Rift Valley fever virus (RVFV), a mosquito-borne phlebovirus, has been detected in Madagascar since 1979, with occasional outbreaks. In 2008 to 2009, a large RVFV outbreak was detected in Malagasy livestock and humans during two successive rainy seasons. To determine whether cases were due to enzootic maintenance of the virus within Madagascar or to importation from the East African mainland, nine RVFV whole genomic sequences were generated for viruses from the 1991 and 2008 Malagasy outbreaks. Bayesian coalescent analyses of available whole S, M, and L segment sequences were used to estimate the time to the most recent common ancestor for the RVFVs. The 1979 Madagascar isolate shared a common ancestor with strains on the mainland around 1972. The 1991 Madagascar isolates were in a clade distinct from that of the 1979 isolate and shared a common ancestor around 1987. Finally, the 2008 Madagascar viruses were embedded within a large clade of RVFVs from the 2006–2007 outbreak in East Africa and shared a common ancestor around 2003 to 2004. These results suggest that the most recent Madagascar outbreak was caused by a virus likely arriving in the country some time between 2003 and 2008 and that this outbreak may be an extension of the 2006–2007 East African outbreak. Clustering of the Malagasy sequences into subclades indicates that the viruses have continued to evolve during their short-term circulation within the country. These data are consistent with the notion that RVFV outbreaks in Madagascar result not from emergence from enzootic cycles within the country but from recurrent virus introductions from the East African mainland.
on the heels of a substantial RVFV outbreak in 2006 to 2007 on the East African mainland, predominantly in Kenya, Tanzania, and Somalia (3, 17, 30), and occurred during the time when RVFV cases were reported from Mayotte, the Republic of the Comoros, and South Africa between 2007 and 2009 (27, 28).

Although the predictive model developed for the Horn of Africa failed to show heightened risk for RVFV infection in Madagascar in 2008 (5), a nationwide cross-sectional serological survey showed extensive and widespread RVFV exposure at that time (1). Preliminary phylogenetic analyses using partial sequences showed that Malagasy viruses were similar to those found circulating during the 2006–2007 East African outbreak; however, the initial source of the 2008 Madagascar outbreak remained unclear (1). The purpose of this study, therefore, was to generate whole genomic sequences from 2008 Malagasy outbreak samples to determine whether cases most likely represented enzootic maintenance within Madagascar or epizootic/epidemic spread or importation from East Africa.

**TABLE 1. GenBank accession numbers and collection information for the 9 RVFVs sequenced in this study**

| SPB no. | ID no.       | Species         | Sample                      | Mo      | Yr       | District    | S segment   | M segment   | L segment   |
|---------|--------------|-----------------|-----------------------------|---------|----------|-------------|-------------|-------------|-------------|
| 200803162 | 0212-08      | Human           | Isolate (serum)             | January | 2008     | Taolagnaro  | JF311386    | JF311377    | JF311368    |
| 200803163 | 0428-08      | Human           | Isolate (serum)             | February| 2008     | Anjozorobe  | JF311387    | JF311378    | JF311369    |
| 200803164 | 0619-08      | Human           | Isolate (serum)             | March   | 2008     | Ankazobe    | JF311388    | JF311379    | JF311370    |
| 200803165 | 0846-08      | Human           | Isolate (serum)             | March   | 2008     | Manjakandriana | JF311389    | JF311380    | JF311371    |
| 200803166 | S-91-41      | Human           | Isolate (serum)             | February| 1991     | Antananarivo | JF311390    | JF311381    | JF311372    |
| 200803167 | ZF-06        | Bovine          | Isolate (embryo liver)      | February| 1991     | Antananarivo | JF311391    | JF311382    | JF311373    |
| 200803168 | 0406-08      | Bovine          | Embryo liver                | February| 2008     | Marinarivo  | JF311392    | JF311383    | JF311374    |
| 200803169 | 0413-08      | Bovine          | Embryo liver                | February| 2008     | Antsirabe II| JF311393    | JF311384    | JF311375    |
| 200803170 | 0855-08      | Bovine          | Serum                       | March   | 2008     | Manjakandriana | JF311394    | JF311385    | JF311376    |

* SPB, Special Pathogens Branch; ID, identification.

**RESULTS**

**Phylogeny.** Tree topologies based on all of the available RVFV S, M, and L sequences are shown in Fig. S1, S2, and S3 in the supplemental material, respectively. For the sake of clarity, abbreviated trees, including relevant S (Fig. 1), M (Fig. 2), and L (Fig. 3) segment sequences generated for this study, are displayed here. Tree topologies were consistent with those generated for all previously available RVFV genomic RNA segments (3, 4). The 1979 Madagascar virus is most closely associated with viruses from the mid- to late 1970s from Zimbabwe and falls within the clade containing samples from the large Egyptian outbreak of that time. The two 1991 Madagascar isolates are members of a different clade, along with three additional 1991 Madagascar RVFVs and viruses from Kenya (1997 to 1998), Somalia (1998), South Africa (1999 to 2000), Saudi Arabia (2000), and East Africa (2006 to 2007). All seven of the 2008 Madagascar RVFVs, and two viruses from the 2008 outbreak in South Africa, were embedded within the larger 2006-2007 East African clade, specifically within the lineage previously termed Kenya-I (3). None of the viruses sampled from Madagascar or South Africa in 2008 fell within the Kenya-II lineage. Instead, most formed subclades with other 2008 viruses (i.e., Mdg3164 and Mdg3168; Mdg3165, and Mdg3170) within the larger Kenya-I lineage.

**Evolutionary rates and time to MRCA.** The addition of the Madagascar RVFV sequences resulted in mean molecular evolutionary rates (and 95% highest posterior density [HPD] intervals) similar to those previously generated by Bird et al. (3,
4): for the S segment, $4.16 \times 10^{-4}$ (95% HPD interval, $2.78 \times 10^{-4}$ to $5.47 \times 10^{-4}$) nucleotide substitutions/site/year; for the M segment, $5.09 \times 10^{-4}$ (95% HPD interval, $3.38 \times 10^{-4}$ to $6.75 \times 10^{-4}$) nucleotide substitutions/site/year; and for the L segment, $4.33 \times 10^{-4}$ (95% HPD interval, $2.73 \times 10^{-4}$ to $5.98 \times 10^{-4}$) nucleotide substitutions/site/year.

The 1979 Madagascar RVFV shares a MRCA with viruses from Zimbabwe around 1971 to 1972 (Fig. 1 to 3). All RVFVs detected since the early 1980s in East Africa, Madagascar, and the Middle East are contained in a single clade and share a MRCA in the 1960s (S segment) (Fig. 1) or 1970s (M and L segments) (Fig. 2 and 3). The MRCA of the clade containing the 1991 Madagascar RVFVs occurred between 1986 and 1988 (Fig. 1 to 3), whereas viruses in the 2006–2008 East Africa/Kenya I lineage, including the 2008 Madagascar and South Africa RVFV samples, share a MRCA approximately 3 to 5 years before the 2008 Madagascar outbreak (2003 to 2005). The subclades containing solely Madagascar sequences appear to be more recent, having arisen less than a year prior to their detection.

**DISCUSSION**

Genomic findings from this study revealed that the viruses circulating in Madagascar in the late 1970s did not give rise to the 1991 or 2008 Malagasy RVFV outbreaks, eliminating the role of this first isolate in enzootic maintenance and the successive outbreaks. Three distinct importation events, from the East African mainland to Madagascar, appear likely: one in the early 1970s, another in the mid- to late 1980s, and the most recent between 2003 and 2008. In all three instances, considerable RVFV activity was detected on the African mainland prior to the detection of these viruses in Madagascar. This pattern is particularly evident with the large sweeping outbreak in Egypt (1978 to 1979) prior to the Madagascar 1979 virus.
The timing of the events associated with the recent outbreak in Madagascar also supports RVFV importation from East Africa, possibly even during the 2006–2007 outbreak. Although most cases were reported from January through April 2008 (1), epidemiologic evidence has linked the 2008–2009 Malagasy outbreak to that occurring on the mainland a few years earlier. Specifically, livestock abortion was observed in one Malagasy district (Toliara II) beginning in early 2007, and a retrospective investigation revealed that RVFV had been circulating in the livestock population at least since December 2007 (1). The same retrospective investigation demonstrated widespread exposure to RVFV among Malagasy slaughterhouse workers since at least 2007, as evidenced by the presence of antibodies in serum samples (1). The combination of the genetic and serological data would be consistent with the view that in Madagascar, large outbreaks of RVF are associated with viruses introduced from the mainland, but the virus activity likely ramps up over the preceding year or two and likely persists at decreasing levels for a year or two following the large outbreak. Unfortunately, disease surveillance is insufficiently sensitive to detect these pre- and postoutbreak periods of virus activity. Increased surveillance and genetic characterization of interepizootic virus isolates would shed light on this issue. RVFV activity during an interepizootic/interepidemic period is not surprising, especially in the years immediately preceding (3) or following (31) an outbreak. Even when the virus fails to become established in the long term, it is likely that there is a temporary enzootic state with short-term circulation for a couple of years surrounding a RVFV outbreak.

Similar patterns of disease spread have been observed in other areas. For instance, in Mayotte, RVFV cases in humans and cattle in 2007 and 2008 also appeared to be an expansion of the East African outbreak (27). In addition, outbreaks of other pathogens, including the 2003–2004 East Coast fever outbreak in Comoros and the 2005–2006 Chikungunya virus outbreak in the islands of Mayotte, Réunion, Seychelles, Mauritius, and Madagascar are thought to have originated on the East African mainland, specifically in Kenya and Tanzania (8, 25, 26). These cases provide additional illustrations of the potential for infectious agents to be imported from coastal areas.

**FIG. 2.** Bayesian coalescent analysis of RVFVs based on the M segment. The maximum clade credibility tree is shown with the year of the MRCA, in boldface, at each node. Viruses from Madagascar and nodes containing associated MRCA estimates are shown in red. KI, Kenya-I lineage; KII, Kenya-II lineage.
countries in Africa to islands located off the African continent and highlight the risk of subsequent establishment and circulation in a new environment. This is especially important given that replication-competent vectors of several viruses can already be found in these areas (12, 23, 27).

While infected mosquitoes have certainly contributed to the widespread dispersal of RVFV, the role of mosquitoes in the importation of RVFV into Madagascar is unknown. Given the 400 km or more of open water between Madagascar and the African mainland, it is highly improbable, though not impossible, that wind-borne mosquitoes could be the origin of the imported virus. More likely, virus-infected mosquitoes could have been unwittingly transported in air or sea shipments to Madagascar. However, the most likely route remains importation of virus-infected cattle or other livestock. In nearby Comoros, the outbreak of East Coast fever in 2003 and 2004 was attributed to the importation of cattle from Tanzania (8). Only limited information is available regarding the cattle trade to and from Madagascar, with some recent records documenting exportation of ruminants to the Comoros archipelago (1) and importation of live cattle from New Zealand (United Nations Commodity Trade Statistics Database, 2010 [http://comtrade.un.org/db/]). No official records are available to substantiate the importation of cattle from the African mainland to Madagascar; however, the legal and illegal importation of livestock and goods is a common practice throughout the region and has already been implicated as the possible cause of a case of RVFV in Comoros in 2007 (27). Once infected livestock are introduced into an area, the disease spreads rapidly, particularly in countries, such as Madagascar, where the large-scale movement of cattle is common.

Detailed examination of the phylogenetic trees indicates considerable movement of virus-infected livestock or mosquitoes on the African mainland. It is obvious that during 2006 to 2008, the Kenya-I lineage (Fig. 1; see also Fig. S1 in the supplemental material) was distributed across the large distances separating Kenya, Tanzania, Madagascar, and South Africa. In addition, some of the Central African viruses are dispersed in different RVFV lineages, as are Zimbabwe lineages and South African viruses. Clearly, distant movements of RVFVs are not infrequent events.

In conclusion, the generation of whole genomic sequences and advances in Bayesian coalescent methods enabled us to conclude that the reported RVFV events in Madagascar can...
be attributed to importation or expansion from mainland Af-
rica. These findings underscore the importance of maintaining
accurate and complete import/export records and remaining
vigilant for the signs and symptoms of RVFV infection, includ-
ing unexpected deaths and/or abortions, in livestock and hu-
mans.

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