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Arboviruses Isolated From Mosquitoes Collected in Uganda, 2008–2012

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Abstract

A large number of arthropod-borne viruses are endemic to East Africa. As a part of the process of undertaking a systematic characterization of the mosquito fauna of Uganda, we examined mosquitoes collected from 2008 through early 2012 for known and novel viruses. In all, 8,288 mosquito pools containing 157,554 mosquitoes were tested. Twenty-nine isolations of 11 different viruses were made from mosquitoes of nine distinct species and from pools identified only to genus *Culex*. Identified viruses were from family *Togaviridae*, alphaviruses Sindbis and Babanki viruses; family *Rhabdoviridae*, hapaviruses Mossuril and Kamese viruses; family *Flaviviridae*, flaviviruses West Nile and Usutu viruses; family *Phenuiviridae*, phlebovirus Arumowot virus; and family *Peribunyaviridae*, orthobunyaviruses Witwatersrand, Pongola, and Germiston viruses. In addition, a novel orthobunyavirus, provisionally named Mburo virus, was isolated from *Coquillettidia metallica* (Theobald). This is the first report of Babanki, Arumowot, and Mossuril virus isolation from Uganda.

Key words: arbovirus, mosquito, Uganda

Arthropod-borne pathogens have contributed significantly to the emergence and reemergence of infectious diseases in recent decades (Rosenberg 2015). Of particular concern to global health are areas of the world that pose a high risk for emerging infectious disease (EID) events due to growing human populations, changes in land use altering the interface between humans and wildlife, increasing global travel and commerce, and climate change. Based on these and other criteria, East Africa has been identified as a region of high risk for EID events in the future (Jones et al. 2008). These events are frequently attributed to zoonotic viruses circulating in wildlife that infect humans via transmission by arthropods, with zoonoses from wildlife representing over 60% of all EIDs (Jones et al. 2008). Uganda, in particular, has been the site of multiple EID events, with >20 new arthropod-borne viruses (arboviruses) having been isolated there in the past 70 yr (Karabatsos 1985).

To assess the current level of known and novel arbovirus circulation in Uganda, the U.S. Centers for Disease Control and Prevention (CDC) and the Uganda Virus Research Institute (UVRI) initiated a collaborative survey beginning in 2008 with the goals of collecting and describing the current mosquito fauna of Uganda and screening for arboviruses circulating in mosquitoes, rodents, bats, and other small mammals. Previous publications have reported results from this survey, including descriptions of the mosquitoes collected, prevalence of antibodies to arboviruses in vertebrate sera, identification of host blood from engorged mosquitoes, and descriptions of viruses isolated from bird and bat hosts (Mutebi et al. 2012; Crabtree et al. 2013; Kading et al. 2013a,b; Ledermann et al. 2014). We report here the results of surveillance for arboviruses in mosquitoes collected from 21 sites in Uganda between November 2008 and February 2012.

Twenty nine (29) virus isolates were obtained from 8,288 mosquito pools (1,57,554 mosquitoes), comprising 11 arboviruses from four virus families. Isolates belong to the families *Togaviridae* (genus *Alphavirus*, $n = 8$), *Peribunyaviridae* (genus *Orthobunyavirus*, $n = 13$), *Phenuiviridae* (genus *Phlebovirus*, $n = 2$), *Flaviviridae* (genus *Flavivirus*, $n = 3$), and *Rhabdoviridae* (genus *Hapavirus*, $n = 3$). We report the first isolation of Arumowot virus, Mossuril virus, and Babanki virus from Uganda and additionally describe one novel virus, provisionally named Mburo virus, identified to be genetically most closely related to viruses in the Tete serogroup of the *Orthobunyavirus* genus, *Peribunyaviridae*.

Materials and Methods

Mosquitoes were collected using CO_2-baited CDC light traps and CDC gravid traps as described elsewhere (Godsey et al. 2012,

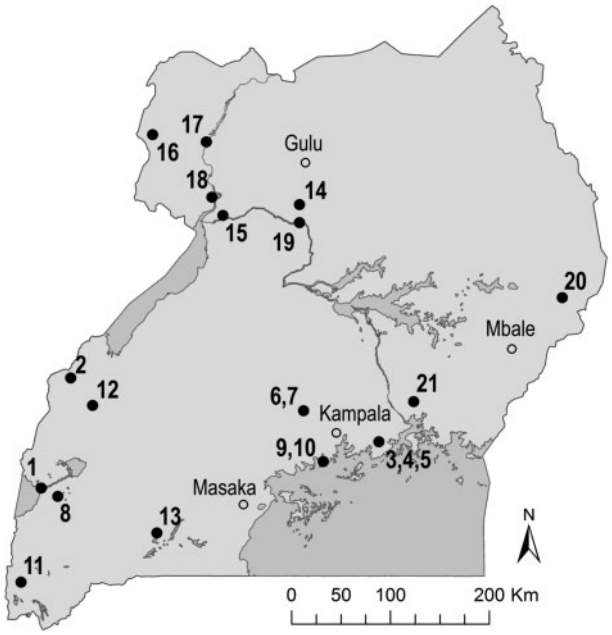

Fig. 1. Map of Uganda showing mosquito collection sites. Collection sites (•) as well as cities and other locales (○) are indicated. Study site numbers correspond to Table 2.

Mutebi et al. 2012). Collections were made at 21 sites throughout Uganda between November 2008 and February 2012 (Fig. 1). Mosquitoes were identified to species using available keys (Edwards 1941, Gillies and DeMeillon 1968, Gillett 1972, Gillies and Coetzee 1987, Jupp 1996), and pooled by trap date, location, and type as well as species, sex, gravidity, and bloodmeal presence (≤25 mosquitoes per pool).

The infection status of mosquito pools was determined by trituration of mosquitoes in 2-ml conical microcentrifuge tubes with 1.3 ml BA-1 medium (1× Medium 199 with Earle's salts, 1% bovine albumin, 100 U/ml penicillin, 100 mg/ml streptomycin, 1.5 µg/ml gentamycin, and 1.5 µl/ml amphotericin B) and one copper bead per tube using a Qiagen Tissuelyser (Qiagen, Valencia, CA). Triturated mosquito preparations were clarified by centrifugation at 9,000 rpm at 4 °C for 20 min. Clarified homogenate supernatants were stored at −80 °C. Mosquito homogenates were tested for infectious virus by plaque titration on Vero cells in six-well plates as previously described using 100 µl of each clarified mosquito pool supernatant and with second overlays applied at 4 d postinfection (Miller et al. 1989). Plates were observed for plaques for 15 d following infection. Plate wells in which plaques were observed were harvested as previously described and viruses were amplified by infecting T-25 flasks of Vero cells with 25 µl of each plaque isolate (Crabtree et al. 2009). Flasks were observed and supernatants harvested when cytopathic effects were evident.

Viral RNA was extracted from amplified virus supernatants and identification of virus isolates was attempted by reverse transcription-polymerase chain reaction (RT-PCR) and partial Sanger dideoxy sequencing as previously described using arbovirus group-specific primers for alphaviruses, orthobunyaviruses, and flaviviruses (Kuno et al. 1998, Powers et al. 2001, Bryant et al. 2005, Lambert and Lanciotti 2008). Partial nucleic acid sequences were compared with those in the GenBank database using the BLAST program to identify isolates. Isolates that were negative by group-specific RT-PCR were tested by immunofluorescence assay (IFA) using National Institutes of Health polyvalent mouse hyperimmune ascitic fluids (grouping fluids) as previously described, to identify an arbovirus serogroup to which the isolates might be related (Sang et al. 2006). Positive IFA results were then used to direct virus-specific RT-PCR using primers designed from published related virus sequences. In cases where IFA was also negative, or where positive IFA followed by virus-specific RT-PCR did not produce positive results, isolates were sequenced using next-generation sequencing (NGS) on an Ion Torrent Personal Genome Machine (Life Technologies) as described elsewhere (Kading et al. 2013b, Ledermann et al. 2014). Genetic relatedness of assembled sequences derived from NGS was identified by using the BLAST program at the National Center for Biotechnology Information. Sequences were verified and sequence gaps filled in by using Sanger dideoxy sequencing as necessary. Phylogenetic relationships among amino acid sequences were determined by maximum likelihood analysis using the LG + G + I + F model on MEGA6 software (Tamura et al. 2013).

Results

Twenty-nine virus isolations were made from pools of *Aedes* (*Aedimorphus*) *tarsalis* (Newstead) (1 isolate), *Coquillettidia* (*Cq.*) *fuscopennata* (Theobald) (1), *Cq.* (*Cq.*) *metallica* (Theobald) (1), *Culex* (*Cx.*) *antennatus* (Becker) (1), *Culex* (*Cx.*) *neavei* Theobald (2), *Culex* (*Cx.*) *perfuscus* (Edwards) (8), *Culex* (*Eumelanimyia*) *rubinotus* Theobald (10), *Culex* (*Cx.*) *univittatus* Theobald (1), *Culex* species (3), and *Mansonia* (*Mansonoides*) *africana* (Theobald) (1) (Table 1). Viruses were isolated from 8 of the 21 collection sites included in the survey: Mweya, Queen Elizabeth National Park (QENP) (1 isolate), Sempaya, Semliki National Park (SNP) (8), Nkokonjeru (1), Luwawa Forest Reserve (LFR) (2), Segganga (10), Kibale National Park (KNP) (1), Lake Mburo National Park (LMNP) (3), and Jinja (3) (Fig. 1; Table 2). All viruses were isolated from mosquitoes collected using CDC light traps.

Eleven different viruses were identified in this study (Table 1). Sindbis virus (SINV; 97.6% BLAST identity) was isolated from one pool each of *Cx. neavei* and *Ma. africana*, both from the LMNP collections (June 2010). Babanki virus (BBKV; 98–99.8% BLAST identities) was isolated from six mosquito pools: five pools of *Cx. perfuscus* and one pool of *Culex* species collected at Sempaya, SNP (November 2008 and June 2009) and Kikusa, Nkokonjeru (February 2009), respectively. Pongola virus (PGAV; 97% BLAST identity) was isolated from one pool each of *Ae. tarsalis* and *Cq. fuscopennata*, both collected at Nansomba Village, LFR (March 2009). Germiston virus (GERV; 92.7–93.4% BLAST identities) was isolated from four pools of *Cx. rubinotus* collected at Luvunvu village, Segganga (February 2009). A novel orthobunyavirus, provisionally named Mburo virus (MBUV), was isolated from a pool of *Cq. metallica* collected at the LMNP site (February 2012). Arumowot virus (AMTV; 97.3% BLAST identity) was isolated from one pool each of *Cx. antennatus* and *Culex* species, both collected at Jinja (February 2012). Six isolates of Witwatersrand virus (WITV; 80% nucleotide BLAST identity) were obtained from *Cx. rubinotus* collected at Luvunvu village, Segganga (February 2009). We isolated West Nile virus (WNV; 98.6% BLAST identity) from a pool of *Cx. neavei* collected at Mweya, QENP (June 2009), and Usutu virus (USUV; 98% BLAST identity) from one pool each of *Cx. univittatus* and *Culex* species, collected at Jinja (February 2012) and KNP (June 2010), respectively. We made two isolates of Mossuril virus (MOSV; 93–96% BLAST identities) and one of Kamese virus (KAMV; 98.2%

Table 1. Number of isolates of each virus by collection site

Collection site	Togaviridae		Peribunyaviridae				Phenuivirus	Flaviviridae		Rhabdoviridae	
	Alphavirus		Orthobunyavirus				Phlebovirus	Flavivirus		Hapavirus	
Species	Babanki	Sindbis	Pongola	Germiston	Mburo	Witwatersrand	Arumowot	West Nile	Usutu	Mossuril	Kamese
Mweya, Queen Elizabeth National Park											
Culex neavei	–	–	–	–	–	–	–	1		–	–
Sempaya, Semliki National Park											
Culex perfuscus	5	–	–	–	–	–	–	–	–	2	1
Nkokonjeru, Kikusa											
Culex species	1	–	–	–	–	–	–	–	–	–	–
Luwawa Forest Reserve, Nansomba Village											
Aedes tarsalis	–	–	1	–	–	–	–	–	–	–	–
Coquillettidia fuscopennata	–	–	1	–	–	–	–	–	–	–	–
Segganga, Luvunvu Village											
Culex rubinotus	–	–	–	4	–	6	–	–	–	–	–
Kibale National Park											
Culex species	–	–	–	–	–	–	–	–	1	–	–
Lake Mburo National Park											
Culex neavei	–	1	–	–	–	–	–	–	–	–	–
Mansonia africana	–	1	–	–	–	–	–	–	–	–	–
Coquillettidia metallica	–	–	–	–	1	–	–	–	–	–	–
Jinja, Kakira Sugar Works											
Culex antennatus	–	–	–	–	–	–	1	–	–	–	–
Culex species	–	–	–	–	–	–	1	–	–	–	–
Culex univitattus	–	–	–	–	–	–	–	–	1	–	–

BLAST identity), all from pools of *Cx. perfuscus* collected at Sempaya, SNP (June 2009). Nucleic acid sequences from these isolates were obtained by using RT-PCR, with primers designed from published sequence of the KAMV polymerase gene (KAMV-184F, T ATGAAAAGTGGAACAATCATC; KAMV-404R, CCTTTCT GGCCGTCCCAAC).

As mentioned above, a novel orthobunyavirus, provisionally named Mburo virus, was isolated from a pool of *Cq. metallica* mosquitoes collected at the LMNP site. To characterize this new virus, we used IFA with National Institutes of Health grouping fluids. The IFA results suggested the virus was serologically related to the orthobunyaviruses (data not shown). Next-generation sequencing was then used to obtain partial sequences from the S and L segments of the virus. BLAST comparison of the 248 amino acid nucleocapsid sequence and the 2,262 amino acid RNA-dependent RNA polymerase (RdRP) sequence suggested that the virus was most similar to viruses in the Tete serogroup and the closely related Oyo virus (OYOV) of the *Orthobunyavirus* genus, family *Peribunyaviridae* (nucleocapsid: >90% coverage, 42–46% identity to Tete serogroup, 45% identity to OYOV; RdRP: 99% coverage, 62–63% identity to Tete serogroup, 55% identity to OYOV). Amino acid sequence maximum likelihood alignment with other orthobunyaviruses confirmed this relationship (Fig. 2).

Discussion

Arboviruses comprise a diverse group of human and animal pathogens that may cause mild to severe illness and result in significant public health and economic consequences. The number of EID events in recent decades that are attributable to zoonotic arboviruses calls for increased regular surveillance to predict and control future emergence or reemergence of viruses causing infectious diseases. This study was designed to gather baseline information regarding the circulation of arboviruses throughout Uganda by sampling potential arbovirus vector mosquitoes that may be part of zoonotic cycles. Because many EID events have resulted as a consequence of increased human population and land use, collection sites for this study were selected to allow surveillance at the interface of wildlife areas and human habitation as well as in villages and undisturbed habitats.

We report the isolation of 11 arboviruses, including one novel virus and three viruses never before isolated from Uganda. Six of these viruses—BBKV, SINV, PGAV, GERV, WNV, and USUV—have been isolated from humans and associated with febrile illness, often accompanied by rash and arthritis. West Nile virus has also been shown to cause more severe disease including CNS symptoms (Davis et al. 2006). Of the known viruses isolated in this study, three—BBKV, AMTV, and MOS—have not been reported previously from Uganda. Additionally, five of the known arboviruses we isolated were obtained from mosquitoes of species not previously associated with these viruses: PGAV from *Cq. fuscopennata*, WNV from *Cx. neavei*, USUV from *Cx. univitattus*, and MOSV and KAMV from *Cx. perfuscus*.

The six isolates that were identified as Witwatersrand virus were compared with GenBank sequences at both the nucleotide and amino acid levels. At the nucleotide level, BLAST identities were 80% compared with the single Witwatersrand virus represented in GenBank that was isolated in 1958. However, amino acid comparisons yielded BLAST identities of 94%, indicating that although changes had occurred in the nucleotide sequence over the 50+ yr between these isolations, the amino acid sequence was highly conserved.

We report herein the isolation of a novel virus that we have provisionally named Mburo virus (MBUV). This virus is genetically

Table 2. Mosquito collection sites and numbers tested

Site no.	Site location	Latitude/Longitude	Collection dates	No. of mosquitoes tested	No. of pools tested
1	Mweya, Queen Elizabeth National Park	0° 11′ S, 29° 54′ E	Nov. 2008	8,540	377
			June 2009	13,355	615
			Jan. 2010	6,630	326
2	Sempaya, Semliki National Park	0° 49′ N, 30° 10′ E	Nov. 2008	4,259	236
			June 2009	5,177	267
			Jan. 2010	1,418	114
3	Busitwe, Nkokonjeru	0° 14′ N, 32° 57′ E	Feb. 2009	3,928	229
4	Kikusa, Nkokonjeru	0° 14′ N, 32° 58′ E	Dec. 2008	275	15
			Feb. 2009	1,447	80
5	Kyambogo, Nkokonjeru	0° 13′ N, 32° 58′ E	Dec. 2008	1,468	88
			Jan. 2009	1,471	76
6	Nansomba Village, Luwawa Forest Reserve, Segganga	0° 31′ N, 32° 18′ E	Feb. 2009	4,188	240
7	Lununvu Village, Segganga	0° 32′ N, 32° 17′ E	Feb. 2009	9,449	445
8	Maramagambo, QENP	0° 16′ S, 30° 03′ E	June 2009	488	45
			Jan. 2010	890	96
9	Kitubulu Forest Reserve, Entebbe	0° 07′ N, 32° 47′ E	June 2009	1,807	86
			Jan. 2010	12,145	563
10	Uganda Virus Research Institute, Entebbe	0° 07′ N, 32° 46′ E	Jan. 2010	21	13
11	Bwindi Impenetrable Forest National Park	01° 03′ S, 29° 43′ E	Jan. 2010	586	53
			June 2010	943	79
12	Kibale National Park	0° 34′ N, 30° 22′ E	June 2010	2,851	166
13	Lake Mburo National Park	0° 36′ S, 30° 57′ E	June 2010	20,809	1,016
14	Chobe, Murchison Falls National Park	2° 24′ N, 32° 13′ E	Jan. 2011	3,658	191
			June 2011	7,451	407
15	Paraa, Murchison Falls National Park	2° 18′ N, 31° 33′ E	Jan. 2011	7,476	368
			June 2011	1,906	183
16	Sunguru, Arua	3° 01′N, 30° 48’E	Jan. 2011	317	46
			June 2011	466	74
17	Rhino Camp	2° 58′ N, 31° 24′ E	Jan. 2011	18,927	841
18	Pakwach	2° 28′ N, 31° 27′ E	June 2011	16	9
19	Karuma	2° 15′ N, 32° 14′ E	June 2011	84	7
20	Sipi, Mt. Elgon National Park	1° 33′ N, 34° 38′ E	Jan. 2012	3,928	352
21	Jinja, Kakira Sugar Works	0° 36′ N, 33° 17′ E	Feb. 2012	11,180	585
	Total			157,554	8,288

most closely related to viruses in the Tete serogroup and OYOV of the *Orthobunyavirus* genus, family *Peribunyaviridae*. Several viruses of the Tete serogroup are among the relatively small number of orthobunyaviruses that are known to be transmitted by or have been isolated from ticks. Further, many members of this serogroup have been repeatedly isolated from birds (Shchetinin et al. 2015). It is therefore not surprising that MBUV was isolated from *Cq. metallica*, as *Coquillettidia* species are known to be ornithophilic (Williams et al. 1958, Mukwaya 1972, Rickenbach et al. 1974, Chandler and Highton 1976, Beier et al. 1990, Crabtree et al. 2013). In a previous study in Uganda, all identified bloodmeals from *Coquillettidia* species mosquitoes were exclusively from birds, except those from *Cq. fuscopennata*. Additionally, using the methods of Crabtree et al. (2013), we identified the bloodmeals of six *Cq. metallica* collected in Uganda, five of which were birds (M.B.C., unpublished data). The single reported isolation of OYOV was made from the blood of a pig in 1964 (R.B. Tesh, personal communication). No arthropod association for OYOV has yet been documented. Mburo virus may also be related to Sedlec virus (SEDV; Hubalek et al. 1990). Sedlec virus was not included in our analyses owing to the limited sequence information available. However, based on partial sequence analysis, the L segment of SEDV appeared to be similar to OYOV and the Tete serogroup, but distinct from MBUV (data not shown), while the S segment was more closely related to the Simbu serogroup–Leanyer virus clade (Bakonyi et al. 2013). Further study is needed to determine whether MBUV may also be tick-borne and what its vertebrate host range might be. Regrettably, owing to a freezer malfunction, the isolate was lost and additional characterization will not be possible until the virus is isolated again in the future.

Although a relatively low number of isolates were obtained from these mosquito collections given the large number of mosquitoes tested, the number was not unexpected when compared with reports of other arbovirus surveillance studies in the region. In 2009, Crabtree, et al. reported the isolation of 27 virus strains from 2,651 mosquito pools collected during an outbreak of Rift Valley fever virus (RVFV) in Kenya (2006–2007; Crabtree et al. 2009). Environmental conditions during this period were conducive to a very high abundance of mosquito vectors, and as a result, increased interaction between mosquitoes and humans or nonhuman hosts, thereby supporting increased virus transmission (Nguku et al. 2010). Similarly, in a study by Ochieng et al. (2013) conducted in Kenya from 2007 to 2012, mosquito collections were made only during wet seasons when mosquito population density was high, again resulting in increased host and vector contact supporting increased virus transmission. During that study, 83 virus isolates were obtained from 15,890 mosquito pools, many of which were floodwater *Aedes*. In our study, collections were made at most sites during multiple seasons, both dry and wet, and as a result, mosquito population density was quite low for some collections. Additionally, some collection sites were close to bodies of water (lakes, rivers, and swamps), resulting in collection of a high number of *Coquillettidia* mosquitoes which are not known to vector many arboviruses (Karabatsos 1985, Mutebi et al. 2012). In addition to the effect of

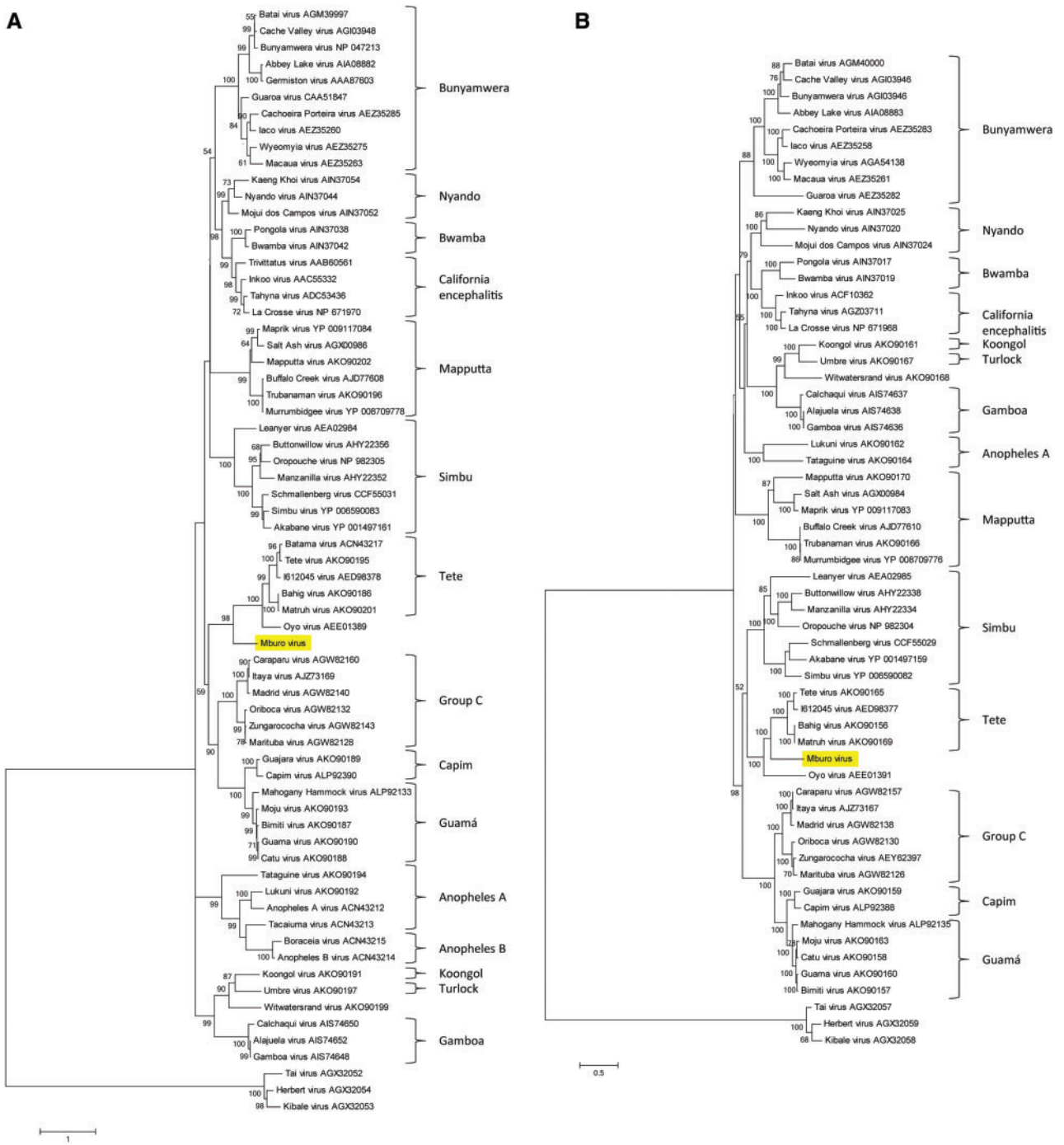

Fig. 2. Amino acid sequence-based phylogenies of orthobunyavirus nucleocapsid and RNA-dependent RNA polymerase. Full-length amino acid sequences of nucleocapsid (**A**) and RNA-dependent RNA polymerase (**B**) were obtained from GenBank and aligned with the derived sequences of Mburo virus (highlighted; GenBank MF074140 and MF074141, respectively). Phylogeny was determined by maximum likelihood analysis using the LG + G + I + F model. The trees were rooted using three members of the *Herbevirus* genus, family *Peribunyaviridae* (Marklewitz et al. 2013). Serogroups are shown at the right for reference.

habitat on the composition of mosquito collections, trapping methods also play an important role. In the study mentioned above by Ochieng et al. mosquitoes were collected using both CO_2-baited CDC light traps and human bait collections, resulting in collection of a higher number of anthropophilic mosquitoes which may be more likely to transmit viruses to humans. Owing to study design constraints, our survey did not include human-baited collections.

Our collections also comprised many naïve or newly emerged specimens as indicated by 1) the presence in some collections of relatively higher numbers of males which emerge before females, 2) the low proportion of engorged specimens collected (0.74%), and 3) the collection of a relatively small proportion of gravid specimens (0.85%). These younger mosquitoes would be less likely to have taken a first bloodmeal and therefore would not have had the opportunity to acquire a virus from a host.

Comparison of viruses isolated in our study with those of the two Kenya studies mentioned above suggests that similar viruses are circulating in both countries. Viruses isolated in all three studies

include SINV, BBKV, PGAV, and WNV. In our study, the greatest number of viruses isolated were from the family *Peribunyaviridae* ($n = 13$), followed by the *Togaviridae* ($n = 8$); in both Kenya studies, the family most represented in the virus isolations was the *Togaviridae*, followed by *Peribunyaviridae* and *Flaviviridae* (Crabtree et al. 2009) or *Flaviviridae* and *Peribunyaviridae* (Ochieng et al. 2013).

The results of this surveillance study are important if we are to detect, prevent, and control outbreaks of emerging and reemerging infectious diseases caused by arboviruses. The number and identity of isolated viruses that cause febrile illness will suggest directions for human diagnostic testing to determine arboviral agents causing febrile illnesses.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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