Sequential Rift Valley Fever Outbreaks in Eastern Africa Caused by Multiple Lineages of the Virus

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Background. During the Rift Valley fever (RVF) epidemic of 2006–2007 in eastern Africa, spatial mapping of the outbreaks across Kenya, Somalia, and Tanzania was performed and the RVF viruses were isolated and genetically characterized.

Methods. Following confirmation of the RVF epidemic in Kenya on 19 December 2006 and in Tanzania on 2 February 2007, teams were sent to the field for case finding. Human, livestock, and mosquito specimens were collected and viruses isolated. The World Health Organization response team in Kenya worked with the WHO's polio surveillance team inside Somalia to collect information and specimens from Somalia.

Results. Seven geographical foci that reported hundreds of livestock and >25 cases in humans between December 2006 and June 2007 were identified. The onset of RVF cases in each epidemic focus was preceded by heavy rainfall and flooding for at least 10 days. Full-length genome analysis of 16 RVF virus isolates recovered from humans, livestock, and mosquitoes in 5 of the 7 outbreak foci revealed 3 distinct lineages of the viruses within and across outbreak foci.

Conclusion. The findings indicate that the sequential RVF epidemics in the region were caused by multiple lineages of the RVF virus, sometimes independently activated or introduced in distinct outbreak foci.

Rift Valley fever (RVF) virus is a mosquito-borne virus associated with epidemics in livestock and humans [1]. Since the 1970s, periodic epidemics of RVF associated with heavy rainfall and flooding have been reported in an increasing number of countries of eastern and southern Africa where the virus became endemic, including Kenya, Somalia, Sudan, Tanzania, Zimbabwe, South Africa, and Madagascar [2–8]. The RVF epidemics have also occurred in countries where virus activity was not previously detected, such as Egypt in 1977, Mauritania in 1983, and Saudi Arabia and Yemen in 2000 [2, 4, 7]. In these naïve ecologies, propagation of the epidemic appears to be the result of spread of the newly introduced virus across nonimmune livestock and human populations through animal movement and mosquito vectors. This conclusion is supported by molecular epidemiological data showing that RVF virus strains recovered during epidemics in naïve ecologies belonged to a single lineage with minimal genetic diversity [9, 10]. For example, comparison of 6 RVF virus isolates from the Egyptian epidemic of 1977 identified a single lineage of virus with <.33% nucleotide (nt) and <.1% amino acid (aa) sequence differences, and 3 RVF virus isolates from the Mauritanian epidemic of 1983 had similarly low genetic differences [9]. In contrast, the mechanisms associated with propagation of the epidemic in ecologies where the virus is endemic are not

well understood; there are minimal data on the genetic diversity of RVF virus strains recovered from various areas of endemic countries during an epidemic [9–11]. A study that compared RVF virus isolates from livestock in Kenya during the 2006–2007 epidemic suggested the presence of multiple lineages of the viruses [12]. However, due to inadequate surveillance in livestock, the extent of the epizootic in livestock was not well documented.

Severe RVF epizootics in countries where the virus is endemic occur after a period of between 3 and 7 years [13]. For instance, in Kenya, where the highest number of epizootics have been reported to date, the average interepizootic period is 3.6 years, a period likely representing the time required for the immunity of livestock populations to decrease to levels that are permissive to virus spread [13]. Recurrent RVF epidemics in regions where the virus is endemic may be due to amplification of virus residing in each ecological zone, the virus being maintained through either persistence in eggs of floodwater *Aedes* mosquito species or via low-level cycling among vertebrates. Alternatively, as in naïve ecologies, a single virus lineage may be introduced and amplified from the first epicenter of the epidemic and subsequently spread across the geographic region.

The RVF epidemic of 1997–1998 that affected Kenya, Somalia, and Tanzania was characterized by outbreaks that started in the North Eastern Province of Kenya in November 1997 and ended with cases reported from the north central region of Tanzania in June 1998 [14]. Spatial mapping of these outbreaks and molecular analysis of viruses involved in the outbreaks were not performed due to late confirmation and response to the epidemic. Another RVF epidemic occurred in 2006–2007 in these 3 countries, once again starting from the North Eastern Province of Kenya and continuing to Tanzania, where the last livestock and human cases were reported in June 2007 [14–17]. During an 8-month period, >1100 suspected cases in humans resulting in 350 deaths were reported across the 3 countries, as well as thousands of livestock abortions and deaths [14, 17]. The early detection of the epidemics in each country enabled us to perform spatial mapping of the sequential outbreaks across 3 countries and to isolate and genetically characterize RVF virus strains from most of the outbreak foci.

METHODS

Tracking RVF outbreaks in the 3 countries
Following confirmation of the RVF epidemic in Kenya on 19 December 2006, teams led by the Kenya Ministry of Health (KMOH) and including local and international partners were sent to various parts of the country the following day for active case finding and response. The epidemic in Tanzania was confirmed on 2 February 2007 and case finding initiated immediately. Linelists of human RVF cases in Kenya were collected by KMOH and in Tanzania by the Ministry of Health and Social Work. Descriptions of the human RVF epidemics in Kenya and

Tanzania have been published [14–16]. The World Health Organization (WHO) offices in Kenya received reports of suspected RVF cases in Somalia on 19 December 2006. Because of political instability in Somalia, there were no international public health teams in the country to respond to the RVF epidemic. The WHO epidemic response team in Nairobi, Kenya, worked closely with its polio surveillance team already in Somalia and with Médecins Sans Frontières to collect information and specimens from Somalia. Livestock surveillance was passive in all 3 countries, with no accurate data on the extent of the cases in livestock herds. However, a general picture of extent of the livestock epizootic in Kenya has been described [18].

Rainfall monitoring
Normalized difference vegetation indices (NDVIs) and rainfall maps for 10-day periods from November 2006 through July 2007 were obtained from the Africa Data Dissemination Service (http://earlywarning.cr.usgs.gov/adds/datatheme.php, 19 April 2010). The NDVI is a commonly used measure of vegetation cover or greenness. It has a range from –1, where there is little vegetation (and therefore greenness) to +1, which corresponds to intense greenness [8].

RVF virus isolation from human specimens
The Kenya Medical Research Institute/Centers for Disease Control and Prevention (KEMRI/CDC) laboratory in Nairobi served as the diagnostic center for suspected cases in humans of RVF disease in Kenya, Somalia, and Tanzania. Serum specimens that tested positive for viral RNA by real-time Reverse Transcription-polymerase chain reaction (real-time RT-PCR) and/or IgM antibodies by enzyme-linked immunosorbent assay were used for virus isolation using Vero cells in T25 tissue culture flasks as described elsewhere [19]. In brief, 95% confluent Vero cells were inoculated with 100 μL of human sera diluted 1:4 in minimum essential medium and incubated at 37°C and 5% carbon dioxide for up to 12 days for development of RVF virus–induced cytopathic changes. The virus-containing cell supernatant was aliquoted into cryovials and stored at −80°C for viral nucleic acid extraction and genetic sequencing. Most specimens that tested positive for both viral RNA and antibody (particularly IgG antibodies) were negative for virus isolation. Table 1 shows the human RVF virus isolates used for genome sequencing in this study, including the following 8 human RVF viruses: KEN/Gar-004/06 (GenBank accession no. HM586975 for S segment, HM586964 for M segment, HM586953 for L segment), KEN/Gar-008/06 (GenBank accession no. HM586976, HM586965, HM586954), KEN/Kil-006/07 (GenBank accession no. HM586977, HM586966, HM586955), KEN/Mal-032/07 (GenBank accession no. HM586978, HM586967, HM586956), KEN/Bar-032/07 (GenBank accession no. HM586979, HM586968, HM586957), KEN/Bar-035/07 (GenBank accession no. HM586980, HM586969, HM586958), TAN/Tan-001/07 (GenBank accession no. HM586981, HM586970, HM586959),

Table 1. History of the sixteen RVFV isolates from distinct outbreak foci during the 2006-07 epidemic

Virus strain	Date isolated	Patient isolated from	Location isolated*	GenBank accession number (Reference)
KEN/Gar-004/06	12/24/06	Human male, 50, serum, survived	1, Fafi-Jarajila	HM586975 for S, HM586964 for M, HM586953 for L.
KEN/Gar-008/06	12/25/06	Human male, 25, serum, died	1, Bura-Guujo	HM586976 for S, HM586965 for M, HM586954 for L
KEN/Gar-Msq131B-04/06	December, 2006	Aedes mosquito	1,	HM586983 for S, HM586972 for M, HM586961 for L.
KEN/Gar-Cap/06	December, 2006	Caprine	1,	EU574068 for S, EU574040 for M, EU574014 for L.
KEN/Gar-Bov/06	December, 2006	Bovine	1,	EU574086 for S, EU574055 for M, EU574029 for L.
KEN/Kil-006/07	1/03/07	Human female, 85, serum, survived	2, Bahari	HM586977 for S, HM586966 for M, HM586955 for L.
KEN/Mal-032/07	1/07/07	Human female, ??,	2,	HM586978 for S, HM586967 for M, HM586956 for L.
KEN/KLF-Msq091/07	January, 2007	Aedes mosquito	2,	HM586984 for S, HM586973 for M, HM586962 for L.
KEN/Msa-Bov/07	January, 2007	Cow	2,	EU574071 for S, EU574043 for M, EU574016 for L.
KEN/Bar-032/07	2/06/07	Female, 14, serum, survived	3, Ichamus	HM586979 for S, HM586968 for M, HM586957 for L.
KEN/Bar-035/07	2/07/07	Male, 22, serum, died	3, Kiserian	HM586980 for S, HM586969 for M, HM586958 for L.
KEN/Bar-Msq187-09/07	February, 2007	Aedes mosquito	3,	HM586974 for M, HM586963 for L
KEN/Kaj-Ovi/07	February, 2007	Ovine	3,	EU574080 for S, EU574050 for M, EU574024 For L.
KEN/Bar-Ovi/07	February, 2007	Ovine	3,	EU574059 for S, EU574033 for M, EU574006 for L.
TAN/Tan-001/07	2/10/07	Human male, 30, liver, died	6, Tanga	HM586981 for S, HM586970 for M, HM586959 for L.
TAN/Dod-002/07	3/01/07	Human female, 55, liver, died	7, Dodoma	HM586982 for S, HM586971 for M, HM586960 for L.

NOTE. *Location of isolation is indicated by number of outbreak focus as indicated in Figure 1 and the exact administrative sub-location. There were no isolates from locations 4 and 5.

and TAN/Dod-002/07 (GenBank accession no. HM586982, HM586971, HM586960), all of which were isolated at the KEMRI/CDC laboratory.

RVF virus isolation from mosquitoes

RVF virus isolates from mosquitoes were cultured at the US Army Medical Research Institute of Infectious Diseases' high containment laboratory at Frederick, Maryland. Mosquito pools were triturated in a 1.5-mL microcentrifuge tube containing one 4.5-mm spherical copper bead and .75 mL of Eagle minimum essential medium containing 10% fetal bovine serum, 10,000 units/mL of penicillin, and 100 10Mg/mL of streptomycin antibiotics, by vortexing the tube for 1–2 min. The mosquito homogenate was clarified by centrifugation in a microcentrifuge at $4°C$ and 12,000 rpm for 5 min. The clarified mosquito homogenate was transferred to a clean microcentifuge tube and stored on ice or frozen at $-70°C$. Clarified mosquito homogenate (100 μL) was also inoculated into Vero cell cultures and

monitored for cytopathic effects or cytopathic changes. Mosquito RVF virus isolates used for genome sequencing and analysis in this study were KEN/Gar-Msq131B-04/06 (GenBank accession no. HM586983 for S segment, HM586972 for M segment, HM586961 for L segment), KEN/KLF-Msq091/07 (GenBank accession no. HM586984, HM586973, HM586962), and KEN/Bar-Msq187-09/07 (GenBank accession no. HM586974, HM586963) (Table 1).

Viral nucleic acid extraction and genome sequencing

Total RNA was extracted from an aliquot of supernatant from infected Vero cell cultures or mosquito homogenates using the specific QIAamp RNA extraction kit (Qiagen). Reverse-transcription PCR assays were performed using RVF virus–specific forward and reverse primers published elsewhere [9]. Three PCR reactions targeting the S, M, and L segments of the RVF virus genome were performed. The PCR products were gel purified, and automated sequencing was performed on an ABI 3100 Prism Genetic Analyzer (Applied Biosystems). Data were collected and analyzed using the ABI data collection software (version 2.0) and sequence analysis software (version 5.1.1). Further data analyses including nt sequence editing and prediction of aa sequences were accomplished using the EditSeq and SeqMan programs (Lasergene analysis software, DNASTAR). To confirm sequences in specific loci, new primers were designed and used to perform additional amplifications and sequencing. For comparison, previously published nt sequences of 5 RVF viruses isolated from livestock during the same 2006–2007 epidemic (KEN/Gar-CAP/06 [GenBank accession no. EU574068 for S segment, EU574040 for M segment, EU574014 for L segment], KEN/Gar-BOV/06 [GenBank accession no. EU574086, EU574055, EU574029], KEN/Msa-BOV/07 [GenBank accession no. EU574071, EU574043, EU574016], KEN/Kaj-OVI/07 [GenBank accession no. EU574080, EU574050, EU574024], and KEN/Bar-OVI/07 [GenBank accession no. EU574059, EU574033, EU574006]) were obtained from GenBank [12].

The Punta Toro virus M gene sequence (GenBank accession no. DQ363407) was obtained for use as an outgroup [20] in subsequent analyses. Nucleotide sequence alignments for each segment were performed using ClustalX [21, 22]. Aligned sequences were compared and dendograms generated using both distance and maximum likelihood methods in PAUP*4.0 (Phylogenetic Analysis Using Parsimony) [23]. Bootstrap analysis was performed with 2000 random samplings using neighbor-interchange pertubation algorithm within PAUP [24].

RESULTS

Spatial mapping of outbreaks among humans

During the 8-month period of the epidemic (November 2006 to June 2007), 1116 suspected cases in humans of RVF were reported in Kenya (n = 700), Somalia (n = 107), and Tanzania (n = 309), resulting in an estimated 354 deaths. Across the 3 countries, 7 outbreak foci that recorded ≥25 confirmed cases in humans and hundreds of livestock cases were identified (Figure 1). The onset of livestock and human cases in each country was preceded by severe rainfall and flooding as shown in Figure 2. As demonstrated by the mean NDVI maps, the 3 countries were dry in early October 2006; however, by November, there was increased groundcover in Kenya and Somalia, where outbreaks were immediately reported (Figure 2). Rainfall had stopped by the end of January in Kenya and Somalia, but at that time Tanzania had heavy rainfall and started to report cases of RVF until June when the rains ended there.

The first outbreak focus was reported in Garissa and Ijara districts in the North Eastern Province of Kenya from 26 November 2006 to 20 February 2007 (Figure 1, Region 1), resulting in 305 cases in humans and 86 deaths. The second outbreak focus in Kilifi and Malindi districts in the Coast Province of Kenya (Figure 1, Region 2) occurred between 26 December 2006 and 15 February 2007, resulting in 88 cases in humans and 25 deaths. The third and fourth outbreak foci were reported in Somalia between 7 January 2007 and 15 February 2007 in the Middle and Lower Juba regions (Figure 1, Region 3) and the Gedo region (Figure 1, Region 4), respectively. In the Juba region, 76 cases in humans and 38 deaths were recorded, and in the Gedo region, 26 cases in humans and 7 deaths were reported.

Cases of RVF in humans were reported from Baringo district in the Rift Valley Province of Kenya (Figure 1, Region 5) between 5 February 2007 and 30 March 2007, resulting in 174 cases and 13 deaths. The last 2 outbreak foci were located in Tanzania; one in Arusha region (Figure 1, Region 6) starting 27 January 2007 and ending 24 March 2007 that reported 25 cases in humans and 15 deaths (Figure 1, Region 6), and another in Dodoma region between 1 February 2007 and 2 June 2007 that resulted in 85 cases in humans and 42 deaths (Figure 1, Region 7).

Genetic analysis of RVF virus strains from different outbreak foci

Table 1 shows the RVF virus isolates from the 2006–2007 epidemic used for genetic sequence comparison. Complete genome sequencing was performed on 8 human and 3 mosquito RVF virus isolates from the 3 foci in Kenya and 2 in Tanzania, and the nt and predicted aa compared to each other and published sequences of RVF virus isolates from infected livestock in Kenya during the same epidemic are shown (Table 1, Figure 1). No viruses were isolated from specimens collected from Somalia.

The pairwise nt sequence comparison of the 16 human, livestock, and mosquito RVF virus isolates from the 2006–2007 epidemic showed low genetic diversity, with an overall nt

sequence variation of .69% at L segment, .7% at M segment, and .86% at S segment. The overall aa sequence variation was .27% at L, .52% at M, and .57% at S segments. Phylogenetic comparison revealed 3 distinct lineages of RVF virus isolates involved in the 2006–2007 epidemic in eastern Africa (Figure 3). The Kenya 1 lineage viruses consisted of 2 sublineages (Kenya 1a and Kenya 1b) and included isolates from humans, livestock, or mosquitoes recovered from Regions 1, 2, and 5. The Kenya 1a sublineage included 3 livestock isolates (KEN/Bar-Ovi/07, KEN/Kaj-Ovi/

07, and KEN/Msa-Bov/07) [12], 1 human isolate (KEN/Gar 008/06), and 1 mosquito isolate (KEN/Bar-Msq 187-09/07). The Kenya 1b sublineage consisted of 5 human (KEN/Gar-004/06, KEN/Bar-032/07, KEN/Bar-035/07, KEN/Mal-032/07, and KEN/Kil-006/07) and 2 mosquito (KEN/Gar-Msq 131B-04/06 and KEN/KLF-Msq 091/07) RVF virus strains from Regions 1, 2, and 5. The Kenya 2 lineage included 2 livestock viruses (KEN/Gar-BOV/06 and KEN/Gar-CAP/06) from Region 1 (Figure 3). The Tanzania 1 lineage consisted of the 2 human isolates (TAN/
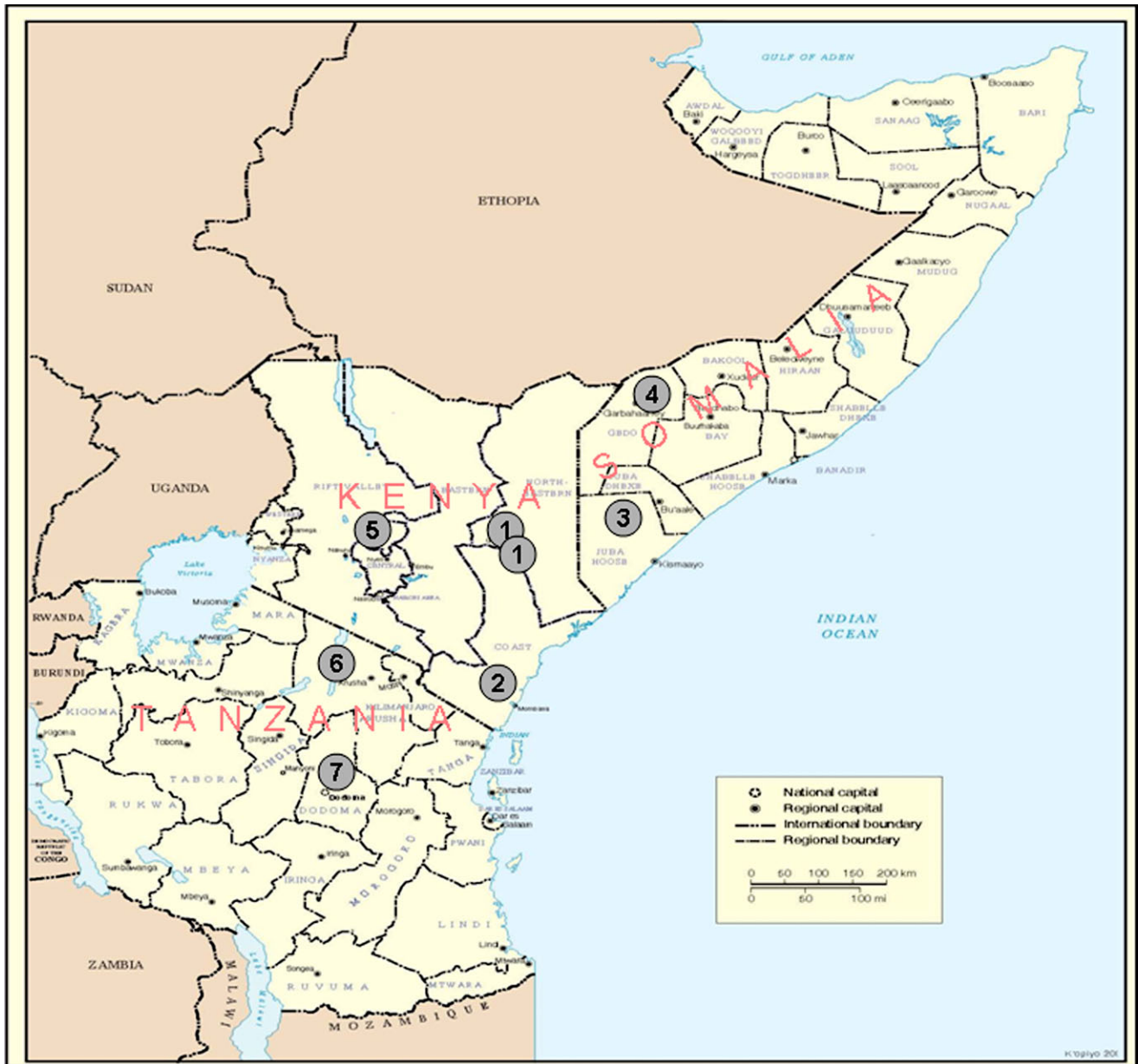
Figure 1. Map of eastern Africa showing the locations of the 7 sequential RVF virus disease outbreaks in order of chronologic appearance of cases in humans, 2006–2007. Region 1 is located in Garissa and Ijara districts in the North Eastern Province of Kenya that were also major epicenters of the RVF virus disease outbreak of 1997–1998. Region 2 is located in Kilifi and Malindi districts in the Coast Province of Kenya, and Regions 3 and 4 are located in Juba and Gedo in Somalia. Region 5 is located in Baringo district in Rift Valley Province, Kenya; Region 6 is located in Arusha district, Tanzania; and Region 7 is in the Dodoma district, Tanzania.

Tan-001/07 and TAN/Dod-002/07) from Regions 6 and 7, respectively (Figure 3). The reliability of the phylogenetic trees was confirmed by performing bootstrap analysis. The Kenya 1a and 1b lineage viruses clustered together with an overall bootstrap value of 98% but with sublineage bootstrap values of 58%–94% (Figure 3). The Kenya 2 and Tanzania 1 lineage viruses clustered in their respective groups with bootstrap values of 98%–100%. The clustering of the same viruses within each of 3

lineages was maintained when separate trees for S, M, and L segments were constructed (data not shown). Comparison of isolates from different foci identified Region 1 (North Eastern Province) as the region that had the highest diversity of viruses with isolates belonging to Kenya 1a, Kenya 1b, and Kenya 2 lineages.

Comparison of the aa differences across the 6 individual RVF virus proteins revealed a number of aa substitutions,
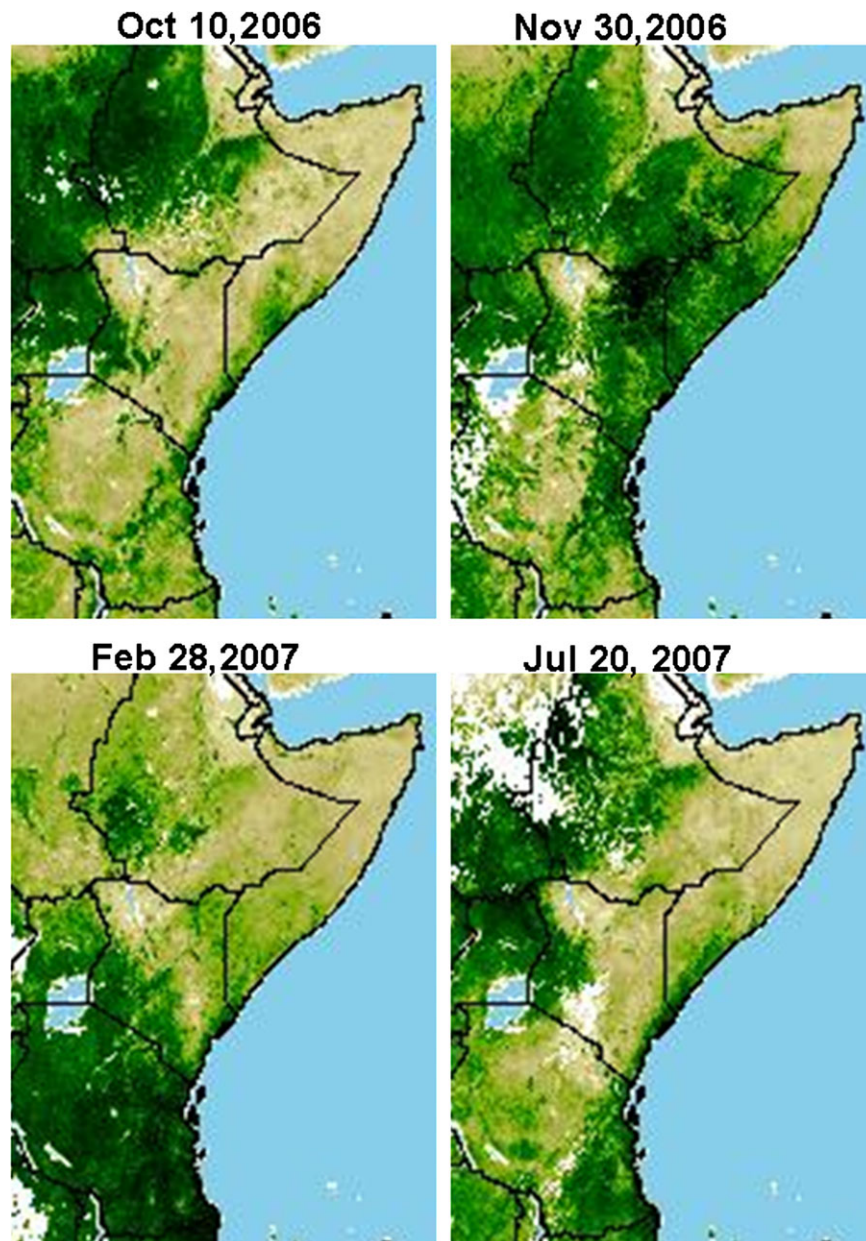
Figure 2. Maps showing the color-coded mean normalized difference vegetative index (NDVI) for East Africa between October 2006 and July 2007. Intensity of green color density indicates vegetation cover. The NDVI is an indicator of rainfall and has been shown to correlate with RVF outbreaks in distinct regions. Map 1 shows the region on 10 October 2006, 6 weeks before onset of the RVF epidemic, when Kenya, Somalia, and Tanzania were dry. The 30 November map represents when rainfall started in Regions 1–4 in Kenya and Somalia. The 28 February 2007 map represents a time point when cases were reported in Tanzania. The 30 July map represents a time point when the eastern Africa region was dry, similar to the 10 October 2006 situation, and there were no more cases of RVF in any of the regions.

particularly in the 2 major surface proteins G1 and G2. Using the first isolate of the epidemic, KEN/Gar 004/06, as the reference strain, we found an average range of .15%–1.82% aa differences within NP (254 aa), NSs (264 aa), NSm (135 aa), G1 (534 aa), G2 (507 aa), and L (2092 aa) proteins. The NSm protein was the most conserved, averaging .15% substitution, whereas G1 protein, the surface glycoprotein, which is involved in virus attachment and entry into cells, was the most variable, averaging 1.82% (range, 1.3%–2.2%) substitutions among the 2006–2007 viruses.
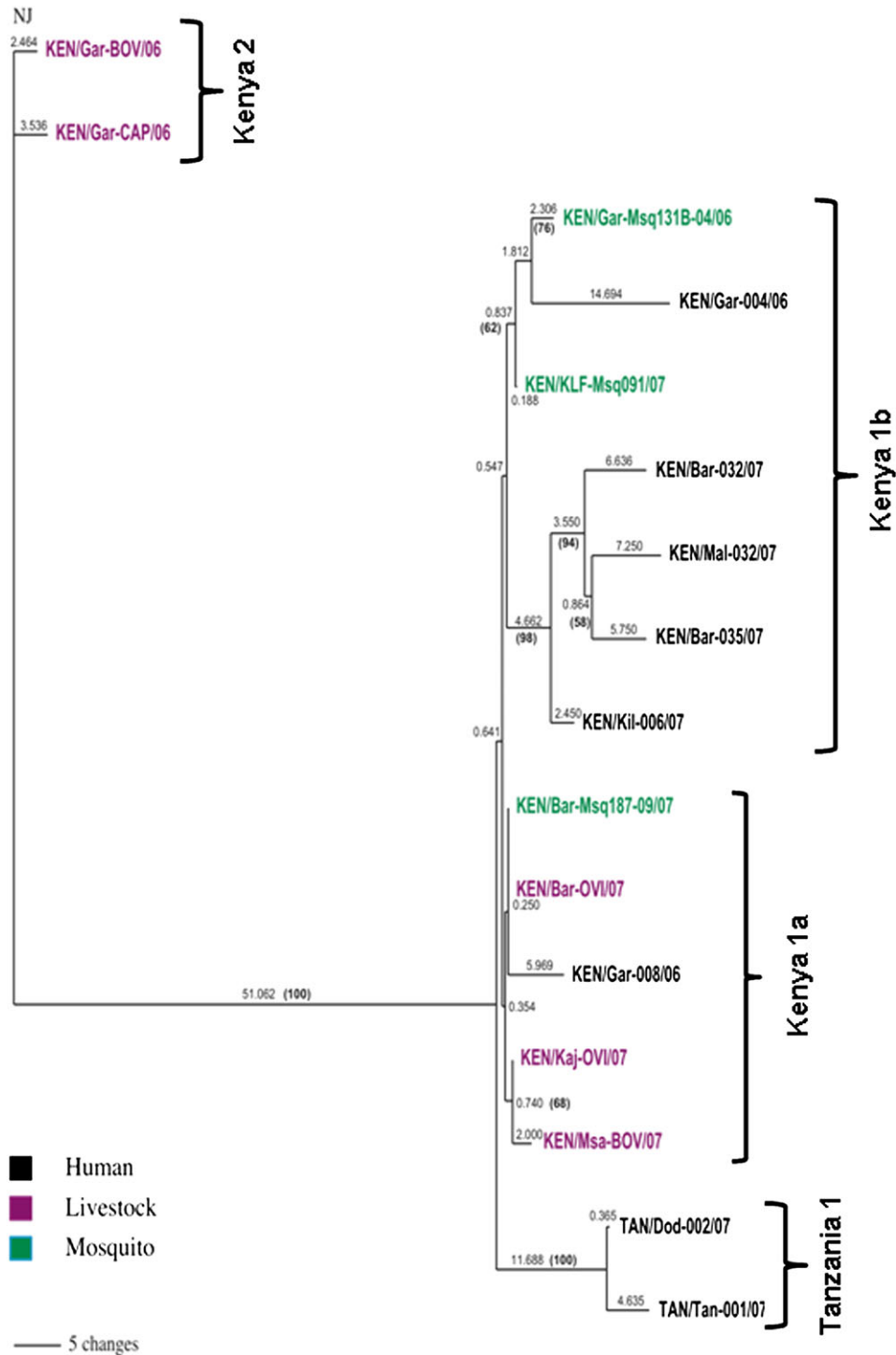
Figure 3. Phylogenetic analysis of the complete genomes of 16 RVF viruses isolated from humans, livestock, and mosquitoes during the 2006–2007 epidemic in eastern Africa. The result revealed 3 distinct lineages of viruses.

Isolates from distinct foci had identical aa substitution patterns, suggesting local spread of a single virus lineage (Figure 4). For example, the isolates from Tanzania, TAN/Tan-001/07 and TAN/Dod-002/07, had 6 of 7 consecutive aa substitutions between positions 419 and 428 of the G1 protein, which were not present in any of the Kenya isolates. Similarly, the 2 Garissa human isolates, KEN/Gar-004/06 and KEN/Gar-008/06, had Arg at position 296 of G1, whereas all the other isolates from other regions had Gly in that position (Figure 4).

Comparison of 2006–2007 isolates with older RVF virus isolates

When compared with older RVF virus isolates (Table 2), all the 16 viruses from the 2006–2007 epidemic clustered together as shown in Figure 5, closest to viruses from the 2 most recent Kenya isolates of 1998 and 1980 and the Saudi Arabia isolate of 2000. In contrast, the 2006–2007 viruses were more distant from the older RVF virus isolates including Entebbe 44 and Kenya 1965, suggesting a continuous but slow evolution clock for the RVF virus. The 2006–2007 isolates had unique aa substitutions
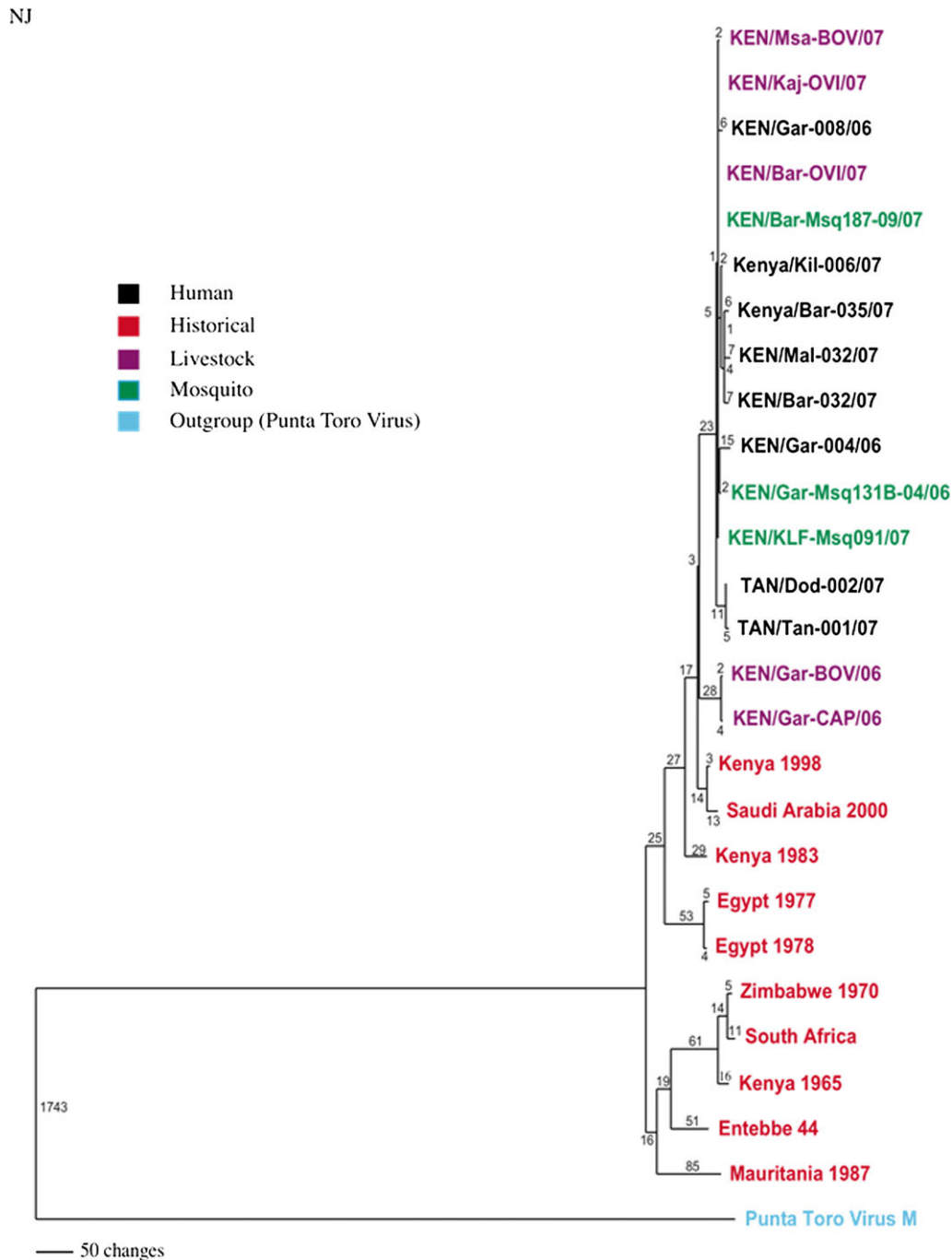
Figure 4. Phylogram comparing the 16 RVF virus strains isolated from the 2006–2007 epidemic in eastern Africa with older RVF viruses.

at positions 280 (Phe to Tyr), 436 (Val to Ile), 442 (Ile to Thr), 449 (Ile to Val), and 506 (Val to Ala) of the G1 protein, different from all historical viruses including Kenya 1998 and Saudi 2000.

DISCUSSION

Both the 1997–1998 and 2006–2007 RVF epidemics in eastern Africa were characterized by sequential outbreaks that appeared across Kenya, Somalia, and Tanzania [14, 25, 26]. The localized outbreaks started in Kenya, followed by Somalia, before being reported in Tanzania, a chronology associated primarily with the pattern of heavy rainfall and flooding in the region during El Niño weather patterns. Apart from heavy rainfall, other factors likely responsible for the localized occurrence of RVF outbreaks in specific areas include flat topology of the area and water-retaining soil types that support flooding, dense bush cover, high *Aedes* mosquito populations, and high livestock populations [15, 27].

We identified 3 lineages of virus responsible for the 2006–2007 epidemic in 5 of the 7 outbreak foci, suggesting activation and amplification of virus residing in these ecological zones. This finding of foci-specific viral lineages in humans is supported by a similar finding in livestock [12], as well as by the unlikely spread of virus between foci because of their being hundreds of miles apart with no reported movement of livestock between them. The de novo initiation of RVF outbreaks in an endemic region may occur if the virus is maintained in the eggs of *Aedes* mosquito species that hatch in large numbers during flooding, or if the virus is maintained through low-level cycling between vertebrates within that ecosystem during an interepidemic period [28]. The finding that RVF virus isolates from 1 focus (Region 1) belonged to 2 different lineages also supports multiple introductions of the virus in 1 locality. Interestingly, the 2 livestock viruses in the Kenya 2 lineage isolated from Region 1 were distinct from any other human viruses isolated from the region, indicating that these viruses did not spread widely to the human population in the region during the 2006–2007 epidemic. On the other hand, detailed aa sequence analyses revealed homologous substitution among viruses from 1 focus (KEN/Gar-004/06 and KEN/Gar-008/06 from Region 1) and among viruses from different foci (TAN/Tan-001/07 from Region 6 and TAN/Dod-002/07 from Region 7), suggesting that amplification and spread of a single virus lineage within and across regions may have occurred. Obviously, the Tanzania 1 lineage virus may have independently emerged in both Tanga (Region 6) and Dodoma (Region 7). Thus, during an RVF outbreak in an endemic region, both viruses spread from 1 focus to the next, and de novo emergence of dormant virus in the ecological zones likely occurred.

Fears had been expressed that the virus was becoming more virulent, following the Arabian Peninsula epidemic of 2000–2001 that reported case fatality rates of 12.4%–14% among identified cases, and the eastern Africa epidemic of 2006–2007 in Kenya and Tanzania that reported case fatality rates of >22.5% [15, 16] . Whereas comparison of the genetic

Table 2. History of the Older (Before 2006) RVFV Isolates Selected for Comparative Genetic Analysis

Virus Name	Source	Geographic Region	Year	GenBank Accession No.	Rationale for Selection
Kenya 1998	Human	Kenya	1998	L - DQ375400M - DQ380196S - DQ380169	Endemic ecology
Kenya 1983	*Aedes macintoshi*	Kenya	1983	L – DQ375402M – DQ380198S – DQ380171	Endemic ecology
Kenya 1965	Bovine	Kenya	1965	L – DQ375427M – DQ380190S – DQ380176	Endemic ecology
Saudi Arabia 2000	Human	Saudi Arabia	2000	L – DQ375401M – DQ380197S – DQ380170	Most recent outbreak
Egypt 1977	Human	Egypt	1977	L – DQ375406M – DQ380200S – DQ380149	Naïve ecology
Egypt 1978	Human	Egypt	1978	L – DQ375412M – DQ380207S – DQ380152	Naïve ecology
Mauritania 1987	Human	Mauritania	1987	L – DQ375396M – DQ380184S – DQ380178	Naïve ecology
Entebbe 44	*Eretmapodites* mosquito (pool)	Uganda	1944	L – DQ375429M – DQ380191S – DQ380156	Oldest isolate
Zimbabwe 1970	Bovine	Zimbabwe	1970	L – DQ375426M – DQ380188S – DQ380174	Southern Africa isolate
South Africa 1975	Human	South Africa	1975	L – DQ375428M – DQ380189S – DQ380175	Southern Africa isolate
Punta Toro (Balliet)	Human	Panama	1966	DQ363407	Outgroup

composition of viruses involved in the 2006–2007 epidemic with the older RVF virus isolates (1944–2002) did not reveal major genetic changes in the virus, it is important to keep in mind that virulence of the virus may be altered by substitution of only a few key aa. Our finding that key viral components such as the G1 glycoprotein of RVF virus (involved in virus attachment and entry into cells) have as high as 2.2% aa substitution suggests that monitoring such changes may be important in identifying the emergence of potentially more virulent viruses. This relatively high mutation rate of G1 protein, an integral membrane protein of the virus that is a major target of host neutralizing antibodies, may be due to sustained immunological pressure from long-lasting neutralizing antibodies in infected vertebrates. It is important to note that the reported increase in death during recent RVF epidemics likely represents a bias in case finding in which severe RVF cases were identified whereas most mild cases went undetected [15].

In conclusion, the findings of this study confirm the presence of different lineages of the RVF virus, both within the same outbreak foci and across geographically distant outbreak foci during the 2006–2007 RVF epidemic involving 3 eastern African countries. These findings support the concept of reemergence of resident populations of endemic viruses in each outbreak foci, probably via either spontaneous hatching of infected *Aedes* mosquito larvae or expansion of a resident virus that was maintained through low-level cycling among vertebrates and possibly humans. Clearly, virus spread through livestock or human movement during an epidemic can occur, which makes banning of livestock movement an important intervention. However, our study suggests that banning livestock movement alone may not prevent the occurrence of RVF outbreaks in other permissive ecosystems in a country.

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Majority        LKSFDISQCPKIGGHGSKKCTGDAAFCSAYECTAQYANAYCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI
                ---------+---------+---------+---------+---------+---------+---------+---------+
                        250       260       270       280       290       300       310       320
                ---------+---------+---------+---------+---------+---------+---------+---------+
KEN/Gar-004/06  LKSFDISQCPKIGGHGRKKRTGDAAFCSAYECTAQYANAYCSRANGSGIVQIQVSRVWKKPLCVGYERVVVKRELSAKPI   320
KEN/Gar-008/06  LKSFDISQCPKIGGHGSKKCTGDAAFCSAYECTAQYANAYCSHANGSGIVQIQVSRVWKKPLCVGYERVVVKRELSAKPI   320
KEN/Kil-006/07  LKSFDISQCPKIGGHGSKKCTGDAAFCSAYECTAQYANAYCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI   320
KEN/Mal-032/07  LKSFDISQCPKIGGHGSKKCTGDAAFCSAYECTAQYANAYCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI   320
KEN/Bar-032/07  LKSFDISQCPKIGGHGSKKCTGDAAFCSAYECTAQYANAYCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI   320
KEN/Bar-035/07  LKSFDISQCPKIGGHGSKKCTGDAAFCSAYECTAQYANAYCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI   320
TAN/Tan-001/07  LKSFDISQCPKIGGHGRKKCTGDAAFCSAYECTAQYANAYCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI   320
TAN/Dod-002/07  LKSFDISQCPKIGGHGRKKCTGDAAFCSAYECTAQYANAYCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI   320
Kenya 9800523   LKSFDISQCPKIGGHGSKKCTGDAAFCSAYECTAQYANAFCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI   320
Saudi 2000-10911 LKSFDISQCPKIGGHGSKKCTGDAAFCSAYECTAQYANAFCSHANGSGIVQIQVSGVWKKPLCVGYERVVVKRELSAKPI  320

Majority        AHCPPQDPCLVHGCIVCAHGLINYQCHTALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK
                ---------+---------+---------+---------+---------+---------+---------+---------+
                        410       420       430       440       450       460       470       480
                ---------+---------+---------+---------+---------+---------+---------+---------+
KEN/Gar-004/06  AHCPPQDPCLVHGCIVCAHGLINNQSHSALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
KEN/Gar-008/06  AHCPPQDPCLVHGCIVCAHGLINYQCHTALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
KEN/Kil-006/07  AHCPPQDPCLVHGCIVCAHGLINYQCHTALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
KEN/Mal-032/07  AHCPPQDPCLVHGCIVCAHGLINYQCHTALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
KEN/Bar-032/07  AHCPPQDPCLVHGCIVCAHGLINYQCHTALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
KEN/Bar-035/07  AHCPPQDPCLVHGCIVCAHGLINYQCHTALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
TAN/Tan-001/07  AHCPPQDPCLVHGCIVCAQAWIISQWHTALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
TAN/Dod-002/07  AHCPPQDPCLVHGCIVCAQAWIISQWHTALSAFVVIFVFSSTAIICLAVLYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
Kenya 9800523   AHCPPQDPCLVHGCIVCAHGLINYQCHTALSAFVVVFVFSSIAIICLAILYRVLKCLKIAPRKVLNPLMWITAFIRWVYK   480
Saudi 2000-10911 AHCPPQDPCLVHGCIVCAHGLINYQCHTALSAFVVVFVFSSIAIICLAILYRVLKCLKIAPRKVLNPLMWITAFIRWVYK  480

Majority        KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA
                ---------+---------+---------+---------+---------+-------+
                        490       500       510       520       530
                ---------+---------+---------+---------+---------+-------+
KEN/Gar-004/06  KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
KEN/Gar-008/06  KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
KEN/Kil-006/07  KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
KEN/Mal-032/07  KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
KEN/Bar-032/07  KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
KEN/Bar-035/07  KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
TAN/Tan-001/07  KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
TAN/Dod-002/07  KMVARVADNINQVNREIGWMEGGQLALGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
Kenya 9800523   KMVARVADNINQVNREIGWMEGGQLVLGNPAPIPRHAPIPRYSTYLMLLLIVSYASA   537
Saudi 2000-10911 KMVARVADNINQVNREIGWMEGGQLVLGNPAPIPRHAPIPRYSTYLMLLLIISYASA  537
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Figure 5. Predicted amino acid alignment of the partial G1 proteins (amino acid 241-537) for selected RVF virus isolates from the 2006–2007 epidemic. Amino acid substitutions in the 2006–2007 RVF virus isolates compared with 2 other, more recent isolates, the Kenya 1998 and Saudi Arabia 2000 RVF virus isolates, are shown.

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