PCR) using the following primers: 5′-ATGTTCAAGGTCCTCATAATTAC-3′ and 5′-TTAATGATCAAAGAACCTATCATCAC-3′. The PCR product was cloned into the pMD18-T vector and amplified in *Escherichia coli* DH 5α. The plasmids with G gene were sequenced using Sanger sequencing method by BGI (BGI, China)

2.2. Phylogenetic reconstruction of BEFV

In addition to the G gene of Shandong/China/2011, available global BEFV G gene sequences (including East Asian, Middle Eastern, and Australian BEFV, $n = 138$) were retrieved from GenBank (Table S1) and aligned with ClustalW (Thompson et al., 1997) for the phylogenetic reconstruction of global BEFV. Xia's test was performed to measure substitution saturation in the set of aligned sequences (Xia et al., 2003). Before phylogenetic trees were reconstructed, the best-fit models of nucleotide substitution were sought using the model selection program implemented in MEGA6 (Tamura et al., 2013). Bayesian phylogenetic inference was performed with the BEAST software package (version 1.83; (Drummond and Rambaut, 2007)) with the general time reversible (GTR) nucleotide substitution model and gamma distributed 4 ($\Gamma 4$) according to a recent report (Trinidad et al., 2014).

2.3. Recombination analysis

Recombinants were identified applying methods previously described (He et al., 2012). Briefly, aligned sequences were scanned for potential mosaic isolates using the RDP software package (version 3.0; (Martin et al., 2005)). The gene sequence similarity of mosaics and their putative parents were searched in GenBank and graphically displayed with Simplot (Lole et al., 1999). Finally, incongruent phylogenetic relationships of different gene regions delimited by a crossover point were used to identify the recombination event. The robustness of tree nodes was tested with the bootstrap method (1000 replicates). Any node with a bootstrap value >70% was considered to be robust.

2.4. Estimates of the dynamic size of effective population of global BEFV

The dynamic size of effective population, the mean evolutionary rate, and the times back to the most recent common ancestors (tMRCA) were calculated using the Bayesian Markov chain Monte Carlo (MCMC) method implemented in the BEAST software package (version 1.8.3; (Drummond and Rambaut, 2007)). As described before (Trinidad et al., 2014), Bayesian MCMC analyses were performed using: (1) the relaxed molecular clock models; (2) the GTR nucleotide substitution models and a gamma-distributed among-site rate variation with four rate categories ($\Gamma 4$); (3) Bayesian Skyline plot as a prior tree with 10 groups. Bayesian MCMC analysis was run for 100 million states and sampled every 1000 states. Posterior probabilities were calculated with a burn-in of 10 million states and checked for convergence using Tracer, version 1.6.1 (http://tree.bio.ed.ac.uk).

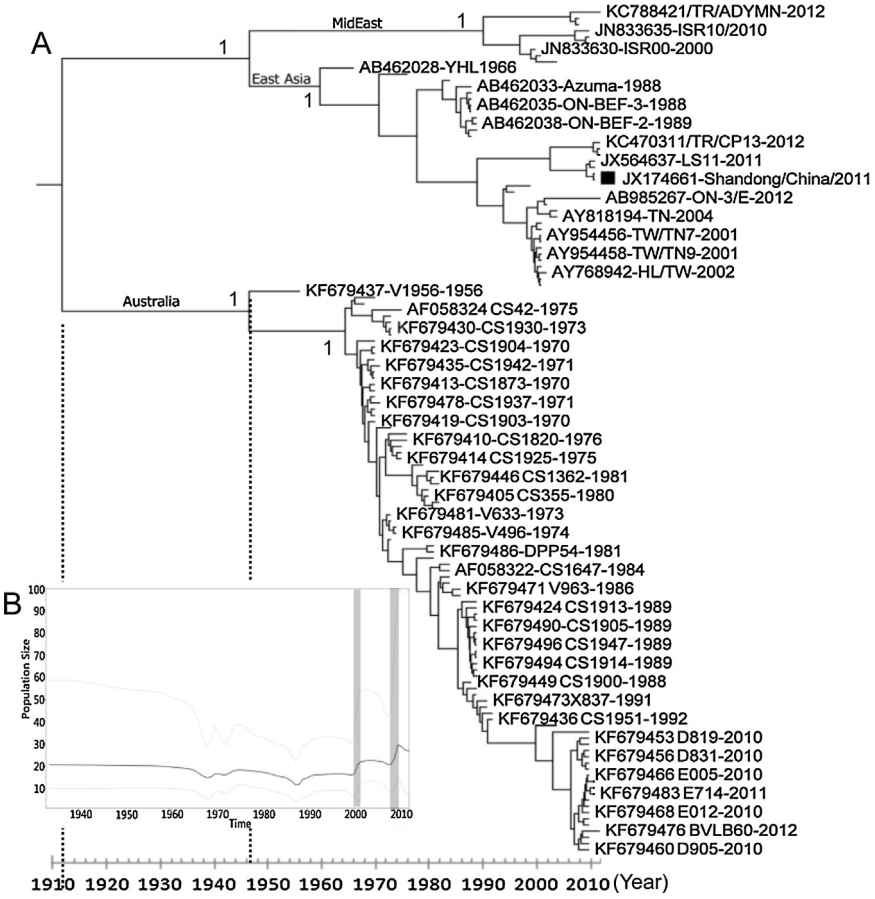

Fig. 1. Global BEFVs phylogenetic history and dynamic of their effective population (A) BEFV phylogenetic history based on sequence alignment after the position 483 of G gene open reading frame (ORF). The evolutionary history was inferred using the Bayesian MCMC method. The tMRCA of each branch was shown in *x* axis. The Shandong isolate is marked with ■. The Bayesian posterior probabilities of several key branches are shown on/under these branches. (B) The dynamic size of BEFV effective population from 1933 to 2012. The thick solid line is the median estimate, and the light color lines show the 95% HPD limits.