Mosquito-borne arboviruses in Uganda: history, transmission and burden

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Abstract

Mosquito-transmitted arboviruses constitute a large proportion of emerging infectious diseases that are both a public health problem and a threat to animal populations. Many such viruses were identified in East Africa, a region where they remain important and from where new arboviruses may emerge. We set out to describe and review the relevant mosquito-borne viruses that have been identified specifically in Uganda. We focused on the discovery, burden, mode of transmission, animal hosts and clinical manifestation of those previously involved in disease outbreaks. A search for mosquito-borne arboviruses detected in Uganda was conducted using search terms “Arboviruses in Uganda” and “Mosquitoes and Viruses in Uganda” in PubMed and Google Scholar in 2020. Twenty-four mosquito-borne viruses from different animal hosts, humans and mosquitoes were documented. The majority of these were from family *Peribunyaviridae*, followed by *Flaviviridae*, *Togaviridae*, *Phenuiviridae* and only one each from family *Rhabdoviridae* and *Reoviridae*. Sixteen (66.7%) of the viruses were associated with febrile illnesses. Ten (41.7%) of them were first described locally in Uganda. Six of these are a public threat as they have been previously associated with disease outbreaks either within or outside Uganda. Historically, there is a high burden and endemicity of arboviruses in Uganda. Given the many diverse mosquito species known in the country, there is also a likelihood of many undescribed mosquito borne viruses. Next generation diagnostic platforms have great potential to identify new viruses. Indeed, four novel viruses, two of which were from humans (Ntwetwe and Nyangole viruses) and two from mosquitoes (Kibale and Mburo viruses) were identified in the last decade using next generation sequencing. Given the unbiased approach of detection of viruses by this technology, its use will undoubtedly be critically important in the characterization of mosquito viromes which in turn will inform other diagnostic efforts.

Key words: history, mosquito-borne arboviruses, outbreaks, Uganda

Introduction

Mosquito-borne arboviruses constitute an important proportion of emerging infectious diseases that are a global threat to the human and animal populations (1-5). About 167/300 (55.7%) of the viruses in "The Arthropod borne viruses of vertebrates", a book that gives an account of the activities of the Rockefeller Foundation program were listed as transmitted by mosquitoes (6). The rest of the viruses are transmitted by either ticks, mites, sandflies or biting midges. Over 530 arboviruses are listed in the CDC Arbovirus catalogue, with majority of the mosquito borne viruses found in the *Togaviridae, Flaviviridae, Rhabdoviridae*, *Reoviridae* families; as well as families in the recently created order *Bunyavirales* (7, 8).

Surveillance for mosquito-borne viruses and vector species in Uganda began in the mid 1930s and were carried out by the Yellow Fever Research Institute (YFRI), the Medical Department of Uganda Protectorate and the Rockefeller Foundation (9, 10). The main objective at that time was to ascertain whether yellow fever virus (YFV, family *Flaviviridae*) was actively transmitted in East Africa and if so determine the extent of spread eastwards from West Africa (11). In the course of these investigations, West Nile virus (WNV, family *Flaviviridae*) and Bwamba virus (BWAV, family *Peribunyaviridae*) were isolated from North-Western Uganda and Western Uganda (12, 13). Further countrywide surveys to determine the endemicity of yellow fever led to the discovery of many other viruses including Semliki Forest virus (SFV, family *Togaviridae*), Bunyamwera virus (BUNV, family *Peribunyaviridae)*, Ntaya virus (NTAV, family *Flaviviridae*) and Uganda S (UGSV, family *Flaviviridae*) (14-18). By 1947, a total of 10 viruses had been isolated and characterized (19, 20). In 1950, the mandate of the YFRI -now the Uganda Virus Research Institute (UVRI)- was expanded to search for all possible viruses that might be endemic in the region. Since then, over

20 arboviruses were described (11, 21). Some of the viruses locally described in Uganda have emerged in other parts of the world and evolved into additional genotypes and lineages associated with morbidity and mortality (22, 23). Although a number of viruses were described in the past, recent technological advances in diagnostic tools have led to an increased number of viruses and outbreaks identified, which could not be identified by available traditional methods (24-27). Here we aimed to update and summarize the current knowledge of mosquito-borne arboviruses that have been identified in Uganda. This is of interest both from a historical point of view, given the importance of the country in the history of arbovirology, but also informative for current preventive efforts in the region.

Overview and analysis of published records

A search for all mosquito-borne arboviruses detected in Uganda was conducted by inserting the search terms "Arboviruses in Uganda" or "Mosquitoes and Viruses in Uganda" into PubMed (https://pubmed.ncbi.nlm.nih.gov) and Google Scholar (https://scholar.google.com/), last accessed on 13th July 2020. Only original research articles that involved work done in Uganda were eligible for review. The first search term ("Arboviruses in Uganda") yielded a total of 56 publications 16 of which were eligible, and the second search term ("Mosquitoes and Viruses in Uganda") yielded 111 articles, 49 of which were eligible. We excluded all articles that were not primary research articles and those that listed no arboviruses. We also cross-checked for arboviruses in other secondary databases including the International Committee of Taxonomy for Viruses, the Centers for Disease Control and Prevention Arbovirus-Catalog and Virus Pathogen Database and Analysis Resource.

A total of 24 mosquito-borne viruses -WNV, BWAV, YFV, BUNV, NTAV, SFV, UGSV, Zika virus (ZIKV, family *Flaviviridae*), chikungunya virus (CHIKV, family *Togaviridae*), Rift Valley fever virus (RVFV, family *Phenuiviridae*), o'nyong nyong virus (ONNV, family *Togaviridae*), Nyando virus (NDOV, family *Peribunyaviridae*), Orungo virus (ORUV, family *Reoviridae*), Babanki virus (BBKV, family *Togaviridae*), PGAV (family *Peribunyaviridae*), Sindbis virus (SINV, family *Togaviridae*), Germiston virus (GERV, family *Peribunyaviridae*), Usutu virus (USUV, family *Flaviviridae*), Tanga virus (TANV, family *Peribunyaviridae*), Wesselsbron virus (WSLV, family *Flaviviridae*), Arumowot virus (AMTV, family *Phenuiviridae*), Mossuril virus (MOSV, family *Rhabdoviridae*), WITV (family *Peribunyaviridae*), and Kamese virus (KAMV, family *Rhabdoviridae*) from different animal hosts including humans and mosquitoes were documented. Out of the 24 viruses, 8 *(*33.3 %*)*, were from the family *Peribunyaviridae*, followed by 7 (29.2 %) *Flaviviridae*, 5 (20.8 %) *Togaviridae*, 2 (8.3 %) *Phenuiviridae* and 1 (4.2 %) each from the *Rhabdoviridae* and *Reoviridae*. 16/24 (66.7 %) are associated with febrile illnesses in humans with 8 of these majorly vectored by *Aedes* species, 5 by *Culex* species and 3 by *Anopheles* species (Figure 1, Supplementary Table 1).

10 (41.7%) of these viruses (WNV, BWAV, BUNV, ZIKV, UGSV, NTAV, SFV, ONNV, ORUV and KAMV were first described locally in Uganda (12, 13, 18, 19, 28-30). 6 of the viruses (WNV, BWAV, ONNV, ZIKV, YFV and RVFV) are public health threats, as they have been previously associated with disease outbreaks either within or outside Uganda (3, 31-35). YFV and CHIKV, although not first isolated in Uganda, are highly endemic and associated with several outbreaks in the country (4, 36-40). This information is summarized in Table 1.

In recent years more viruses were identified, in line with increased surveillance and improved detection methods. Discovery data relate to specific areas of Uganda, as indicated in Figure 2.

The figure also shows that earlier studies were mainly conducted in the Western and Central regions. This was partly due to the yellow fever endemicity described in these areas at the time. Out of the five viruses associated with outbreaks (Table 1), RVFV had the highest number of papers recorded (13), followed by YFV (8), ONNV (3) and fewer reported for CHIKV (2) and BWAV (2). The majority of these outbreaks were documented in the Central and Western regions of Uganda. Transmission to humans occurs following the bite of infected *Aedes*, *Culex* or *Anopheles* mosquito species. BWAV and ONNV, transmitted by *An. gambiae* and *An. funestus*, show a geographical distribution limited only to the African continent. Below we summarize the number of disease outbreaks, the prevalence and case fatality associated with selected mosquito borne arboviruses.

Viruses previously involved in disease outbreaks in Uganda

Bwamba virus

BWAV (family: *Peribunyaviridae*, genus: *Orthobunyavirus*), a virus transmitted by *An*. *gambiae* and *An. funestus* mosquitoes, was first reported during an epidemic in a small village setting in Bwamba County, Western Uganda (12, 20, 41). The virus was isolated from African labourers working on the road construction project to Bwamba county. It was isolated by inoculation of human serum into mice, during this epidemic, nine strains of the virus were isolated (42). Victims presented with low grade pyrexia for 2 to 5 days, headache, backache and this was followed by rapid recovery (19). Later, it was isolated from *Aedes* and *Mansonia* species in the same county (19, 33, 41). Infection is characterized by meningitis, myocarditis, diarrhea, headache, skin rash and joint pains lasting for 4 to 5 days (12, 41). The general prevalence of BWAV in Uganda was

estimated to be around 57.8 %, however this may vary from place to place (19). A survey in 1952 within the local people of the East African region showed 70.5 % seropositivity in Bwamba county while in Tanga region of Tanzania it was 80.1 % (42). Since then, little was heard of BWAV until forty years later, when three strains were isolated in South-Western Uganda. Isolation was made in people, one was a refugee from North Eastern Tanzania, another a health worker at UVRI working with the Rakai project on HIV, while the third strain was from a pool of *An. funestus* mosquitoes (33). Although the disease is wide spread, it often presents with mild symptoms sometimes mistaken for malaria and no fatalities have ever been documented. An animal reservoir has not been well characterized, but antibodies to BWAV have been found in several animal hosts including birds, monkeys, donkeys, rodents and domestic animals (41).

West Nile virus

WNV (family: *Flaviviridae*, genus: *Flavivirus*), a neurotropic virus antigenically related to Japanese encephalitis virus (JEV) and St. Louis encephalitis virus (SLEV), was first described in a febrile female patient in the West Nile district of Uganda (13). Initial serological testing of the patient serum for YFV antibodies was negative, however intra-cerebral inoculation of serum in new born mice led to the isolation of a new virus, later named WNV after the West Nile district (13). While conducting extensive ecological studies in Egypt, Williams and Taylor described the role of birds in the maintenance of WNV in nature (43, 44). In 1955, sera collected from wild birds and tested revealed neutralizing antibodies to WNV in Uganda (45). Serological tests in several animal hosts confirmed domestic fowl, migratory birds and equines as possible reservoirs of WNV (46). The incubation period for disease in humans ranges from 2 to 14 days (47). Infection is characterized by the onset of fever, headache, backache, anorexia, neurological disorders, conjunctival

inflammation, myalgia, arthralgia, skin rash which may persist up to one week, lymphadenopathy and myocarditis (47). In severe cases, it may present with evidence of encephalitis manifested by tremors, stiff neck, loss of vision, paralysis, coma and death. WNV is maintained in an enzootic cycle by *Culex* mosquitoes and some *Coquillettidi*a species, while birds are amplifying hosts with equines sometimes being incidental hosts (Figure 1). During routine mosquito collections and sero-surveys in Uganda, the virus has been isolated from pools of *Cq. metallica*, *Cq. aurites* and *Cx. neavei* and humans (48-50). During blood feeding, *Culex* mosquitoes acquire infection from animal reservoirs, and on subsequent feedings, the mosquito secretes saliva which contains the virus to infect other animal hosts. Spillover to human hosts occurs when infected mosquito vectors that have acquired infection from animal/bird hosts and pass it on to humans on subsequent feedings. The virus has now been detected in many parts of Africa, Middle East and Europe and in 1999 was described for the first time in North America (3, 51).

Yellow fever virus

YFV (family: *Flaviviridae*, genus: *Flavivirus*) is of specific importance as much arbovirus research in Uganda was initiated specifically because of this pathogen. It is a mosquito-borne virus that may cause hemorrhagic fever and jaundice with a high associated mortality (40)**.** Country wide surveys to determine the mosquito vectors, transmission and biology of YFV were started in the mid 1930s. During those surveys, one of the three well described YFV transmission cycles, the sylvatic cycle, in which *Ae. africanus* is involve*d*, was discovered in western Uganda (10, 52). In this cycle also known as the jungle or forest cycle, YFV is maintained endemically with *Ae. africanus* as the main vector for transmission between simian hosts such as monkeys, chimpanzees, baboons and bush babies (Figure 1). Once in a susceptible monkey population, the

183 virus may at times cause an epizootic resulting in death of monkeys. *Ae. africanus* is mainly active
184 between sunset and sunrise and often bites at $\geq$ 50 ft above the ground. The other two YFV
185 transmission cycles are the urban and intermediate cycles. The urban cycle involves
186 anthropophilic *Ae. aegypti aegypti* populations which prefer human blood, oviposit their eggs in
187 artificial containers and mainly bite indoors. However, records show that significant variations
188 exist within the *Ae. aegypti* populations. In Uganda, *Ae. aegypti* populations are mainly zoophilic
189 (*Ae. aegypti formosus*), outdoor dwelling and less competent to virus transmission, thus the
190 urban YFV transmission cycle has not been documented in Uganda (53, 54).
191 The YFV intermediate cycle in Uganda involves *Ae. bromeliae* a sub species of *Ae. simpsoni*
192 complex that inhabits and breeds in garden plantations (mainly banana and *Colocasia*). During
193 feeding, *Ae*. *bromeliae* can be infected with YFV from infected monkeys and other non-human
194 primates that have raided garden plantations in search for food and transmit it to humans during
195 subsequent feedings (55). Published literature on the history of yellow fever outbreaks in
196 Uganda, the distribution of YFV vectors and the country wide serosurveys since the inception of
197 yellow fever activities are summarized in Table 2.
198 The first human case of YFV described in Uganda was a 27 year old female African from Bwamba
199 county in Western Uganda (10). She presented with symptoms of meningitis including severe
200 headache, high temperature (40.3^{o}C) and neck pain. Virus isolation attempts revealed *Ae.*
201 *simpsoni* as the main mosquito vector, with *Eretmopodites chrysogaster* and *Ae. africanus*
202 implicated in virus transmission (10). The first documented YFV outbreak in Uganda was in 1941
203 in Bwamba county. A serological survey in this area showed 28.6 % of the human population had
204 been infected (10). In order to prevent further spread of the disease East wards to Toro district,

a mass vaccination program was instituted by the Medical Department of the Uganda Government. In 1950, a survey in the West Nile district showed that 18 % of the population in Midigo, with 36 % of the monkeys had been exposed to the YFV (56). Since then, several autochthonous outbreaks have been reported mainly in the western and central regions of the country. In 1952, a fatal case due to YFV in a European worker occurred around Fort Portal in Western Uganda (57). Infection was detected in *Ae. africanus* and monkey hosts near the residence of the deceased individual. In 1964, another fatal outbreak occurred in Central Uganda, 25 miles away from Kampala on the Hoima road(58). At the time of death, the victim had deep jaundice and gross albuminuria. YFV was confirmed through isolation from the cerebral-spinal fluid, histological examination of the liver tissue; and isolation from three pools of *Ae. africanus* (58, 59). A countrywide survey showed YFV range from 1/103 (0.97 %) in North-Eastern through 6.4% in Central to 34.2 % in Western Uganda (60). In 1943, an epizootic occurred in non-human primates on Bukasa islands where the prevalence of YFV was 88.9 % (61). Related studies done in 1972 showed that another epizootic had occurred in the monkey population in the Zika Forest, where the prevalence was 40 % (62). In 2010, an outbreak of a viral hemorrhagic fever (VHF) characterized by high fever, convulsions, vomiting, bleeding through body orifices and finally death occurred in Northern Uganda (40). Serological and molecular testing for the common circulating VHFs including Ebola, Marburg, RVFV and Crimean Congo Hemorrhagic Fever at Special pathogens UVRI tested negative. Further screening of samples at partner institution CDC (Atlanta, GA, USA) using NGS revealed 92 % homology to YFV (27, 40). Further laboratory tests - carried out after this confirmation- at UVRI; including IgM, PRNT and PCR using primers developed to target the YFV East African genotype- revealed 7.5% of the suspected cases to be

positive (27, 40). Case fatality during this outbreak was (45 deaths) 24.9% (40). The latest documented YFV outbreak occurred in the central and south-western parts of Uganda, in which case fatality was 33 % (63) . Although in other parts of the world, YFV was controlled through massive vaccination campaigns and control of vector species, this remains a challenge as vaccination is not mandatory in Uganda other than in international travelers. Molecular studies have shown two YFV genotypes in Uganda, the East African and East/Central African genotypes. The prevalence and risk of YFV transmission in Uganda varies with the distribution of YFV vectors. The uneven distribution of YFV across Uganda may be due to the *Ae. simpsoni* populations. Some populations of *Ae. simpsoni* (*Ae. bromeliae*) mosquitoes from Western Uganda are more anthropophilic than other *Ae. simpsoni* (*Ae. lilii*) populations from other parts of the country (55, 64).

Clinical manifestation in humans vary from mild undifferentiated febrile illness to severe disease and occurs in two phases (65). The incubation period for YFV varies from 3 to 6 days but sometimes may go up to 14 days (47). During the infection phase, the virus multiplies and circulates within the blood. The patient may experience non-specific symptoms such as fever confused with malaria, typhoid or viral hepatitis (66). When the infection stage enters the intoxication phase, the virus leaves the blood and replicates in the liver, spleen, heart and lymph nodes. This phase may be characterized by chills, nausea, anorexia, convulsions, myalgia, vomiting, dehydration, prostration, hemorrhage, hepatitis with jaundice and central nervous system involvement (40, 47) (Supplementary Table 1).

Zika virus

ZIKV (family: *Flaviviridae*, genus: *Flavivirus*), is a mosquito-borne emerging virus related to dengue virus (DENV), YFV, WNV and Japanese encephalitis virus (JEV) (67). The disease was first isolated from serum of a sentinel rhesus monkey (Rhesus 766) caged in the canopy of Zika Forest in Uganda in April 1947 (29). The virus was named after the place where the isolation was made. The following year in January (1948), it was isolated from a pool of 86 *Ae. africanus* mosquitoes caught on a tree platform in Zika Forest (29). Between the time of ZIKV isolation from the caged rhesus monkey and January when mosquito collections were done and tested, all the remaining caged 5 rhesus monkeys had developed antibodies to ZIKV (19). A serological survey conducted around the population living close to the forest and Entebbe region areas revealed ZIKV antibodies (68). In other areas of the country such as Bwamba county, antibody prevalence to ZIKV was as high as 20 % (19). Dick demonstrated that ZIKV caused lesions in the skeletal muscles and myocardial injury in five-day old mice. In 1956, Weinbren isolated a strain of ZIKV from a pool of *Ae. africanus* mosquitoes collected from Lunyo Forest (69). Serological surveys through complement fixation tests confirmed the presence of neutralizing antibodies against ZIKV in Uganda and Tanzania. Between 1961 and 1963, 12 isolates of the virus were obtained from pools of *Ae. africanus* (70). In 1970, McCrae and colleagues isolated ZIKV from a pool of *Ae. africanus* and *Ae. apicoargenteus* mosquitoes collected during the weekly routine collections conducted in Zika Forest (4). Although no documentation about ZIKV epidemics in Uganda, epizootics had probably occurred in the Entebbe peninsula in 1948 and 1956, and thereafter several epizootics were documented in the years 1962 to 1963, and 1969 to 1970 (4, 71). Since then, there have been no reports about ZIKV, however recent serosurveys suggest evidence of human exposure

in Central Uganda (72). Reasons for the absence of ZIKV in countries where it was earlier identified remain poorly understood, however the low infections in humans were partly attributed to the catholic feeding style of *Ae. africanus* the principal vector which prefers monkeys to humans(19). In Africa, where diverse forms of *Aedes* species exist, limited studies have been conducted to identify the likely competent vector species, however the virus has on several occasions been isolated from *Ae. africanus* a sylvatic mosquito species. Other species likely to transmit ZIKV include *Ae. vitattus*, *Ae. opok*, *Ae. bromeliae* and *Ae. luteocephalus* however few competence experiments have been conducted to confirm whether they are vectors (67). Outside Africa, an Asian lineage is in existence (73). Many of the issues related to outbreak in the Americas and Zika congenital syndrome have been expertly reviewed elsewhere (74, 75) and do not need to be expanded on here.

Rift Valley fever virus

RVFV (family: *Phenuiviridae;* genus: *Phlebovirus*) is a re-emerging zoonotic viral disease that primarily affects ruminants such as goats, sheep and cattle. Infection is characterized by abortions and still births in adult animals and high mortality in young animals. Animals get infected through the bite of infected mosquito or tick vectors. Infected young adults may suffer an acute febrile disease with prostration while the young ruminants grow weak, fail to stand to suckle and eventually may die within 12 to 24 hours depending on species. Epizootics in animals are often triggered by persistent heavy rainfall which leads to flooding, which triggers hatching of infected mosquito populations to initiate RVFV transmission. In Uganda, the first report of RVFV was in wild mosquitoes collected from Semliki forest, in Western Uganda, during the collection a dead buffalo and a sick buffalo calf, thought to have been infected with RVFV were

observed in the catchment area (76). In 1956, Mims reported the presence of RVFV neutralizing antibodies in *Arvicanthi*s rodents, however further virus isolation attempts contradicted his findings (71, 77). The second report was a successful virus isolation was from a sampled calf in Entebbe (71). For over 30 years, little information was documented about RVFV in animals until 2007 when a study done in the Central region revealed 10% RVFV immunoglobulin M (IgM) neutralizing antibodies in domestic animals (78). These findings were aligned with an outbreak in 2016 in South-Western Uganda in which a serosurvey revealed that RVFV prevalence was highest in cattle (26.9%) followed by goats (6.5%) and finally in sheep (5.7%) (79). Between 2016 and 2018, over 10 fatal RVFV outbreaks were reported in domestic animals in over 15 districts, with spillover into the human population (34, 79).

RVFV was first described in 1930 in Kenya, during that time a new virus named after the Rift Valley Province of Kenya isolated from sheep was described, later on fatal human cases were reported in different parts of Kenya (80). During the YFV surveys, a dead red buffalo thought to have died of RVFV in the mosquito collection area was observed in Bwamba county in 1944. Entomological studies led to further isolation of RVFV in mosquitoes *Ae. tarsalis*, *Ae. circumluteolus* and *Eretmopodites* species in the Semliki Forest, Western Uganda *(76).* The second successful virus isolation of RVFV was in 1955, from *Ae*. *africanus* and *Ae. circumluteolus* in Lunyo village, Entebbe (19, 71). Since then, several virus isolations were made from sentinel collections of *Ae. africanus* and in 1968 following an outbreak in Entebbe region, RVFV was isolated from *Ma. africana* (71). Following the multiple mosquito surveys, *Ae. africanus*, *Ae. albocephalus, Ae. dendrophilus, E. chrysogaster* and *E. inornatus Ae. africanus*, *Ma. africana*, *Cq. fuscopennata* and *Ma. uniformis, Cx. antennatus, Cx. rubinotus*, *Ae. mcnitoshi*, *Ae. circumluteolus*,

315 *Ae*. *tarsalis* and *Ae. ochraceus* are known vectors of RVFV (71, 76) (Figure 1). Despite the
316 isolations from multiple species, limited competence experiments have been documented to
317 confirm the vectorial capacity of the diverse mosquito species.
318 Transmission of RVFV is frequently by *Aedes* and *Culex* species. Infected *Aedes* species lay
319 infected eggs in small standing pools of water, remaining viable for long periods of drought.
320 Rainfall and flooding stimulate the eggs to hatch leading to a high infected mosquito population.
321 The emerging infected *Aedes* primarily feed on cattle leading to virus amplification, this provides
322 source of virus for the secondary mosquito vectors, the culicines. As the population of the
323 infected mosquitoes build up, culicines as well as aedines transmit infection to the susceptible
324 ruminants such as goats and sheep (81).
325 In Uganda, historical records show several outbreaks in the human population in the 1950s, many
326 of these occurred in Western and Central regions of Uganda. From 1960 to 1967, over 8
327 successful RVFV isolations were made in febrile patients coming from the villages near Entebbe
328 area reporting to the EAVRI clinic (71). The largest outbreak at the time was in 1968 in the
329 Entebbe region when RVFV was isolated from 7 febrile patients who had complained of fever,
330 general body weakness and arthralgia (71). From the 1970s onwards, RVFV remained unreported
331 in Uganda and the country was classified as a low-risk nation until 2016, when an outbreak
332 occurred in South-Western Uganda. Infection was confirmed by detection of RVFV RNA in both
333 human and livestock samples (34).
334 Humans often get infected when they come into contact with animal tissues and fluids such as
335 blood, body organs of infected animals, drinking milk from infected animals as well as via
336 mosquito bites. Within a period of two to six days, it can present as an acute influenza-like illness

characterized with transient fever, mental confusion, shivering, headache, photophobia, severe muscle and joint pains, convulsions, hallucinations, anorexia, nausea, vomiting and epistaxis. This may eventually progress to the hemorrhagic form characterized with liver impairment, jaundice, vomiting of blood, passing blood in urine and faeces, bleeding in the nose and gums (34, 82) (Supplementary Table 1).

Although factors responsible for RVFV re-emergence have been extensively investigated elsewhere, there is limited data on the factors driving the recurrence of RVFV outbreaks in Uganda however the increased recurrence is partly attributed to livestock movement including animal products, high density of mosquito vectors and increased El Niño rains (79). More recently, improved surveillance methods have led to a higher detection frequency of the disease. RVFV can be identified through detection of antibody IgG and IgM ELISA, detection of virus antigen by immunofluorescent assays (IFA), virus isolation in cell culture or intracerebral inoculation of weanling mice, or detection of viral RNA by reverse transcription PCR (RT-PCR).

Chikungunya virus

CHIKV (family: *Togaviridae;* genus: *Alphavirus*) is a re-emerging mosquito-borne alphavirus that belongs to the Semliki Forest group and is serologically related to ONNV (83, 84). Locally, the word chikungunya is a Tanzanian Kimakonde term which means immobilization of the elbow joints or “that which folds one up.” The disease due to the bite of an infected *Aedes* species was first reported in the Makonde plateau of Tanzania, in 1953 (85). In 1955, CHIKV was isolated from a mosquito catcher working in Zika Forest in Uganda (39). The victim suffered from an illness characterized by high fever, headache, coryza, severe pain in the joints and back. It was later isolated from a pool of 78 *Ae. africanus* mosquitoes collected on a tree platform in Zika Forest.

In 1961, a 120 feet steel tower that had been put in Mpanga Forest was transferred to Zika forest to boost mosquito collections. During those collections on the steel tower, CHIKV virus was isolated from several *Ae. africanus* populations, mosquito collectors and febrile patients reporting at Entebbe clinic where the prevalence of CHIKV was estimated at 2.8% (37).

Transmission occurs following a bite by an infected *Aedes* mosquito. Once in the human body, the virus replicates in the skin, disseminates to vital organs including liver, muscle joints, lymphoid tissue and brain (86). It may remain in blood for five to seven days during which it is available to re-infect other mosquitoes. Within three to twelve days, the patient may present with a rapid onset of fever (38.9^{o}C to 40.6^{o}C), often characterized with an irritating maculo-papular rash on the trunk, postorbital pain, severe joint and muscle pain often confused as ONNV or DENV infection.

In rural settings where the virus has been described in Uganda, CHIKV circulates in an enzootic cycle involving sylvatic species such as *Ae. africanus* and *Ae. furcifer*, however it has also been recovered from several other mosquito species including *Cq. fuscopenata, Ma. uniformis, Ma. africana, Ae. taylori* and *Cx. pipiens fatigans* (87, 88).

The disease is one of the most prevalent arboviruses with a seroprevalence of about 46.9% in North Eastern Uganda, however this magnitude requires careful interpretation as the high magnitude could be due to the cross reactivity in diagnostics between CHIKV and ONNV which are closely related and co-circulate within the region (89, 90). In a related study conducted across the country, the seroprevalence of CHIKV was 31.7% (90). Two outbreaks have been documented in Uganda, in the Zika Forest in 1968 and in Mukono district in 1982 (84, 91). Since then, little was heard of CHIKV until recently, when it was described in international travelers coming from

the Asian countries (92). Over the years, three genotypes with differences in virulence have been reported in different parts of the world, these include the East/Central/South Africa genotype, the Asian genotype and the West African genotype (93). In addition to the above genotypes, recent phylogenetic analyses show multiple CHIKV lineages (94, 95).

O'nyong-nyong virus

ONNV (family: *Togaviridae;* genus*: Alphavirus*) causes febrile illness and is characterized by joint pains hence the name o'nyong-nyong, a Luo term for severe joint pains. The disease was first described and isolated in 1959 from a 40-year-old female in Northern Uganda presenting with fever, severe joint pains, backache, headache and anorexia (28). Later, it was isolated from *Anopheles* mosquitoes (96). Hemagglutination assays, complement fixation tests and other serological assays showed cross reactivity of ONNV with CHIKV and SFV (97). Cell culture studies showed that the virus replicates in various cell types with cytopathic effects in BHK-21 (hamster kidney) and Vero (green monkey kidney) cells (98). It is estimated that by the end of 1959, over 750,000 people had been infected (99). Between 1959 and 1962, ONNV caused a large outbreak that spread to cover all the countries in Eastern Africa including Uganda, Kenya, Tanzania, Malawi, Sudan and Belgian Congo with over 2 million people infected (100). A second outbreak occurred in South-Western Uganda and spread into Northern Tanzania in 1996 (32, 101). Seroprevalence studies revealed an infection rate of 121/391 (30.9%), 74 of whom were ONNV laboratory confirmed while the 47 had presumptive evidence of ONNV infection in the human population (102). Entomological surveys revealed that *An*. *funestus* mosquitoes as the main vectors for ONNV (101). Serologically, ONNV belongs to the Semliki Forest group and is a variant of Igbo Ora virus from Nigeria (103). Symptoms for ONNV infection include moderate fever

(38.9^{o}C) which lasts about five days, a rash which begins from face and extends to the trunk and hands, swollen lymphadenitis, backache, headache, anorexia, severe joint pains which lasts for a period of about 6 to 8 days and conjunctivitis (103).

Current laboratory testing and the role of new next generation platforms in virus discovery

Traditional arbovirus testing includes serology, reverse transcription polymerase chain reaction (RT-PCR), immunofluorescence assay (IFA) and virus isolation (104). Although these methods are often used in virus identification, they have limitations. Serology based methods involve testing for immunoglobulin M antibodies (IgM) which are the first antibody response to virus infection. Although the method is quite useful in reporting infection to related viruses, it may not distinguish a previous from active infection, at times give false positives in areas where vaccination was conducted and often suffer from cross reactivity of closely related viruses (104, 105). Issues of cross reactivity may be overcome by plaque neutralization tests using paired acute and convalescent samples. Since the former takes a long period of time of close to 14 days, molecular testing using RT-PCR is often done during outbreak episodes. Although RT-PCR is sensitive, the method can only detect viruses that have been previously described. Immunofluorescence assays are conducted using arbovirus grouping fluids or virus specific antibodies, the method uses fluorophores to identify antigens in infected cells. The challenge with the method is that use of a monovalent or polyvalent antibody gives results of a related virus group and therefore further identification is needed. Virus isolation remains a gold standard and optimal method for identifying a virus. The method involves inoculation of specimen such as serum or cerebrospinal fluid or crushed tissue on to a confluent monolayer of mammalian or

insect cell lines to monitor for cytopathic effects (CPE). The challenge with this is cytopathic effects require careful interpretation as some may result from sample toxicity, which in turn may result in false positives. Some viruses are undetectable soon after the onset of symptoms, a stage when antibody titers have risen; and some viruses fail to grow on particular cell lines.

Strengths and limitations of next generation sequencing in virus discovery

Recent advances in diagnostic tools have accelerated virus discovery and outbreak investigations in different hosts including arthropod vectors, mammals and humans. Metagenomic next generation sequencing is a catch-all term for unbiased sequencing using a range of high-throughput technologies. It can be used to detect both previously described and novel pathogens and does not require prior knowledge of the pathogen genetic sequence (106). In Uganda, it has been used to discover several new viruses from different animal host and vector species. Four novel viruses described in Uganda in the last decade were identified using NGS (24-26). Two (Kibale virus and Mburo virus) out of the four viruses were detected in mosquitoes, while the other two (Nyangole virus and Ntwetwe virus) were detected in febrile patients. Phylogenetic studies suggest that some viruses are likely to be vectored not only by mosquitoes but also other arthropods including ticks and midges. The technology has potential to improve outbreak investigations to identify pathogens that could otherwise not be detected or investigated, by using available routine diagnostic tools (27). To give one example, a study detection of emerging and novel viral infections associated with febrile illnesses in returning travellers was based on this methodology (25, 107). In areas where mosquitoes feed on different vertebrate hosts, the tool has also been used to investigate multiple sources of host blood feeding by detecting the

DNA of the host in the mosquito vector (108). NGS has additionally been used to study the insect microbiome, which may affect competence of mosquitoes to transmit viruses (109, 110). With increased human population and related activities in once uninhabited locations, the risk of zoonotic transmission to humans is high. Therefore, highly sensitive and unbiased tools such as NGS should be adopted for early virus detection in such "hotspot" areas of zoonotic transmission. This has the potential to facilitate early containment of outbreaks and development of appropriate interventions against arboviruses. Further studies are needed to adopt new diagnostic tools to investigate the role of mosquitoes in the transmission of arboviruses. At present, the tool remains expensive in terms of reagents, equipment and maintenance especially for low- and middle-income countries (LMICs). In addition, the large volumes of data generated by NGS platforms require sophisticated computational infrastructure for storage and analysis. Nonetheless in time, NGS technologies are likely to be increasingly relevant also in more challenging settings, and support investigations.

Conclusion

Historically, there is a high burden and endemicity of arboviruses in Uganda, which we document here. The majority of these are classified in the family *Peribunyaviridae* followed by *Flaviviridae*, *Togaviridae* and *Phenuiviridae.* Viruses in the *Reoviridae* and *Rhabdoviridae* families are also prevalent. Given the many diverse mosquito species already described in Uganda there is a likelihood of many undescribed mosquito borne viruses. In an era of emerging viruses, some of which are becoming a global threat, more sensitive tools need to be developed to supplement

the already existing ones for early detection. The summary provided here will help to give context to new discoveries and can support research efforts in the future.

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Author contributions

Conceptualization – A.K. and M.M.N.; funding acquisition – A.K, E.C.T. and M.M.N.; supervision – A.K, E.C.T., F.M. and J.L.; visualization – A.S. and M.M.N.; writing – original draft – M.M.N.; writing – review and editing – A.K., A.S., E.C.T., F.M., J.L., M.E. and M.M.N.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Bouree P. [a New Pandemic: Zika Virus Infection]. Rev Prat. 2016;66(6):641-7.
2. Cugola FR, Fernandes IR, Russo FB, Freitas BC, Dias JL, Guimaraes KP, et al. The Brazilian Zika virus strain causes birth defects in experimental models. Nature. 2016;534(7606):267-71.
3. Hayes CG. West Nile virus: Uganda, 1937, to New York City, 1999. Ann N Y Acad Sci. 2001;951:25-37.

4. McCrae AW, Kirya BG. Yellow fever and Zika virus epizootics and enzootics in Uganda. Trans R Soc Trop Med Hyg. 1982;76(4):552-62.
5. Hrnjakovic Cvjetkovic IB, Cvjetkovic D, Patic A, Nikolic N, Stefan Mikic S, Milosevic V. Chikungunya - a Serious Threat for Public Health. Med Pregl. 2015;68(3-4):122-5.
6. Theiler M, Downs WG. The arthropod-borne viruses of vertebrates. An account of the Rockefeller Foundation virus program, 1951-1970. The arthropod-borne viruses of vertebrates An account of The Rockefeller Foundation Virus Program, 1951-1970. 1973.
7. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, et al. Taxonomy of the order Bunyavirales: update 2019. Archives of Virology. 2019;164(7):1949-65.
8. Karabatsos N. International catalogue of arboviruses, including certain other viruses of vertebrates, American Society of Tropical Medicine and Hygiene. San Antonio, TX. 1985.
9. Kirya BG. A yellow fever epizootic in Zika forest, Uganda, during 1972: Part 1: Virus isolation and sentinel monkeys. Trans R Soc Trop Med Hyg. 1977;71(3):254-60.
10. Mahaffy A, Smithburn K, Jacobs H, Gillett J. Yellow fever in western Uganda. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1942;36(1):9-20.
11. Sempala SD. Institute profile: the Uganda virus research institute. Trends in Microbiology. 2002;10(7):346-8.
12. Smithburn K, Mahaffy A, Paul J. Bwamba fever and its causative virus. The American Journal of Tropical Medicine and Hygiene. 1941;1(1):75-90.
13. Smithburn K, Hughes T, Burke A, Paul J. A neurotropic virus isolated from the blood of a native of Uganda1. The American journal of tropical medicine and hygiene. 1940;1(4):471-92.
14. Smithburn K, Haddow A, Mahaffy A. A neurotropic virus isolated from Aedes mosquitoes caught in the Semliki forest. The American journal of tropical medicine and hygiene. 1946;1(2):189-208.
15. Smithburn K, Haddow A. Ntaya virus. A hitherto unknown agent isolated from mosquitoes collected in Uganda. Proceedings of the Society for Experimental Biology and Medicine. 1951;77(1):130-3.
16. Kokernot R, Smithburn K, De Meillon B, Paterson H. Isolation of Bunyamwera Virus from a Naturally Infected Human Being and Further Isolations from Aedes (Banksinelld) circumluteolus Theo. American Journal of Tropical Medicine and Hygiene. 1958;7(6):579-84.
17. Smithburn K, Haddow A. Semliki Forest virus: I. Isolation and pathogenic properties. The Journal of Immunology. 1944;49(3):141-57.
18. Dick GW, Haddow AJ. Uganda S virus; a hitherto unrecorded virus isolated from mosquitoes in Uganda. I. Isolation and pathogenicity. Trans R Soc Trop Med Hyg. 1952;46(6):600-18.
19. Dick G. Epidemiological Notes on Some Viruses isolated in Uganda (Yellow Fever, Rift Valley Fever, Bwamba Fever, West Nile, Mengo, Semliki Forast, Bunyamwera, Ntaya, Uganda S and Zika Viruses). Transactions of the Royal Society of Tropical Medicine and Hygiene. 1953;47(1):13-43.
20. Carrington LB, Wills B. Lessons from history: viral surveillance in 1940s East Africa: Epidemiological notes on some viruses isolated in Uganda, GWA Dick, Transactions of the Royal Society of Tropical Medicine and Hygiene, 1953; 47 (1): 13–48. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2018;112(9):413-4.
21. Crabtree MB, Kading RC, Mutebi JP, Lutwama JJ, Miller BR. Identification of host blood from engorged mosquitoes collected in western Uganda using cytochrome oxidase I gene sequences. J Wildl Dis. 2013;49(3):611-26.
22. Al-Qahtani AA, Nazir N, Al-Anazi MR, Rubino S, Al-Ahdal MN. Zika virus: a new pandemic threat. J Infect Dev Ctries. 2016;10(3):201-7.
23. Chancey C, Grinev A, Volkova E, Rios M. The global ecology and epidemiology of West Nile virus. Biomed Res Int. 2015;2015:376230.

24. Marklewitz M, Zirkel F, Rwego IB, Heidemann H, Trippner P, Kurth A, et al. Discovery of a unique novel clade of mosquito-associated bunyaviruses. Journal of virology. 2013;87(23):12850-65.
25. Edridge AW, Deijs M, Namazzi R, Cristella C, Jebbink MF, Maurer I, et al. Novel orthobunyavirus identified in the cerebrospinal fluid of a Ugandan child with severe encephalopathy. Clinical Infectious Diseases. 2019;68(1):139-42.
26. Ramesh A, Nakielny S, Hsu J, Kyohere M, Byaruhanga O, de Bourcy C, et al. Metagenomic next-generation sequencing of samples from pediatric febrile illness in Tororo, Uganda. PloS one. 2019;14(6):e0218318.
27. McMullan LK, Frace M, Sammons SA, Shoemaker T, Balinandi S, Wamala JF, et al. Using next generation sequencing to identify yellow fever virus in Uganda. Virology. 2012;422(1):1-5.
28. Williams M, Woodall J. O'nyong-nyong fever: an epidemic virus disease in East Africa. II. Isolation and some properties of the virus. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1961;55(2).
29. Dick G, Kitchen S, Haddow A. Zika virus (I). Isolations and serological specificity. Transactions of the royal society of tropical medicine and hygiene. 1952;46(5):509-20.
30. Tomori O, Fabiyi A, Murphy F. Characterization of Orungo virus, an orbivirus from Uganda and Nigeria. Archives of Virology. 1976;51(4):285-98.
31. Haddow AJ, Williams MC, Woodall JP, Simpson DI, Goma LK. Twelve Isolations of Zika Virus from Aedes (Stegomyia) Africanus (Theobald) Taken in and above a Uganda Forest. Bull World Health Organ. 1964;31:57-69.
32. Lanciotti RS, Ludwig ML, Rwaguma EB, Lutwama JJ, Kram TM, Karabatsos N, et al. Emergence of epidemic O'nyong-nyong fever in Uganda after a 35-year absence: genetic characterization of the virus. Virology. 1998;252(1):258-68.
33. Lutwama JJ, Rwaguma EB, Nawanga PL, Mukuye A. Isolations of Bwamba virus from south central Uganda and north eastern Tanzania. Afr Health Sci. 2002;2(1):24-8.
34. Shoemaker TR, Nyakarahuka L, Balinandi S, Ojwang J, Tumusiime A, Mulei S, et al. First Laboratory-Confirmed Outbreak of Human and Animal Rift Valley Fever Virus in Uganda in 48 Years. Am J Trop Med Hyg. 2019;100(3):659-71.
35. Shakib K. Epidemic of Zika virus and maxillofacial surgery. Br J Oral Maxillofac Surg. 2016;54(4):355-7.
36. Lumsden WH. The yellow fever vector situation with reference to Entebbe airport. Bull World Health Organ. 1954;11(3):485-7.
37. McCrae AW, Henderson BE, Kirya BG, Sempala SD. Chikungunya virus in the Entebbe area of Uganda: isolations and epidemiology. Trans R Soc Trop Med Hyg. 1971;65(2):152-68.
38. Simpson DI, Haddow AJ, Williams MC, Woodall JP. Yellow Fever in Central Uganda, 1964. Iv. Investigations on Blood-Sucking Diptera and Monkeys. Trans R Soc Trop Med Hyg. 1965;59:449-58.
39. Weinbren MP, Haddow AJ, Williams MC. The occurrence of Chikungunya virus in Uganda. I. Isolation from mosquitoes. Trans R Soc Trop Med Hyg. 1958;52(3):253-7.
40. Wamala JF, Malimbo M, Okot CL, Atai-Omoruto AD, Tenywa E, Miller JR, et al. Epidemiological and laboratory characterization of a yellow fever outbreak in northern Uganda, October 2010–January 2011. International Journal of Infectious Diseases. 2012;16(7):e536-e42.
41. Gonzales J, Georges A. Bunyaviral fevers: Bunyamwera, Ilesha, Germiston, Bwamba, and Tataguine. The arboviruses: epidemiology and ecology. 1988;2:87-98.
42. Smithburn K. Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa. The Journal of Immunology. 1952;69(2):223-34.
43. Taylor R, Work T, Hurlbut H, Rizk F. A Study of the Ecology of West Nile Virus in Egypt1. The American Journal of Tropical Medicine and Hygiene. 1956;5(4):579-620.
44. Williams M. Virological studies on birds. 1957. p. 1956-57.

45. Williams M. Collection of sera from wild birds and neutralization tests with bird sera. Rep E Afr Virus Res Inst 1955–56. 1956:31-2.
46. Work TH, Hurlbut HS, Taylor R. Indigenous Wild Birds of the Nile Delta as Potential West Nile Virus Circulating Reservoirs1. The American journal of tropical medicine and hygiene. 1955;4(5):872-88.
47. Fields BN, Knipe DM, Howley PM. Fields virology. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2007.
48. Woodall JP, Gillett JD, Corbet PS, Weinbren MP, Williams MC. The isolation of West Nile virus from the bird-biting mosquito Mansonia metallica in Uganda. Ann Trop Med Parasitol. 1961;55:398-402.
49. Mossel EC, Crabtree MB, Mutebi JP, Lutwama JJ, Borland EM, Powers AM, et al. Arboviruses Isolated From Mosquitoes Collected in Uganda, 2008-2012. J Med Entomol. 2017;54(5):1403-9.
50. Williams MC, Simpson DI, Haddow AJ, Knight EM. The Isolation of West Nile Virus from Man and of Usutu Virus from the Bird-Biting Mosquito Mansonia Aurites (Theobald) in the Entebbe Area of Uganda. Ann Trop Med Parasitol. 1964;58:367-74.
51. Martín-Acebes MA, Saiz J-C. West Nile virus: a re-emerging pathogen revisited. World Journal of Virology. 2012;1(2):51.
52. Haddow A, Smithburn K, Mahaffy A, Bugher J. Monkeys in relation to yellow fever in Bwamba County, Uganda. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1947;40(5):677-700.
53. Powell JR, Tabachnick WJ. History of domestication and spread of Aedes aegypti-A Review. Memórias do Instituto Oswaldo Cruz. 2013;108:11-7.
54. Kotsakiozi P, Evans BR, Gloria-Soria A, Kamgang B, Mayanja M, Lutwama J, et al. Population structure of a vector of human diseases: Aedes aegypti in its ancestral range, Africa. Ecology and evolution. 2018;8(16):7835-48.
55. Gillett J. Further studies on the biting behaviour of Aedes (Stegomyia) simpsoni Theobald in Uganda. Annals of Tropical Medicine & Parasitology. 1955;49(2):154-7.
56. Lumsden W, Buxton A. A study of the epidemiology of yellow fever in West Nile District, Uganda. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1951;45(1):53-78.
57. Ross E, Haddow A, Raper A, Trowell H. A fatal case of yellow fever in a European in Uganda. East African medical journal. 1953;30(1):1-11.
58. Tulloch J, Patel K. Yellow fever in central Uganda, 1964. Part II. Report of a fatal case. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1965;59(4).
59. Haddow AJ. Yellow Fever in Central Uganda, 1964. I. Historical Introduction. Trans R Soc Trop Med Hyg. 1965;59:436-40.
60. Henderson BE, Cheshire PP, Kirya GB, Lule M. Immunologic studies with yellow fever and selected African group B arboviruses in rhesus and vervet monkeys. The American Journal of Tropical Medicine and Hygiene. 1970;19(1):110-8.
61. Haddow A, Dick G, Lumsden W, Smithburn K. Monkeys in relation to the epidemiology of yellow fever in Uganda. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1951;45(2):189-224.
62. Kirya B, Okia N. A yellow fever epizootic in Zika Forest, Uganda, during 1972: Part 2: Monkey serology. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1977;71(4):300-3.
63. Kwagonza L, Masiira B, Kyobe-Bosa H, Kadobera D, Atuheire EB, Lubwama B, et al. Outbreak of yellow fever in central and southwestern Uganda, February-may 2016. BMC Infect Dis. 2018;18(1):548.
64. Mukwaya L. Host preference in Aedes simpsoni (Theo.)(Diptera, Culicidae) with special reference to the anthropophilic and non-anthropophilic forms in Uganda. Bulletin of Entomological Research. 1974;64(1):129-39.
65. Vasconcelos PF, Monath TP. Yellow Fever Remains a Potential Threat to Public Health. Vector Borne Zoonotic Dis. 2016;16(8):566-7.

66. Xavier AR, Freitas GS, Santos CF, Januzzi WA, Lacerda GS, Carvalho ER, et al. Yellow fever: laboratorial diagnosis and clinical manifestations. Jornal Brasileiro de Patologia e Medicina Laboratorial. 2018;54(5):296-305.
67. Boyer S, Calvez E, Chouin-Carneiro T, Diallo D, Failloux A-B. An overview of mosquito vectors of Zika virus. Microbes and infection. 2018;20(11-12):646-60.
68. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerging infectious diseases. 2008;14(8):1232.
69. Weinbren M, Williams M. Zika virus: further isolations in the Zika area, and some studies on the strains isolated. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1958;52(3):263-8.
70. Haddow A, Williams M, Woodall J, Simpson D, Goma L. Twelve isolations of Zika virus from Aedes (Stegomyia) africanus (Theobald) taken in and above a Uganda forest. Bulletin of the World Health Organization. 1964;31(1):57.
71. Henderson B, McCrae A, Kirya B, Ssenkubuge Y, Sempala S. Arbovirus epizootics involving man, mosquitoes and vertebrates at Lunyo, Uganda 1968. Annals of Tropical Medicine & Parasitology. 1972;66(3):343-55.
72. Kayiwa JT, Nankya AM, Ataliba IJ, Mossel EC, Crabtree MB, Lutwama JJ. Confirmation of Zika virus infection through hospital-based sentinel surveillance of acute febrile illness in Uganda, 2014-2017. The Journal of general virology. 2018.
73. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic Characterization of Zika Virus Strains: Geographic Expansion of the Asian Lineage. PLoS Neglected Tropical Diseases. 2012;6(2):e1477.
74. Laavanya V, Jayasurya R, Kavitha N, Prabakaran G, Jayakumari S. A Review on Microcephaly Associated with Zika Fever in New Born Babies. Research Journal of Pharmacy and Technology. 2017;10(1):337.
75. Krauer F, Riesen M, Reveiz L, Oladapo OT, Martínez-Vega R, Porgo TV, et al. Zika virus infection as a cause of congenital brain abnormalities and Guillain–Barré syndrome: systematic review. PLoS medicine. 2017;14(1):e1002203.
76. Smithburn K, Haddow A, Gillett J. Rift Valley fever. Isolation of the virus from wild mosquitoes. British Journal of Experimental Pathology. 1948;29(2):107.
77. Mims C. Rift Valley fever protection test surveys. Annual Report of the East African Virus Research Institute. 1956:25.
78. Magona J, Kabi F, Walubengo J, Ssekitto C. Surveillance of Rift Valley fever in cattle, goats and sheep in Uganda. 2009.
79. Tumusiime D, Kihu S, Mutua E, Bett BK. Towards the development of risk-based intervention strategies for Rift Valley fever in Uganda. 2018.
80. Daubney R, Hudson J. Enzootic Hepatitis or Rift Valley Fever. An Un-described Virus Disease of Sheep, Cattle and Man from East Africa. Journal of pathology and bacteriology. 1931;34:545-79.
81. Van den Bergh C. Rift Valley fever virus circulation in livestock and wildlife, and population dynamics of potential vectors, in northern KwaZulu-Natal, South Africa: University of Pretoria; 2019.
82. Mohamed M, Mosha F, Mghamba J, Zaki SR, Shieh W-J, Paweska J, et al. Epidemiologic and clinical aspects of a Rift Valley fever outbreak in humans in Tanzania, 2007. The American journal of tropical medicine and hygiene. 2010;83(2_Suppl):22-7.
83. Pardigon N. The biology of chikungunya: a brief review of what we still do not know. Pathologie Biologie. 2009;57(2):127-32.
84. Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of Chikungunya and O'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. Journal of General Virology. 2000;81(2):471-9.

85. Ross R. The Newala epidemic: III. The virus: isolation, pathogenic properties and relationship to the epidemic. Epidemiology & Infection. 1956;54(2):177-91.
86. Schwartz O, Albert ML. Biology and pathogenesis of chikungunya virus. Nature Reviews Microbiology. 2010;8(7):491-500.
87. Mutebi J-P, Crabtree M, Kading R, Powers A, Lutwama J, Miller B. Mosquitoes of western Uganda. Journal of medical entomology. 2014;49(6):1289-306.
88. Mutebi J-P, Crabtree M, Kading R, Powers A, Ledermann J, Mossel E, et al. Mosquitoes of northwestern Uganda. Journal of Medical Entomology. 2018;55(3):587-99.
89. Rodhain F, Gonzalez JP, Mercier E, Helynck B, Larouze B, Hannoun C. Arbovirus infections and viral haemorrhagic fevers in Uganda: a serological survey in Karamoja district, 1984. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1989;83(6):851-4.
90. Clements TL, Rossi CA, Irish AK, Kibuuka H, Eller LA, Robb ML, et al., editors. Chikungunya and O'nyong-nyong Viruses in Uganda: Implications for Diagnostics. Open forum infectious diseases; 2019: Oxford University Press US.
91. Kalunda M, Lwanga-Ssozi C, Lule M, Mukuye A. Isolation of Chikungunya and Pongola viruses from patients in Uganda. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1985;79(4):567-.
92. Kayiwa JT, Nankya AM, Ataliba I, Nassuna CA, Omara IE, Koehler JW, et al. Dengue fever and chikungunya virus infections: identification in travelers in Uganda–2017. Tropical Diseases, Travel Medicine and Vaccines. 2019;5(1):1-5.
93. Zeller H, Van Bortel W, Sudre B. Chikungunya: its history in Africa and Asia and its spread to new regions in 2013–2014. The Journal of infectious diseases. 2016;214(suppl_5):S436-S40.
94. Spicher T, Delitz M, Schneider AdB, Wolfinger MT. Dynamic molecular epidemiology reveals lineage-associated single-nucleotide variants that alter RNA structure in Chikungunya virus. Genes. 2021;12(2):239.
95. de Bernardi Schneider A, Ochsenreiter R, Hostager R, Hofacker IL, Janies D, Wolfinger MT. Updated phylogeny of Chikungunya virus suggests lineage-specific RNA architecture. Viruses. 2019;11(9):798.
96. Williams M, Woodall J, Corbet PS, Gillett J. O'nyong-nyong fever: an epidemic virus disease in East Africa. VIII. Virus isolations from Anopheles mosquitoes. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1965;59(3).
97. Porterfield J. Cross-neutralization studies with group A arthropod-borne viruses. Bulletin of the World Health Organization. 1961;24(6):735.
98. Vanlandingham DL, Hong C, Klingler K, Tsetsarkin K, McElroy KL, Powers AM, et al. Differential infectivities of o'nyong-nyong and chikungunya virus isolates in Anopheles gambiae and Aedes aegypti mosquitoes. The American journal of tropical medicine and hygiene. 2005;72(5):616-21.
99. Haddow A, Davies C, Walker A. O'nyong-nyong fever: an epidemic virus disease in East Africa. I. Introduction. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1960;54(6).
100. Williams M, Woodall J, Gillett J. O'Nyong-Nyong fever: An epidemic virus disease in East Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1965;59(2):186-97.
101. Lutwama JJ, Kayondo J, Savage HM, Burkot TR, Miller BR. Epidemic O'Nyong-Nyong fever in southcentral Uganda, 1996-1997: entomologic studies in Bbaale village, Rakai District. Am J Trop Med Hyg. 1999;61(1):158-62.
102. Kiwanuka N, Sanders EJ, Rwaguma EB, Kawamata J, Ssengooba FP, Najjemba R, et al. O'Nyong-Nyong Fever in South-Central Uganda, 1996—1997: Clinical Features and Validation of a Clinical Case Definition for Surveillance Purposes. Clinical infectious diseases. 1999;29(5):1243-50.
103. Rezza G, Chen R, Weaver SC. O'nyong-nyong fever: a neglected mosquito-borne viral disease. Pathogens and global health. 2017;111(6):271-5.

104. Kayiwa JT, Nankya AM, Ataliba IJ, Mossel EC, Crabtree MB, Lutwama JJ. Confirmation of Zika virus infection through hospital-based sentinel surveillance of acute febrile illness in Uganda, 2014-2017. J Gen Virol. 2018;99(9):1248-52.
105. Steinhagen K, Probst C, Radzimski C, Schmidt-Chanasit J, Emmerich P, van Esbroeck M, et al. Serodiagnosis of Zika virus (ZIKV) infections by a novel NS1-based ELISA devoid of cross-reactivity with dengue virus antibodies: a multicohort study of assay performance, 2015 to 2016. Eurosurveillance. 2016;21(50):30426.
106. Brinkmann A, Nitsche A, Kohl C. Viral Metagenomics on Blood-Feeding Arthropods as a Tool for Human Disease Surveillance. International journal of molecular sciences. 2016;17(10).
107. Jerome H, Taylor C, Sreenu VB, Klymenko T, Filipe ADS, Jackson C, et al. Metagenomic next-generation sequencing aids the diagnosis of viral infections in febrile returning travellers. Journal of Infection. 2019;79(4):383-8.
108. Logue K, Keven JB, Cannon MV, Reimer L, Siba P, Walker ED, et al. Unbiased characterization of Anopheles mosquito blood meals by targeted high-throughput sequencing. PLoS neglected tropical diseases. 2016;10(3):e0004512.
109. Osei-Poku J, Mbogo C, Palmer W, Jiggins F. Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from K enya. Molecular ecology. 2012;21(20):5138-50.
110. Jupatanakul N, Sim S, Dimopoulos G. The insect microbiome modulates vector competence for arboviruses. Viruses. 2014;6(11):4294-313.
111. Smithburn K, Haddow A. Isolation of yellow fever virus from African mosquitoes. American Journal of Tropical Medicine. 1946;26(3):261-71.
112. Williams MC, Woodall JP, Corbet PS, Gillett JD. O'nyong-Nyong Fever: An Epidemic Virus Disease in East Africa. 8. Virus Isolations from Anopheles Mosquitoes. Trans R Soc Trop Med Hyg. 1965;59:300-6.
113. Nyakarahuka L, de St. Maurice A, Purpura L, Ervin E, Balinandi S, Tumusiime A, et al. Prevalence and risk factors of Rift Valley fever in humans and animals from Kabale district in Southwestern Uganda, 2016. PLOS Neglected Tropical Diseases. 2018;12(5):e0006412.
114. Smithburn KC, Haddow AJ, Lumsden WH. An outbreak of sylvan yellow fever in Uganda with Aedes (Stegomyia) africanus Theobald as principal vector and insect host of the virus. Ann Trop Med Parasitol. 1949;43(1):74-89.

Tables

Table 1: Mosquito-borne arboviruses that have caused outbreaks in Uganda.

Virus	Number of outbreaks	Case fatality (%)	Seroprevalence (%)	Reference
BWAV	2	Not known	9%-57.8%	(19, 33, 41)
YFV	8	24.9%	0.97% to 34.2%	(10, 27, 57-59, 111)
ONNV	3	Not known	44% to 61%	(32, 101, 112)
RVFV	13	1% to 2%	10% to 13%	(19, 113)
CHIKV	1	Not known	46.9%	(37, 39)

Abbreviations: BWAV: Bwamba virus, YFV: yellow fever virus, ONNV: o'nyong nyong virus, RVFV: Rift Valley fever virus, CHIKV: chikungunya virus.

767 **Table 2**: A summary of YFV outbreaks in Uganda.

Year	Region, place	Animal host	Seroprevalence /Case fatality (%)	Reference
1941	Western Uganda, Bwamba county	Humans	28.6%	(10)
1943	Epizootic on Bukasa islands	Monkey population	88.9%	(61)
1946/1947	Western Uganda, Bwamba county	Children $\leq$ 4 years	69.8%	(114)
1950	West Nile district	Humans and monkeys	Humans- 18% Animals- 36%	(56)
1951	Entebbe area	Monkey population	45.5%	(61)
1952	Western Uganda, Fort Portal	Humans	1 fatal case	(57)
1964	Central Uganda, Luwawa Forest	Humans	1 fatal case	(58)
1972	Central Uganda, Zika Forest	Non-human primates	40 %	(62)

2010	North Eastern Uganda, 13 districts	Humans	24.9 % (Case fatality)	(40)
2016	Central and South-western Uganda 7 districts	Humans	33 % (Case fatality)	(63)

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781 Figure 1: Mosquito-borne viruses associated with disease in humans, (a) mosquito vectors, (b)

782 vertebrate hosts

783 Figure 2: Map showing mosquito borne viruses in Uganda
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788 **Table S1:** Mosquito-borne viruses described in Uganda that are associated with fever/febrile illnesses in humans.

Virus name	Other symptoms	Vector species	Vertebrate hosts and or reservoirs	References
WNV	Incubation period of about 2-14 days, fever, headache, backache, vomiting, diarrhea, anorexia, neurologic involvement, conjunctival inflammation, myalgia, arthralgia, skin rash which may persist up to one week,	*Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. neavei*, *Cx. univitattus, Cq. metalica*	Birds, non-human primates, equines, canines and rodents	(47, 48, 50)

	lymphadenopathy and myocarditis			
BWAV	Headache, myalgia, epigastric pain, conjunctival inflammation	*An. gambiae* and *An. funestus* some *Aedes* and *Mansonia* species suggested	Birds, donkeys and monkeys. Rodents such as *Arvicanthis niloticus*, *Varanus niloticus, Boedon fuliginosus* suggested	(12, 33, 41)
BUNV	Rash, brain encephalitis, arthralgia, stiff neck	*An. gambiae, An. funestus, Ae. circumluteolus,*	Birds, rodents, domestic animals, monkeys, chimpanzee	(14, 19)

		Ma. uniformis, Ma. africana		
YFV	Incubation period is 3 to 6 days, fever, chills, nausea, convulsions, myalgia, anorexia, vomiting, dehydration, bleeding, jaundice, headache, backache, prostration	*Ae. africanus, Ae. bromeliae sub species of Ae. simpsoni, Ae. vitattus, Ae. metallicus, Ae. opok, E. chrysogaster, Ma. africana*	Monkeys, chimpanzees, baboons, bush babies	(9, 36, 38, 40, 47)
RVFV	Self-limiting febrile illness, rhinitis, encephalitis, hemorrhagic fever,	*Ae. circumluteolus, Ae. africanus, Ae. dendrophilus*, *Ae. tarsalis, Ma.*	Sheep, goats, humans	(34, 71, 76, 82)

	headache, shivering, mental confusion, photophobia, severe muscle and joint pains, convulsions, hallucinations, anorexia, nausea, vomiting, epistaxis, liver impairment, jaundice, vomiting of blood, passing blood in urine and faeces, bleeding in the nose and gums.	*africana*, *Ma. uniformis*, *Eretmopodites* spp, *Cq. fuscopennata*		

ZIKV	Incubation following exposure of about 2 to 7 days, fever, headache, rash, anorexia, conjunctivitis, myalgia, muscle and joint pains, microcephaly and other neurodevelopmental issues in infants, Guillain-Barré Syndrome in adults, neuropathy and myelitis.	*Ae. aegypti aegypti, Ae. africanus*	Monkeys, humans	(4, 19, 69, 74, 75)
CHIKV	Incubation following exposure 3 to 12 days,	*Ae. africanus*, *Ae. furcifer*, *Ae*. *taylori*,	Non-human primates	(5, 37, 39, 47)

	fever, rash, arthritis, itchy rash, headache, joint and muscle pains, prostration, conjunctival inflammation, myalgia, arthralgia, lymphadenopathy, leukopenia	*Ae*. *luteocephalus*, *Mansonia* spp, *Cx*. *quinquefasciatus*	especially monkeys such as the red tailed and African green monkeys. Reptiles and amphibians have also been suggested	
ONNV	Moderate fever lasting about 5 days, maculopapular rash which erupts 4 to 7 days after onset of symptoms, joint pains	*An. gambiae* and *An. funestus*	Human	(47, 101, 102, 112)

	prostration, headache, conjunctivitis, respiratory involvement, lymphadenitis, arthralgia, cervical lymphadenopathy			
ORUV	Headache, myalgia, vomiting, conjunctival inflammation	*An. funestus, An. gambiae, Ae. dentatus, Cx. perfuscus*	Human	(30)
SFV	Myalgia, arthralgia, headache, brain encephalitis, abdominal	*Ae. abnormalis, Ae. argenteopunctatus, Ae. dentatus, E. grahami*	Human	(19)

	pains, diarrhea, conjunctivitis			
BBKV	Arthralgia, joint pains, rash and arthritis	*Ae. africanus, Ae. simpsoni, Ae. mcintoshi, Ae. ochraceus, Cx. perfuscus*	Birds, humans	(49)
SINV	Arthralgia, muscle and joint pains rash and arthritis	*Cx. univitattus, Ma. africana*	Birds	(49)
PGAV	Joint pains, rash and arthritis	*Ae. tarsalis and Cq. fuscopennata*	Humans	(49, 91)
GERV	Rash and arthritis	*Cx. rubinotus, Cx. theileri*	Rodents such as *Lophuromys*,	(49, 71)

			Arvicanthis and *Rattus* spp	
USUV	Rash and arthritis	*Culex spp*	Birds, humans, cattle and sheep	(8, 49, 71)
WSLV	Myalgia, arthralgia, general body weakness, anorexia, headache, myalgia, arthralgia, still births in ruminants	*Ae. circumluteolus, Ae. mcintoshi*	Goats, cattle, sheep	(8)

789 Abbreviations: WNV: West Nile virus, BWAV: Bwamba virus, BUNV: Bunyamwera virus, YFV: yellow fever virus, RVFV: Rift Valley
790 fever virus, ZIKV: Zika virus, CHIKV: chikungunya virus, ONNV: o'nyong nyong virus, ORUV: Orungo virus, SFV: Semliki Forest virus,
791 PGAV: Pongola virus, BBKV: Babanki virus, SINV: Sindbis virus, GERV: Germiston virus, USUV: Usutu virus, WSLV: Wesselsbron virus

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AAS: African Academy of Sciences

AESA: Alliance for Accelerating Excellence in Science in Africa

AMTV: Arumowot virus

AVI: ArboViral Infection study

BBKV: Babanki virus

BHK-21: Hamster kidney cells

BUNV: Bunyamwera virus

BWAV: Bwamba virus

CDC: Centers for Disease Control and Prevention

CHIKV: chikungunya virus

CPE: cytopathic effects

DENV: Dengue virus

DNA: Deoxyribonucleic acid

EAVRI: East African Virus Research Institute

ELISA: Enzyme Linked Immunosorbent Assay

GERV: Germiston virus

HIV: Human Immunodeficiency virus

IFA: Immunofluorescence assay

IgG: Immunoglobulin G antibodies

IgM: Immunoglobulin M antibodies

JEV: Japanese encephalitis virus

KAMV: Kamese virus

LMIC: Low and middle-income countries

MOSV: Mossuril virus

MUII: Makerere University/UVRI Infection and Immunity Research Training program

NDOV: Nyando virus

NEPAD: New Partnership for Africa's Development Planning and Coordinating Agency

NGS: Next Generation Sequencing

NTAV: Ntaya virus

ONNV: o'nyong nyong virus

ORUV: Orungo virus

PCR: Polymerase chain reaction

PGAV: Pongola virus

PRNT: Plaque Reduction Neutralization Test

RNA: Ribonucleic acid

RT-PCR: Reverse transcription polymerase chain reaction

RVFV: Rift Valley fever virus

SFV: Semliki forest virus

SINV: Sindbis virus

SLEV: St. Louis encephalitis virus

TANV: Tanga virus

UGSV: Uganda S virus

UK: United Kingdom

USUV: Usutu virus

UVRI: Uganda Virus Research Institute

VHF: Viral hemorhagic fever

WITV: Witwatersrand virus

WNV: West Nile virus

WSLV: Wesselsbron virus

YFRI: Yellow Fever Research Institute

YFV: Yellow fever virus

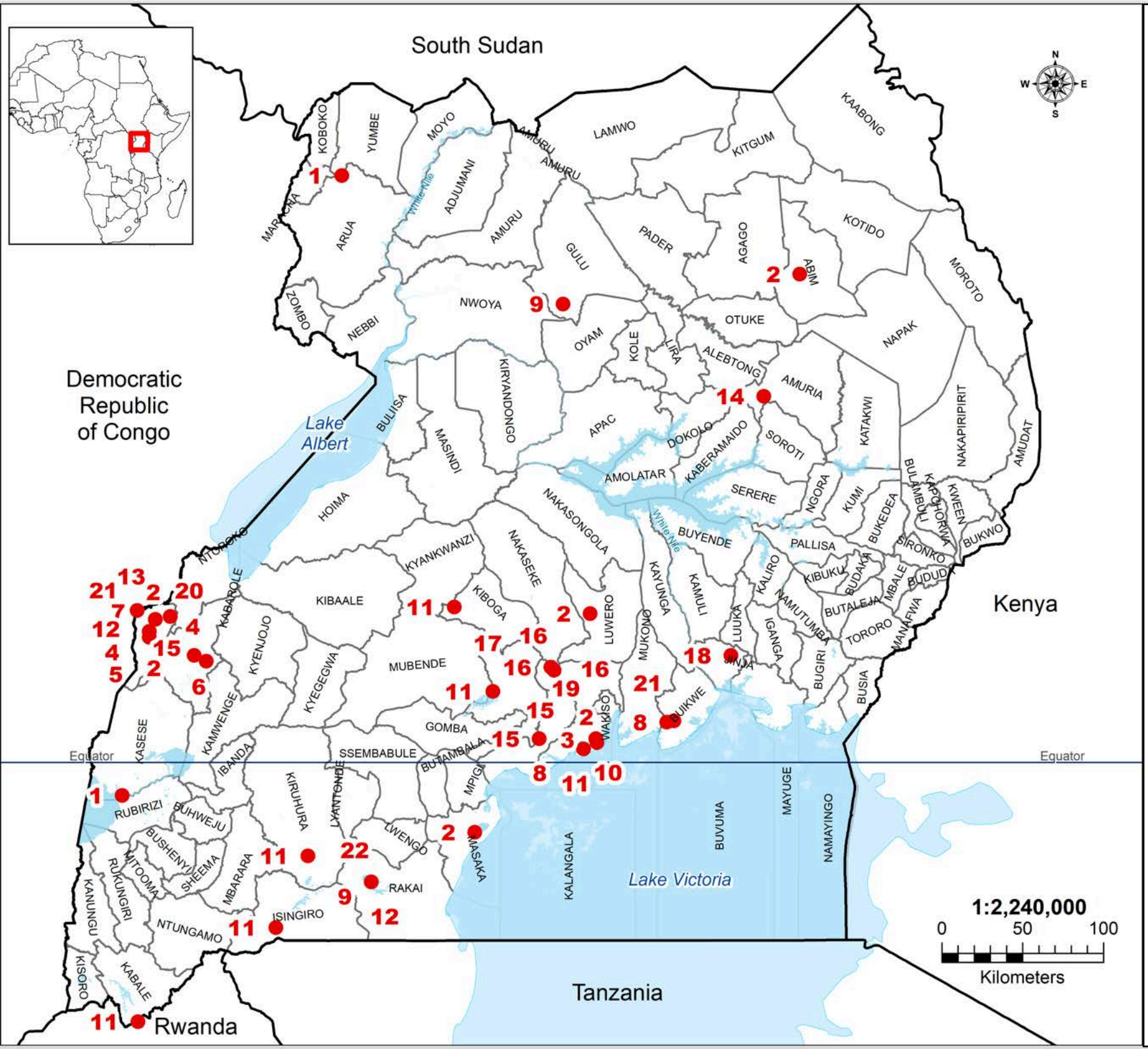
ZIKV: Zika virus

(a)

Mosquito-borne viruses	Mosquito vectors
RVFV	*Ae. circumluteolus, Ae. africanus, Ae. dendrophilus, Ae. tarsalis, Ma. africana, Ma. uniformis, Eretmopodites spp, Cq. fuscopennata*
YFV	*Ae. africanus, Ae. simpsoni, Ae. vitattus, Ae. opok, E. chrysogaster, Ma. africana, Ae. metallicus*
CHIKV	*Ae. africanus, Ae. furcifer, Ae. taylori, Ae. luteocephalus, Mansonia spp, Cx. quinquefasciatus*
WNV	*Cx. pipiens, Cx. quinquefasciatus, Cx. neavei, Cx. univitattus, Cq. metalica*
USUV	*Cx. pipiens, Cx. quinquefasciatus, Cx. neavei, Cx. univitattus, Cq. aurites*
BUNV	*An. gambiae, An. funestus, Ae. circumluteolus, Ma. uniformis, Ma. africana*
BBKV	*Ae. africanus, Cx. perfuscus, Ae. ochraceus, Ae. mcintoshi, Ae. simpsoni*
SFV	*Ae. abnormalis, E. grahami, Ae. dentatus, Ae. argenteopunctatus*
ORUV	*An. funestus, Cx. perfuscus, Ae. dentatus, An. gambiae*
BWAV	*An. gambiae, An. funestus, Aedes spp, Mansonia spp*
ZIKV	*Ae. aegypti aegypti, Ae. africanus*
WSLV	*Ae. circumluteolus, Ae. mcintoshi*
SINV	*Cx. univitattus, Ma. africana*
PGAV	*Ae. tarsalis, Cq. fuscopennata*
ONNV	*An. gambiae, An. funestus*
GERV	*Cx. rubinotus, Cx. theileri*

(b)

Mosquito-borne viruses	Vertebrate hosts
WNV	Birds, Non-human primates, Equines, Canines,Rodents
BUNV	Birds, Rodents, Domestic animals, Monkeys, Chimpanzee
YFV	Monkeys, Chimpanzee, Baboons, Bush babies
USUV	Birds, Humans, Cattle, Sheep
BWAV	Birds, Donkeys, Monkeys, Rodents
WSLV	Goats, Cattle, Sheep
RVFV	Sheep, Goats, Humans
CHIKV	Non-human primates, Reptiles, Amphibians
ZIKV	Monkeys, Humans
BBKV	Birds, Humans
SINV	Birds
SFV	Humans
PGAV	Humans
ORUV	Humans
ONNV	Humans
GERV	Rodents



Virus names

1. WNV
2. YFV
3. ZIKV
4. UGSV
5. NTAV
6. USUV
7. SFV
8. CHIKV
9. ONNV
10. SINV
11. RVFV
12. BWAV
13. BUNV
14. ORUV
15. KAMV
16. WITV
17. GERV
18. AMTV
19. PGAV
20. MOSV
21. BBKV
22. NDOV

788 **Table S1:** Mosquito-borne viruses described in Uganda that are associated with fever/febrile illnesses in humans.

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	lymphadenopathy and myocarditis			
BWAV	Headache, myalgia, epigastric pain, conjunctival inflammation	*An. gambiae* and *An. funestus* some *Aedes* and *Mansonia* species suggested	Birds, donkeys and monkeys. Rodents such as *Arvicanthis niloticus*, *Varanus niloticus, Boedon fuliginosus* suggested	(12, 33, 41)
BUNV	Rash, brain encephalitis, arthralgia, stiff neck	*An. gambiae, An. funestus, Ae. circumluteolus,*	Birds, rodents, domestic animals, monkeys, chimpanzee	(14, 19)

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YFV	Incubation period is 3 to 6 days, fever, chills, nausea, convulsions, myalgia, anorexia, vomiting, dehydration, bleeding, jaundice, headache, backache, prostration	*Ae. africanus, Ae. bromeliae sub species of Ae. simpsoni, Ae. vitattus, Ae. metallicus, Ae. opok, E. chrysogaster, Ma. africana*	Monkeys, chimpanzees, baboons, bush babies	(9, 36, 38, 40, 47)
RVFV	Self-limiting febrile illness, rhinitis, encephalitis, hemorrhagic fever,	*Ae. circumluteolus, Ae. africanus, Ae. dendrophilus*, *Ae. tarsalis, Ma.*	Sheep, goats, humans	(34, 71, 76, 82)

	headache, shivering, mental confusion, photophobia, severe muscle and joint pains, convulsions, hallucinations, anorexia, nausea, vomiting, epistaxis, liver impairment, jaundice, vomiting of blood, passing blood in urine and faeces, bleeding in the nose and gums.	*africana*, *Ma. uniformis*, *Eretmopodites* spp, *Cq. fuscopennata*		

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	prostration, headache, conjunctivitis, respiratory involvement, lymphadenitis, arthralgia, cervical lymphadenopathy			
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	pains, diarrhea, conjunctivitis			
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