



## Effect of precipitation on abundance and molecular diversity of potential vectors for Rift Valley fever virus in Nyandarua County, Kenya

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### Abstract

Rift Valley fever (RVF) is a mosquito-borne viral zoonotic disease that causes significant economic and public health impacts in Kenya. Currently, there is inadequate information on rainfall patterns and mosquito diversity, including the dominant species in Nyandarua. Therefore, this study aimed to determine the rainfall patterns, relative abundance, and diversity of mosquito species that can transmit the RVF virus (RVFV) in Nyandarua. Mosquitoes were purposively collected from all the homesteads with suspect RVF cases in 10 villages using the CDC light trap during the dry (January 2021), long (July 2020), and short (November 2021) rainy seasons. Rainfall data was derived from the Climate-SERV satellite database. The mosquitoes were identified morphologically using a dissecting microscope and their identities, as well as genetic diversity, were determined using sequencing and phylogenetic analysis of the CO1 gene. A total of 97, 328, and 366 mosquitoes were trapped during the dry, long and short rainy seasons, respectively. There was variation in the average daily rainfall between 2015 and 2021 during the three seasons. Of the mosquitoes trapped, 71 (9%) were males while 720 (91%) were females with 26 (4%) mosquitoes being blood-fed. During the three seasons, various species of mosquitoes including *Culex pipiens* (58%), *Culex theileri* (16%), *Culex vansomereni* (3%), *Culex rima* (2%), and *Culex perexiguus* (3%) were identified (Simpson index = 0.4). *Culex pipiens* was the most dominant species in this ecosystem (Shannon index = 1.2). We conclude that the anomalous variations in rainfall patterns may be correlated with the emergence of diverse species of mosquitoes that have the potential to transmit RVFV to animals in Nyandarua.

**Keywords:** Rift Valley fever; precipitation; mosquito diversity; Nyandarua

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### Introduction

Rift Valley fever (RVF) is a mosquito-borne viral zoonotic disease that causes significant economic losses in the livestock sector and is a public health threat of concern in the Horn of Africa,

subtropical Madagascar and the Arab Peninsula (Gerken et al., 2022; Nanyingi et al., 2015; Nyaruaba et al., 2019). RVF disease is caused by the Rift Valley fever virus (RVFV), a single-stranded RNA virus of the order *Bunyavirales* recently reclassified into the new *Phenuiviridae*

family and the genus *Phlebovirus* (Linthicum *et al.*, 2016a). RVFV was first isolated in 1931 from infected sheep in the Naivasha area in Rift Valley, Kenya. During previous explosive outbreaks in the Horn of Africa region, RVF was reported to occur mainly as epizootics in cycles of 8 to 10 years (Breiman, 2010). These outbreaks were mainly associated with the El Nino-Southern Oscillation (ENSO) phenomena, which were characterised by persistent and above-normal rainfall (Anyamba *et al.*, 2010; Bhardwaj, 2013). The ENSO phenomenon occurred in half of the African continent, Saudi Arabia, and Yemen and resulted in RVF outbreaks in these regions (Nanyingi *et al.*, 2015).

The virus is usually transmitted after heavy rainfall by competent mosquitoes of the genera *Aedes*, *Culex*, and *Mansonia* in RVF endemic regions including Kenya (Pepin *et al.*, 2010). The female *Aedes* mosquito is the primary vector that bites and infects susceptible ruminant hosts. The dormant eggs of *Aedes* in the soil hatch within 1 to 2 days after flooding and some of the emerging mosquitoes may be infected, allowing them to transmit RVFV when the eggs develop into adult mosquitoes (Linthicum *et al.*, 1985). Secondary vectors of the virus, which include the *Culex* and *Mansonia*, dominate 30 to 40 days later, thus spreading and amplifying RVFV (Hightower *et al.*, 2012; Linthicum *et al.*, 2016b).

Many species of *Culex* mosquitoes, including *Culex pipiens*, *Cx. theileri*, *Cx. vansomereni*, *Cx. rima*, *Cx. perexiguus*, *Cx. tritaeniorhynchus*, *Cx. annulioris*, *Cx. cinerellus*, *Cx. poicilipes*, *Cx. univittatus*, *Cx. antennatus*, and *Cx. neavei*, have the potential to transmit RVFV (Ajamma *et al.*, 2016; Chiuya *et al.*, 2021; Eifan *et al.*, 2021; Naeem *et al.*, 2016). *Cx. pipiens* complex has been reported to transmit RVFV in Saudi Arabia, North-western Iran, Nigeria, Morocco, Tunisia, Algeria, Egypt, and Botswana (Amraoui *et al.*, 2012; Eifan *et al.*, 2021; El-Rahim *et al.*, 1999; Mohammed *et al.*, 2021; Pachka *et al.*, 2016; Paksa *et al.*, 2019). *Culex* mosquitoes have been reported to be present in the north-eastern and coastal regions of Kenya, making these regions hotspots for RVF outbreaks (Arum *et al.*, 2015). The morphology and genetic diversity of *Aedes* and *Culex* mosquitoes that transmit RVFV have been characterised in Baringo and areas bordering Lake Victoria, including western Kenyan regions such as Busia,

Bungoma, and Kakamega (Ajamma *et al.*, 2016; Chiuya *et al.*, 2021; Ochieng *et al.*, 2016). However, the potential vectors for RVFV and their genetic diversity remain unknown in the Kenyan highlands of Nyandarua County. These high-altitude areas experience high rainfall episodes (Miller *et al.*, 2021; Nicholson, 2017), resulting in high humidity during the rainy season, and this can provide conducive conditions for the larval development of mosquitoes (Asigau & Parker, 2018; Costa *et al.*, 2010; Lumley *et al.*, 2017).

This study aimed to determine the effect of enhanced precipitation variability on the relative abundance and diversity of potential mosquito vectors for RVFV in Nyandarua to guide vector control strategies.

## Materials and methods

### Study area

Nyandarua County covers an area of 3,285.7km<sup>2</sup> (KNBS, 2019) and is located in Central Kenya with minimum and maximum altitudes of 1,114m and 3,973m above sea level, respectively. Nyandarua borders the Laikipia Counties to the north, Nyeri, and Murang'a to the northeast and east respectively. It borders the counties of Kiambu to the south and Nakuru to the west. The annual rainfall range in Nyandarua is between 700 and 1,700mm, and the average temperatures range from 12°C to 25°C (Mwongera *et al.*, 2019). Three subcounties of OI Kalou, Ndaragwa, and Kipipiri which closely border each other were included in the study. A total of five wards were involved, Kaimbaga and Rurii in OI Kalou, Shamata in Ndaragwa, and Wanjohi and Kipipiri wards in Kipipiri. Ten villages in these wards had recently reported cases of abortion in animals and were included in the study. These cases were reported by farmers to the county veterinarians. The surface of the land is always waterlogged and marshy, especially during the wet season. When the rainfall subsides towards the end of the rainy season and during the dry period, stagnant water is left in seasonally waterlogged shallow depressions (dambos). The area has clay loam soil and is mainly covered with guinea grass, king grass, and couch grass along Lake Olbolosat. The grass also covers a large flat portion of non-cultivated land and is used as pasture for grazing livestock.

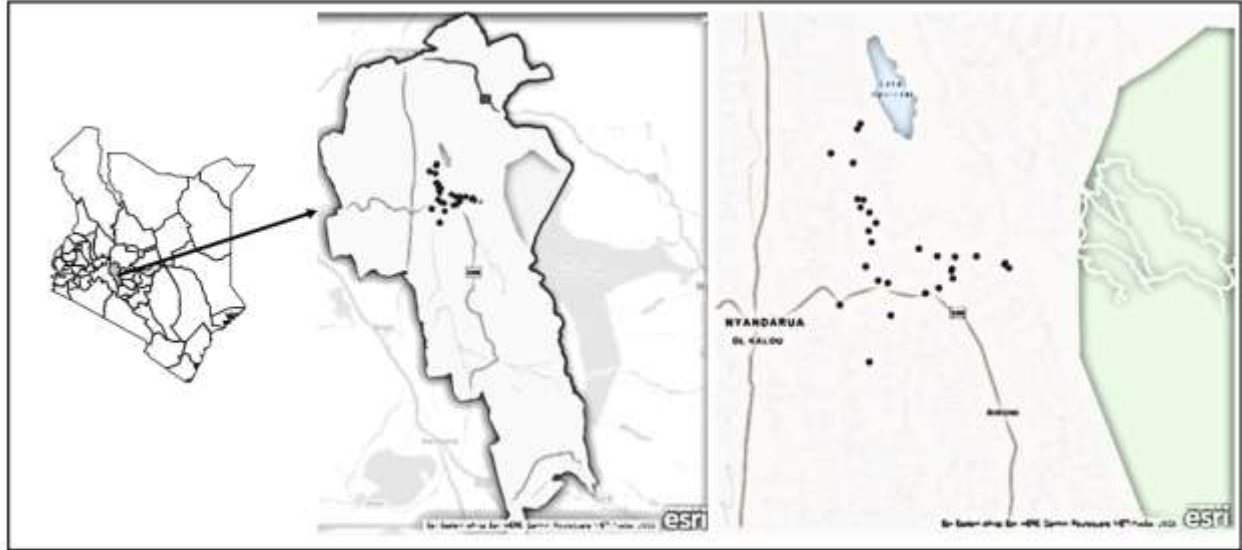


Figure 1. A map showing the sites where the mosquitoes were trapped in Nyandarua Kenya

#### **Study design and mosquito's collection**

The study was carried out between July 2020 and November 2021 in both dry and wet seasons. In particular, initial samples were collected in July 2020 towards the end of the long rainy season; additional samples were collected in January 2021 and November 2021 during the dry and short rainy seasons, respectively. The farms which had reported recent cases of abortion in animals, were purposively sampled for the collection of *Culicidae* mosquitoes. These farms were located in 10 villages of Nyandarua, namely, Magomano, Malewa, Michore, Gichungo, and Kanjogu. The other villages were Huherio, Kiaduba, Kirima, Mugathika, and Mukindu. CDC light traps were placed in animal shelters 1 metre above the ground from 1500 h, and mosquitoes were attracted by animal exhalation, body odor and warmth. The CDC traps were removed at 0600 h at dawn with the collected mosquitoes (Lühken *et al.*, 2014). Mosquitoes were collected once from each of the selected farms with sampling trapping mosquitoes in three farms during the dry season, seven farms during long rains, and 22 farms during the short rainy season. At the same time, questionnaires were administered to livestock farmers, and GPS coordinates were taken using the Epicollect5 application software.

#### **Extraction of rainfall data**

Rainfall data on seasonality and Nyandarua patterns were extracted between January 2015 and December 2021. National Aeronautics and Space Administration - The Integrated Multi-satellite Retrieval for Global Precipitation Measurement (NASA-IMERG) data was used as a source of precipitation in this study (Huffman *et al.*, 2015; Watters & Battaglia, 2019). This product uses a combination of station data and satellite imagery to create a gridded rainfall dataset. Rainfall data was downloaded from the ClimateSERV database (<https://climateserv.servirglobal.net/>) as raster and CSV files using GPS coordinates of the sampled farms to make a polygon shapefile. Monthly average rainfall (mm) was determined and compared for dry, long, and short rainy seasons (Novella and Thiaw, 2013; Shukla *et al.*, 2021).

#### **Morphological identification of mosquito genera**

The trapped mosquitoes were immobilized inside a -20°C freezer for 10 minutes. The frozen mosquitoes were sorted against a white background to separate them from other insects. Thereafter, mosquitoes were morphologically identified using Leica HD4 dissecting microscope based on their sex and genus (Becker *et al.*, 2010; Bram, 1967; Harbach, 1985; Noureldin *et al.*, 2021; Rueda, 2004). The morphological identification of *Anopheles* mosquitoes was carried out using

the key of female Afrotropical *Anopheles* mosquitoes (Coetzee, 2020; Gillies & Coetzee, 1987). The identified mosquitoes were preserved in RNALater (ThermoFisher Scientific) and transported to the Central Veterinary Entomology Laboratory in Kabete, Nairobi. In the laboratory, the preserved mosquitoes were stored at -80°C pending genetic analysis.

#### **Extraction of mosquito DNA**

From each morphologically identified mosquito species, an individual mosquito was selected for DNA extraction. Genomic DNA from a single mosquito was extracted using the ZR Tissue & Insect DNA Kit (Zymo Research, USA) according to the manufacturer's protocol. Briefly, the mosquitoes were crushed and homogenized using ZR bashing beads. Thereafter, the homogenate was lysed with 750 µl of lysis buffer containing beta-mercaptoethanol. The lysate was centrifuged and the supernatant recovered by filtration. Further lysis was performed by adding 1200 µl of genomic lysis buffer to 400 µl of the lysate. This lysate was added to a silica gel membrane to bind mosquito DNA and then briefly centrifuged to recover DNA. The silica gel membranes were washed twice with 200 µl of DNA pre-wash buffer. Further washing was done using 500 µl of gDNA Wash Buffer. The bound DNA was then eluted using a DNA elution buffer. The quality and quantity of the DNA were determined using NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Scientific), at a wavelength of 260 to 280 nm. The extracted DNA was stored at -80°C for subsequent genetic analysis.

#### **Identification of mosquito species by Polymerase Chain Reaction and sequencing**

Polymerase Chain Reaction (PCR) was performed to confirm identity of the species of the genus *Culex*. The 658bp mitochondrial cytochrome oxidase 1 (CO1) gene fragment was amplified with universal primers, including Forward LCO1490: 5'GGTCAACAAATCATAAAGATATTGG3', and Reverse HCO2198: 5'TAAACTTCAGGGTGACCAAAAATCA3' (Laurito *et al.*, 2013; Noureldin *et al.*, 2021). The PCR amplification reaction was performed in a thermal cycler (MultiGene Optimax thermal cycler, Labnet International). The total volume of

the PCR reaction was 25 µl containing 12.5 µl of OneTaq® 2X Master Mix with standard buffer (Biolabs, New England), 0.5 µl of each reverse and forward primer, 2 µl of DNA template, and 9.5 µl of nuclease-free water. The PCR reaction involved an initial denaturation at 94°C for 3 minutes, 40 cycles of denaturation at 94°C for 1 minute, primer annealing at 55°C for 1 minute, followed by primer extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes (Ajamma *et al.*, 2016; Hernandez-Triana *et al.*, 2017). The PCR products were electrophoresed on a 1.5% agarose gel immersed in 1X Tris-Boric acid EDTA (TBE) buffer (Gibco Life Technologies, UK). Subsequently, gel staining was performed with GelRed nucleic acid gel stain (Biotium Inc., Hayward, USA). Amplicon visualization was performed using a DOC PRINT VX5 (Vilbert Lourmat) imaging system. Nuclease-free water was used as the negative control. The seven PCR products were purified and then sequenced at Inqaba Biotec in South Africa using the Sanger method using the same primers targeting the CO1 gene. Mosquito species were identified using the Basic Local Alignment Search Tool for Nucleotide (BLASTn) output of the sequenced CO1 gene. [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE\\_TYPE=BlastSearch](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch)

#### **Phylogenetic analysis of mosquito nucleotide sequences**

To establish the genetic diversity of the mosquitoes, phylogenetic trees were constructed using CO1 gene nucleotides generated from this study. These sequences were aligned with other nucleotides of mosquito DNA previously identified in other areas of Kenya and elsewhere in the world (Ajamma *et al.*, 2016; Alten *et al.*, 2000; Eifan *et al.*, 2021). The Bioedit software was used for obtaining nucleotide consensus sequences after performing alignment of contigs generated by sequencing using the forward and reverse primers. Multiple alignments of nucleotide sequences were performed using the MUSCLE algorithm using MEGA 11 (Tamura *et al.*, 2021). Evolutionary history was inferred using the neighbor-joining method based on the Saitou-Nei model (Saitou and Nei, 1987). The statistical significance of the internal branches of the tree was obtained using the bootstrap with 1000 replicates (Felsenstein, 1985). The

evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004). The genetic diversities of the mosquitoes from different villages were calculated using Simpson and Shannon Weiner Diversity Indices.

**Statistical analysis**

The morphological and genetic diversity of mosquito species were determined using the Simpson (D) and Shannon (H) Weiner Diversity Indices (Rybanov & Aleksandrovna, 2020). The values for the diversity ranged from 0 to 1. The estimates were done for the three seasons; dry, long, and short rains. The Paired t-test for the equality of means for diversity of mosquito species was conducted using the Simpson and Shannon Weiner indices according to the number of traps used and season. Means were calculated for dry, long, and short rainy seasons, to monitor whether mosquito species increased or decreased in each season. The proportions of mosquitoes per species were computed, and their prevalence were also calculated. The statistical analysis was done using R software version 4.1.1 (Team and Team, 2021). Statistically significant differences were determined at  $p < 0.05$ .

**Ethical approval**

Ethical approval with Ref: KNH-ERC/A/373 was granted by the KNH-UON ERC. The

collection of samples in the field was approved by both the Directorate of Veterinary Services and Nyandarua County Director of Veterinary Services in Kenya. Informed consent was obtained from all farmers. Subsequently, the collected data was assigned unique identifiers and anonymised for confidentiality.

**Results**

**Rainfall pattern**

There was variation in the monthly average rainfall pattern from 2015 to 2021 in Nyandarua County for the three seasons (Table 1). The highest monthly mean rainfall during the dry season was recorded in January 2020, and was significantly different with the other years except in January 2016. For the rainfall data obtained in April, which represented the long rainy season, the highest monthly mean rainfall was experienced in 2018 and was significantly different from the mean rainfall recorded in this season from 2015 to 2021. Meanwhile, for the short rainy season, the highest monthly mean rainfall was observed in 2019 and the lowest in 2018, and there was a significant difference from the rainfall recorded during the rest of the study period (Table 1).

Table 1. Mean daily rainfall patterns in January, April, and November from 2015 to 2021

Month/Year	Mean Rainfall (mm) $\bar{x} \pm SEM$	Total rainfall (mm)
Dry season		
January 2015	8.4 $\pm$ 2.02	259.5
January 2016	<sup>a</sup> 58.8 $\pm$ 17.75	1821.6
January 2017	11.4 $\pm$ 8.14	352.2
January 2018	25.2 $\pm$ 6.56	779.8
January 2019	7.7 $\pm$ 1.93	238.8
January 2020	72.3 $\pm$ 17.55	2240.7
January 2021	17.4 $\pm$ 4.42	539.8
Long rainy season		
April 2015	145.5 $\pm$ 25.5	4366.3
April 2016	134.4 $\pm$ 30.6	4032.3
April 2017	50.4 $\pm$ 12.84	1511.3
April 2018	257.3 $\pm$ 37.65	7717.8
April 2019	109 $\pm$ 41.15	3269.0

April 2020	155 ± 32.17	4649.5
April 2021	66.6 ± 14.06	1997.7
Short rainy season		
November 2015	74.3 ± 12.48	2228.9
November 2016	75.9 ± 12.41	2277.5
November 2017	68.1 ± 12.37	2043.9
November 2018	13.6 ± 2.51	407.2
November 2019	134.9 ± 29.19	4048.3
November 2020	46.6 ± 9.64	1399.1
November 2021	33.9 ± 8.79	1006.7
Year		
2015		6854.7
2016		8131.4
2017		3907.4
2018		8904.8
2019		7556.1
2020		8289.3
2021		3544.2

<sup>a</sup> Not significantly different ( $p > 0.05$ )

#### **Identification of mosquito genera**

A total of 791 individual mosquitoes belonging to the two main subfamilies, namely, Culicinae and Anophelinae, were trapped in 10 villages in all homesteads with RVF suspect cases. Specifically, we identified the *Culex* and *Anopheles* genera based on their morphological characteristics. The 150 collected *Anopheles* mosquitoes belonged to *An. gambiae*.

As shown in Table 2, a higher proportion (46.3%) of *Culex* and *Anopheles* mosquitoes were trapped during short rains than during long rains (41.5%). There was no significant difference ( $p = 0.326$ ) between the total number of mosquitoes trapped during the two seasons. However, fewer mosquitoes were trapped during the dry season

and there were no significant differences in the total number of mosquitoes trapped during the short ( $p = 0.817$ ) and long ( $p = 0.330$ ) rainy seasons. When we analyzed the total number of mosquitoes collected in the three seasons, *Culex* was the most abundant and significantly present ( $p = 0.02$ ) compared to *Anopheles*.

During the long rainy season, trapping was carried out in July, which is the last month of this season. The month of July was the best for mosquito trapping, since the amount of rain had subsided and the mosquitoes had started to breed. Nevertheless, no *Anopheles* mosquito was trapped during the dry season with only 150 trapped during the long and short rainy seasons (Table 2).

Table 2. *Culex* and *Anopheles* mosquitoes trapped in various villages during the three seasons

Village	Traps	<i>Culex</i> spp.	<i>Anopheles gambiae</i>	Total (%)
Dry season (January 2021)				
Michore	1	1	0	1
Magomano	1	93	0	93
Malewa	1	3	0	3
Total		97	0	97 (12)
Long rainy season (*July 2020)				

Gichungo	3	117	5	122
Kanjogu	2	94	65	159
Michore	1	27	0	27
Malewa	1	15	5	20
Total		253	75	328 (42)
Short rainy season (November 2021)				
Mukindu	3	32	0	32
Kiaduba	4	75	15	90
Mugathika	3	29	3	32
Gichungo	4	15	5	20
Magomanao	2	64	1	65
Huherio	3	58	41	99
Malewa	2	2	3	5
Kirima	1	16	7	23
Total	32	291	75	366 (46)
Grand total		641	150	791

\* End of the long rainy season

***Culex species identified in the ten villages***

The genus *Culex* species identified by morphological analysis were further analysed by PCR and sequenced to confirm the actual species of *Culex* trapped. Conventional PCR with primers targeting the CO1 gene amplified a specific band corresponding to 658bps. Seven representative samples of the PCR products were sequenced and confirmed by blastn analysis. As shown in Table 3, sequencing and blastn analysis

identified five species of *Culex*. In particular, the sequences of the two identified mosquitoes were homologous to *Cx. pipiens*, with a nucleotide identity of 95.77% and 98.85%. The other two representative sequences were homologous to *Cx. theileri* with a nucleotide identity of 95.00%. The other three remaining sequences were homologous to *Cx. vansomerani*, *Cx. rima*, and *Cx. perexiguus* with an identity of 98.82%, 99.54%, and 99.19% respectively (Table 3).

Table 3. *Culex species identified in villages*

Sample	Accession No. (This study)	Mosquito species	Accession No. (Highest BLASTn match)	% Identity
MS37	ON406277.1	<i>Culex pipiens</i>	MK047311.1	95.77
MS62	ON406278.1	<i>Culex pipiens</i>	MK714002.1	98.85
MS3	ON406275.1	<i>Culex theileri</i>	JN051388.1	95.00
MS8	ON406276.1	<i>Culex theileri</i>	JN051388.1	95.00
MS22	ON422316.1	<i>Culex vansomerani</i>	KU187047.1	98.82
MS39	ON428528.1	<i>Culex rima</i>	KU380471.1	99.54
MS43	ON422317.1	<i>Culex perexiguus</i>	KU380423.1	99.19

Tables 4. *Culex* identified by villages during the three seasons

Village	Traps No.	<i>Culex pipiens</i>	<i>Culex theileri</i>	<i>Culex vansomereni</i>	<i>Culex rima</i>	<i>Culex perexiguus</i>	Total
Dry season (January 2021)							
Michore	1	1	0	0	0	0	1
Magomano	1	93	0	0	0	0	93
Malewa	1	3	0	0	0	0	3
Total		97	0	0	0	0	97
Long rainy season (*July 2020)							
Gichungo	3	0	90	11	0	16	117
Kanjogu	2	94	0	0	0	0	94
Michore	1	27	0	0	0	0	27
Malewa	1	10	0	0	5	0	15
Total		131	90	11	5	16	253
Short rainy season (November 2021)							
Mukindu	3	25	6	0	1	0	32
Kiaduba	4	48	13	7	3	4	75
Mugathika	3	28	1	0	0	0	29
Gichungo	4	2	11	2	0	0	15
Magomanao	2	64	0	0	0	0	64
Huherio	3	46	2	1	9	0	58
Malewa	2	2	0	0	0	0	2
Kirima	1	14	2	0	0	0	16
Total		229	35	10	13	4	291
Grand total		457 (58)	125 (16)	21 (3)	18 (2)	20 (3)	641

\* End of the long rainy season

As shown in Table 3, five different *Culex* species were identified from the ten villages. *Culex pipiens* (58%) was found to be the most dominant species in the ten villages except for Gichungo. *Cx. theileri*, *Cx. perexiguus*, and *Cx. vansomereni* were identified to be the most dominant species in Gichungo village and not in the other villages during the end of the long rainy season. However, in the other villages, these species were not dominant during the peak of the short rainy season. It was also noted that *Cx. pipiens* were collected in all three seasons compared to other species. The second most abundant mosquito was *Cx. theileri* (16%) and *Cx. rima* (2%) was the least abundant species. The mosquito species diversities collected during the three rainfall seasons ranged from 0 to 0.44 for the Simpson index diversity, and from 0 to 1.38 for the

Shannon Weiner index. The Simpson index for mosquito species was the highest (0.44) in November and the lowest (0) during the dry season. However, the Shannon-Weiner index diversity for mosquito species was highest in July (1.38). All trapped mosquitoes showed a diversity of 1.2 (Shannon index), an evenness of 0.67, and a diversity of 0.4 (Simpson index).

#### **Genetic diversity of *Culex* species**

Phylogenetic trees were constructed to establish the genetic diversity of *Culex spp.* The analyses were based on the CO1 gene and grouped the two *Cx. pipiens* DNA sequences from Nyandarua in the same clade similar to *Culex* species identified from Turkey (Figure 2). Other sequences from Hungary, Kenya, and Turkey were grouped into different clades. The Nyandarua *Cx. theileri*



sequences belonged to the same clade as the one from Rusinga Island, but just not as closely related as to the one from Spain (Figure 3). However, the DNA sequences from Greece, Hungary, and Portugal were grouped in a different clade, indicating that they were not genetically related to the ones from Nyandarua. *Cx. perexiguus* was placed in the same clade and very similar to the KU380423.1 strain from Kenya but in a different one from the other DNA sequences from Malawi, Pakistan, and the USA. The similarity of the DNA sequence of *Cx. perexiguus* from Nyandarua and the USA strain was closer than the KU380348.1 from Kenya. This indicates that the Kenyan *Cx. perexiguus* DNA

sequences were genetically distinct from each other. The Nyandarua *Cx. rima* DNA sequence was grouped in the same clade with another sequence from Kenya, indicating that the two Kenyan mosquitoes were genetically related. Furthermore, the Nyandarua *Cx. vansomereni* DNA sequence was also grouped in the same clade with another from Kenya. This indicates that the two Kenyan *Cx. vansomereni* DNA sequences, one from Nyandarua, were genetically similar (Figure 4).

Figure 2: The maximum likelihood tree of *Culex pipiens* reconstructed on the basis of the partial CO1 gene with 1000 bootstrap replicas.

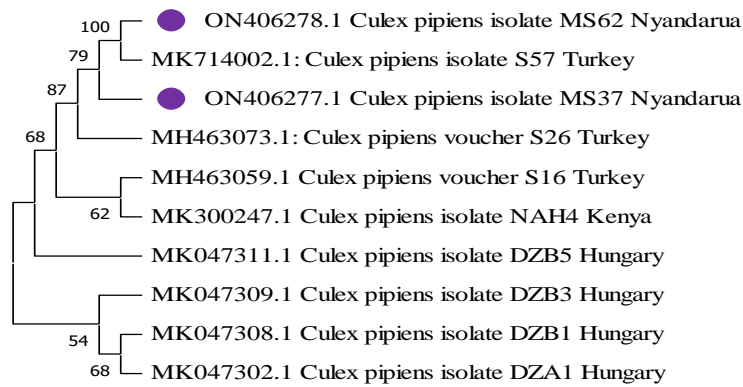


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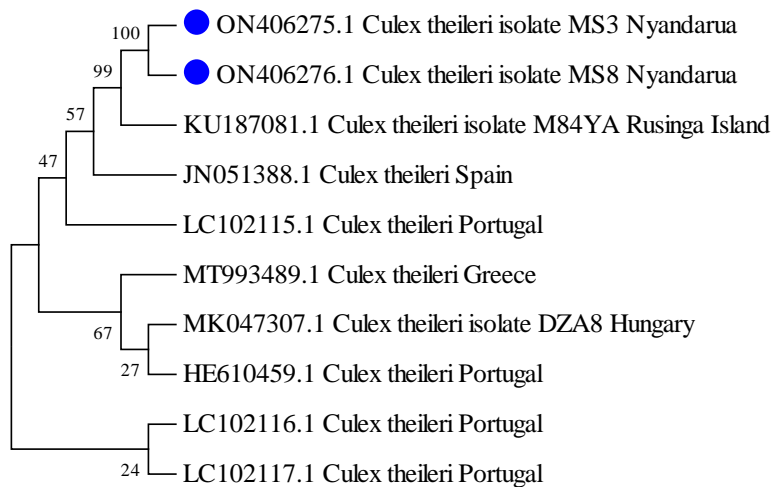


Figure 3. The maximum-likelihood tree of *Culex theileri* reconstructed based on the partial CO1 gene with 1000 bootstrap replicates

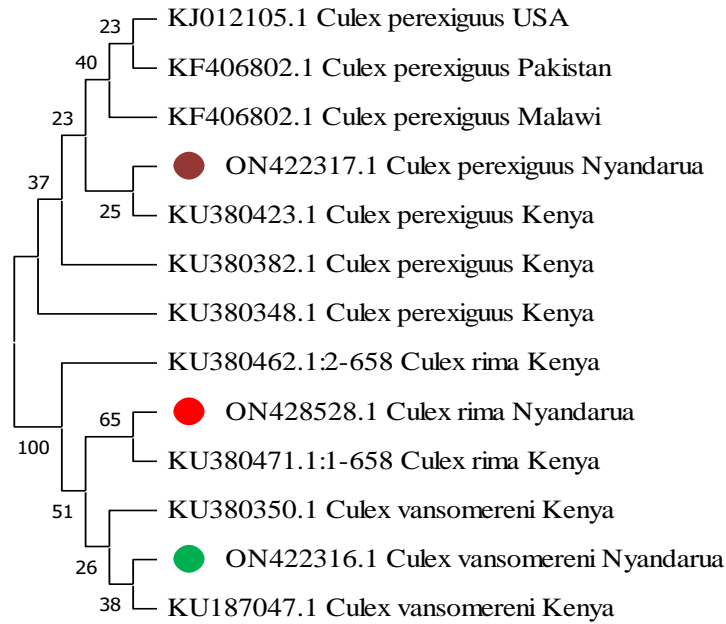


Figure 4. The maximum-likelihood tree of *Culex perexiguus*, *Culex rima*, and *Culex vansomereni* reconstructed based on the partial CO1 gene with 1000 bootstrap replicates

## Discussion

The current study noted anomalous rainfall data variations during the seven years between 2015 and 2021, indicating the possibility of precipitation fluctuations. Similar variations in rainfall were also observed in a study conducted in South Africa (Anyamba *et al.*, 2021). The variations in rainfall may influence the population and distribution of potential mosquito vectors. The increase in mosquito populations in these high-altitude areas can influence the transmission of arboviruses. The variability in rainfall remains one of the drivers that cause the increase in the mosquito population and influences the transmission of RVFV in South Africa, Senegal, and Kenya (Anyamba *et al.*, 2021; Ondiba *et al.*, 2019; Seck *et al.*, 2022). The average monthly rainfall during the three seasons varied, with recorded data indicating an increase or decrease in precipitation. During the three seasons, the study area recorded above or below normal rainfall. High variability was observed in rainfall patterns in 2018 that recorded the highest and lowest rainfall during the long and short rainy seasons, respectively. The variation in rainfall pattern observed in this study appears to play a role in mosquito abundance, with the potential of

increasing the risk of RVFV transmission especially during the rainy seasons (Nguku *et al.*, 2010). The number of traps that collected mosquitoes in the study was higher during the short rains. A large number of farms were attributed to the increase in suspected RVF cases in livestock and the high seroprevalence after previous screening (Pachka *et al.*, 2016; Wanjama *et al.*, 2022). The rainfall variability in Nyandarua could have resulted in the incursion of *Culex* species mosquitoes with a risk of unexpected RVF outbreaks. The high-altitude areas among them Nyandarua were previously perceived to be free of mosquitoes.

Three genera of mosquitoes, *Culex*, *Anopheles*, and *Aedes*, have previously been identified in semi-arid and arid areas and in Tana River County in Kenya (Sang *et al.*, 2017). The current study only identified two genera of mosquitoes, *Culex* and *Anopheles*, and no *Aedes*. *Aedes* mosquitoes are limited to certain areas with environmental and ecological adaptations, compared to high-altitude areas such as Nyandarua (Arum *et al.*, 2015). High-altitude areas receive a higher amount of rainfall compared to semi-arid areas, and this difference could have led to the failure to collect *Aedes* mosquitoes during the study period.

The current study identified five species of *Culex*, namely, *Cx. pipiens*, *Cx. theileri*, *Cx. vansomereni*, *Cx. rima*, and *Cx. perexiguus*. Although, other studies in Kenya identified *Cx. annulioris*, *Cx. cinerellus*, *Cx. cinereus*, *Cx. poicilipes*, *Cx. rubinotus*, *Cx. tigripes*, *Cx. zombaensis* and *Cx. univittatus* in western Kenya (Chiuya *et al.*, 2021) and *Cx. antennatus*, *Cx. bitaeniorhynchus*, *Cx. ethiopicus*, *Cx. neavei*, *Cx. poicilipes*, *Cx. simpsoni*, *Cx. watti*, *Cx. adersianus* and *Cx. (Neoculex) spp.* from islands and the mainland of Lake Baringo and Lake Victoria (Ajamma *et al.*, 2016).

Previous studies have reported the presence of *Cx. pipiens* mainly in semi-arid and arid areas, and this mosquito was associated with RVF outbreaks (Arum *et al.*, 2015). To our knowledge, this is the first study to identify the presence of *Cx. pipiens* which is a potential vector for RVFV in the high-altitude ecosystem of Nyandarua.

*Cx. theileri* was the second most abundant species identified in Nyandarua compared to a previous study in Lake Victoria, Kenya, which identified only two *Cx. theileri* mosquitoes (Ajamma *et al.*, 2016). *Cx. theileri* is among the potential secondary vectors in RVFV transmission (Drouin *et al.*, 2022). Therefore, the identification of *Cx. theileri* in the high-altitude ecosystem of Nyandarua is of importance in this study and warrants further studies.

Phylogenetic analysis showed that the *Cx. pipiens* identified in Nyandarua belonged to the same clade as others from Turkey. Nevertheless, it was comparatively different from those identified in Hungary, Kenya, and other Turkey strains. The analysis showed that *Cx. pipiens* from Hungary clustered in the same clade. However, the mosquito strains collected from Nyandarua were distinct from the Kenya strains and other strains from Turkey. Genetic variation arises from various factors such as the difference in climate or mutations of *Cx. pipiens*.

The study also indicated that *Cx. theileri* identified from Nyandarua belonged to the same clade as that of Rusinga Island and was in a different clade from that of Greece, Hungary, and Portugal. Therefore, the *Cx. theileri* identified from Nyandarua could be a unique strain from others in different geographical areas that were in

separate clades. This was also observed in *Cx. theileri* identified in Rusinga Island in Kenya in a previous study (Ajamma *et al.*, 2016).

Furthermore, *Cx. perexiguus* identified from Nyandarua shared a clade with the same species identified from Baringo, Kenya. The two *Cx. perexiguus* indicated a close relationship with each other and a recent evolutionary history. Nonetheless, *Cx. perexiguus* identified in Nyandarua was in a different clade from others identified in Malawi, Pakistan, and the USA that clustered in one clade. Previous studies showed that *Cx. perexiguus* from Pakistan was in a clade similar to the one identified in Baringo Kenya (Ajamma *et al.*, 2016). Likewise, *Cx. rima* identified from Nyandarua and Rusinga Island in Kenya shared a clade presenting a close relationship and similar ancestry. Furthermore, *Cx. vansomereni* identified from Nyandarua was found to be in a clade similar to other mosquitoes collected from the island and mainland of Lake Victoria. (Ajamma *et al.*, 2016).

## Conclusion

This study presents evidence of the abundance, diversity, and distribution of possible RVFV mosquito vectors attributable to variation in rainfall. Differences in mosquito diversity and distribution of mosquitoes are correlated with dry, long, and short rainy seasons. This variation in rainfall could have contributed to the observed difference in distribution, abundance, and diversity of potential mosquito vectors for RVFV in Nyandarua County. The data collected will help in the initiation of mitigation measures for the prevention, control, and risk prediction of RVF disease in Nyandarua.

## Recommendation

Further research which should focus on longitudinal mosquito studies to check the entire diversity and distribution of all potential RVFV vectors in Nyandarua. Eventually, this will contribute to mapping mosquito species in Nyandarua and different parts of Kenya.

### *Data availability*

The datasets generated during and/or analysed during the current study are included within the article and are available from the corresponding author upon reasonable request. The mosquito CO1 gene sequences obtained in this study have been deposited in the Genbank database with accession numbers ON406275.1 to ON406278.1, ON422316.1, ON422317.1 and ON428528.1.

### References

- Ajamma, Y. U., Villinger, J., Omondi, D., Salifu, D., Onchuru, T. O., Njoroge, L., & Masiga, D. K. (2016). Composition and genetic diversity of mosquitoes (Diptera: Culicidae) on islands and mainland shores of Kenya's Lakes Victoria and Baringo. *Journal of Medical Entomology*, 53(6), 1348-1363.
- Alten, B., Bellini, R., Caglar, S., Simsek, F., & Kaynas, S. (2000). Species composition and seasonal dynamics of mosquitoes in the Belek region of Turkey. *Journal of Vector Ecology*, 25, 146-154.
- Amraoui, F., Krida, G., Bouattour, A., Rhim, A., Daaboub, J., Harrat, Z., & Failloux, A.-B. (2012). *Culex pipiens*, an experimental efficient vector of West Nile and Rift Valley fever viruses in the Maghreb region. *PloS one*, 7(5), e36757.
- Anyamba, A., Damoah, R., Kemp, A., Small, J. L., Rostal, M. K., Bagge, W., & Paweska, J. T. (2021). Climate Conditions During a Rift Valley Fever Post-epizootic Period in Free State, South Africa, 2014-2019. *Frontiers in veterinary science*, 8, 730424-730424.
- Anyamba, A., Linthicum, K. J., Small, J., Britch, S. C., Pak, E., de La Rocque, S., & Chretien, J.-P. (2010). Prediction, assessment of the Rift Valley fever activity in East and Southern Africa 2006-2008 and possible vector control strategies. *The American journal of tropical medicine and hygiene*, 83(2 Suppl), 43.

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- Arum, S. O., Weldon, C. W., Orindi, B., Landmann, T., Tchouassi, D. P., Affognon, H. D., & Sang, R. (2015). Distribution and diversity of the vectors of Rift Valley fever along the livestock movement routes in the northeastern and coastal regions of Kenya. *Parasites & vectors*, 8(1), 1-9.
- Asigau, S., & Parker, P. G. (2018). The influence of ecological factors on mosquito abundance and occurrence in Galápagos. *Journal of Vector Ecology*, 43(1), 125-137.
- Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., & Kaiser, A. (2010). *Mosquitoes and their control*: Springer Science & Business Media.
- Bhardwaj, N. (2013). Rift valley fever; an emerging viral zoonosis. *Adv. Anim. Vet. Sci*, 1(2), 47-52.
- Bram, R. A. (1967). *Contributions to the mosquito fauna of southeast Asia-II. The genus Culex in Thailand (Diptera: Culicidae)*. Retrieved from
- Breiman, R. (2010). Decision-support tool for prevention and control of Rift Valley fever epizootics in the Greater Horn of Africa. *American Journal of Tropical Medicine and Hygiene*, 83(2, Supplement), 75-85.
- Chiuya, T., Masiga, D. K., Falzon, L. C., Bastos, A. D., Fèvre, E. M., & Villinger, J. (2021). A survey of mosquito-borne and insect-specific viruses in hospitals and livestock markets in western Kenya. *PloS one*, 16(5), e0252369.
- Coetzee, M. (2020). Key to the females of Afrotropical Anopheles mosquitoes

- (Diptera: Culicidae). *Malaria Journal*, 19(1), 1-20.
- Costa, E. A. P. d. A., Santos, E. M. d. M., Correia, J. C., & Albuquerque, C. M. R. d. (2010). Impact of small variations in temperature and humidity on the reproductive activity and survival of *Aedes aegypti* (Diptera, Culicidae). *Revista Brasileira de Entomologia*, 54(3), 488-493.
- Drouin, A., Chevalier, V., Durand, B., & Balenghien, T. (2022). Vector Competence of Mediterranean Mosquitoes for Rift Valley Fever Virus: A Meta-Analysis. *Pathogens*, 11(5), 503.
- Eifan, S., Hanif, A., Nour, I., Alqahtani, S., Eisa, Z. M., Dafalla, O., & Kohl, A. (2021). Distribution and Molecular Identification of *Culex pipiens* and *Culex tritaeniorhynchus* as Potential Vectors of Rift Valley Fever Virus in Jazan, Saudi Arabia. *Pathogens*, 10(10), 1334.
- El-Rahim, I. A., El-Hakim, U. A., & Hussein, M. (1999). An epizootic of Rift Valley fever in Egypt in 1997. *Revue scientifique et technique-Office International des Epizooties*, 18(3), 741-748.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *evolution*, 39(4), 783-791.
- Gerken, K. N., LaBeaud, A. D., Mandi, H., L'Azou Jackson, M., Breugelmans, J. G., & King, C. H. (2022). Paving the way for human vaccination against Rift Valley fever virus: A systematic literature review of RVFV epidemiology from 1999 to 2021. *PLoS neglected tropical diseases*, 16(1), e0009852.
- Gillies, M. T., & Coetzee, M. (1987). A supplement to the Anophelinae of Africa South of the Sahara. *Publ S Afr Inst Med Res*, 55, 1-143.
- Harbach, R. E. (1985). *Pictorial keys to the genera of mosquitoes, subgenera of Culex and the species of Culex (Culex) occurring in southwestern Asia and Egypt, with a note on the subgeneric placement of Culex deserticola (Diptera: Culicidae)*. Retrieved from
- Hernandez-Triana, L. M., Brugman, V. A., Prosser, S. W. J., Weland, C., Nikolova, N., Thorne, L., . . . & Johnson, N. (2017). Molecular approaches for blood meal analysis and species identification of mosquitoes (Insecta: Diptera: Culicidae) in rural locations in southern England, United Kingdom. *Zootaxa*, 4250(1), 067-076.
- Hightower, A., Kinkade, C., Nguku, P. M., Anyangu, A., Mutonga, D., Omolo, J., & Ombok, M. (2012). Relationship of climate, geography, and geology to the incidence of Rift Valley fever in Kenya during the 2006–2007 outbreak. *The American journal of tropical medicine and hygiene*, 86(2), 373-380.
- Huffman, G. J., Bolvin, D. T., Braithwaite, D., Hsu, K., Joyce, R., Xie, P., & Yoo, S.-H. (2015). NASA global precipitation measurement (GPM) integrated multi-satellite retrievals for GPM (IMERG). *Algorithm Theoretical Basis Document (ATBD) Version*, 4(26).
- Kenya National Bureau of Statistic, Population and Housing Census 2019.
- Laurito, M., de Oliveira, T. M., Almirón, W. R., & Sallum, M. A. M. (2013). COI barcode versus morphological identification of *Culex* (*Culex*)(Diptera: Culicidae) species: a case study using samples from Argentina and Brazil. *Memórias do Instituto Oswaldo Cruz*, 108, 110-122.
- Linthicum, K., Davies, F., Kairo, A., & Bailey, C. (1985). Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Epidemiology & Infection*, 95(1), 197-209.
- Linthicum, K. J., Britch, S. C., & Anyamba, A. (2016a). Rift Valley fever: an emerging mosquito-borne disease. *Annu Rev Entomol*, 61(1), 395-415.
- Linthicum, K. J., Britch, S. C., & Anyamba, A. (2016b). Rift Valley fever: an emerging mosquito-borne disease. *Annual review of entomology*, 61, 395-415.
- Lühken, R., Pfitzner, W. P., Börstler, J., Garms, R., Huber, K., Schork, N., & Tannich, E. (2014). Field evaluation of four widely used mosquito traps in Central Europe. *Parasites & vectors*, 7(1), 1-11.
- Lumley, S., Horton, D. L., Hernandez-Triana, L. L., Johnson, N., Fooks, A. R., & Hewson, R. (2017). Rift Valley fever virus: strategies for maintenance, survival and

- vertical transmission in mosquitoes. *Journal of General Virology*, 98(5), 875-887.
- Miller, S., Mishra, V., Ellenburg, W. L., Adams, E., Roberts, J., Limaye, A., & Griffin, R. (2021). Analysis of a Short-Term and a Seasonal Precipitation Forecast over Kenya. *Atmosphere*, 12(11), 1371.
- Mohammed, B., Yayo, A., Ajanusi, O., & Lawal, I. (2021). Relative abundance and molecular identification of *Culex pipiens* complex (Diptera: Culicidae), in Kura Local Government Area, North-western Nigeria. *Parasite epidemiology and control*, 14, e00213.
- Mwongera, C., Nowak, A., Notenbaert, A. M., Grey, S., Osiemo, J., Kinyua, I., & Girvetz, E. (2019). Climate-smart agricultural value chains: Risks and perspectives. In *The climate-smart agriculture papers* (pp. 235-245): Springer, Cham.
- Naeem, M., Alahmed, A., Kheir, S., & Sallam, M. (2016). Spatial distribution modeling of *Stegomyia aegypti* and *Culex tritaeniorhynchus* (Diptera: Culicidae) in Al-bahah Province, Kingdom of Saudi Arabia. *Trop Biomed*, 33(2), 295-310.
- Nanyingi, M. O., Munyua, P., Kiama, S. G., Muchemi, G. M., Thumbi, S. M., Bitek, A. O., & Njenga, M. K. (2015). A systematic review of Rift Valley Fever epidemiology 1931-2014. *Infection ecology & epidemiology*, 5(1), 28024.
- Nguku, P. M., Sharif, S., Mutonga, D., Amwayi, S., Omolo, J., Mohammed, O., & Rao, C. (2010). An investigation of a major outbreak of Rift Valley fever in Kenya: 2006-2007. *The American journal of tropical medicine and hygiene*, 83(2 Suppl), 05.
- Nicholson, S. E. (2017). Climate and climatic variability of rainfall over eastern Africa. *Reviews of Geophysics*, 55(3), 590-635.
- Noureldin, E., Dafalla, O., Hakami, A., Jubran, M., Alzhrani, A., Mujally, M., & Eisa, Z. (2021). *Culex* (Diptera: Culicidae) Mosquitoes in Jazan Region, Saudi Arabia, and Their Molecular Identification. *International Journal of Zoology*, 2021.
- Novella, N. S., & Thiaw, W. M. (2013). African rainfall climatology version 2 for famine early warning systems. *Journal of Applied meteorology and Climatology*, 52(3), 588-606.
- Nyaruaba, R., Mwaliko, C., Mwau, M., Mousa, S., & Wei, H. (2019). Arboviruses in the East African Community partner states: a review of medically important mosquito-borne Arboviruses. *Pathogens and Global Health*, 113(5), 209-228.
- Ochieng, A. O., Nanyingi, M., Kipruto, E., Ondiba, I. M., Amimo, F. A., Oludhe, C., & Estambale, B. B. (2016). Ecological niche modelling of Rift Valley fever virus vectors in Baringo, Kenya. *Infection ecology & epidemiology*, 6(1), 32322.
- Ondiba, I. M., Oyieke, F. A., Athinya, D. K., Nyamongo, I. K., & Estambale, B. (2019). Larval species diversity, seasonal occurrence and larval habitat preference of mosquitoes transmitting Rift Valley fever and malaria in Baringo County, Kenya. *Parasites & vectors*, 12(1), 1-14.
- Pachka, H., Annelise, T., Alan, K., Power, T., Patrick, K., Véronique, C., & Ferran, J. (2016). Rift Valley fever vector diversity and impact of meteorological and environmental factors on *Culex pipiens* dynamics in the Okavango Delta, Botswana. *Parasites & vectors*, 9(1), 1-17.
- Paksa, A., Sedaghat, M. M., Vatandoost, H., Yaghoobi-Ershadi, M. R., Moosa-Kazemi, S. H., Hazratian, T., . . . & Oshaghi, M. A. (2019). Biodiversity of mosquitoes (Diptera: Culicidae) with emphasis on potential arbovirus vectors in East Azerbaijan province, northwestern Iran. *Journal of Arthropod-Borne Diseases*, 13(1), 62.
- Pepin, M., Bouloy, M., Bird, B. H., Kemp, A., & Paweska, J. (2010). Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Veterinary research*, 41(6), 61.
- Rueda, L. M. (2004). *Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission*. Retrieved from
- Rybanov, A. A., & Aleksandrovna, M. L. (2020). Data types diversity quantitative indicators in relational databases physical models. *TEM Journal*:

- Technology, Education, Management, Informatics*, 9(3), 862.
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, 4(4), 406-425.
- Sang, R., Lutomiah, J., Said, M., Makio, A., Koka, H., Koskei, E., & Bukachi, S. (2017). Effects of irrigation and rainfall on the population dynamics of Rift Valley fever and other arbovirus mosquito vectors in the epidemic-prone Tana River County, Kenya. *Journal of Medical Entomology*, 54(2), 460-470.
- Seck, I., Lo, M. M., Fall, A. G., Diop, M., Ciss, M., Cêtre-Sossah, C. B., & Coste, C. (2022). Identification of drivers of Rift Valley fever after the 2013–14 outbreak in Senegal using serological data in small ruminants. *PLoS neglected tropical diseases*, 16(2), e0010024.
- Shukla, S., Macharia, D., Husak, G. J., Landsfeld, M., Nakalembe, C. L., Blakeley, S. L., & Way-Henthorne, J. (2021). Enhancing access and usage of earth observations in environmental decision-making in Eastern and Southern Africa through capacity building. *Front. Sustain. Food Syst.* 5: 504063. doi: 10.3389/fsufs.
- Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, 101(30), 11030-11035.
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, 38(7), 3022-3027.
- Team, R. C., & Team, R. C. (2021). R: A Language and Environment for Statistical Computing v4.1.1 (Version 4.1.1).
- Wanjama, J., Aboge, G., Muneri, C., & Nanyingi, M. (2022). Sero-Epidemiological Survey of Rift Valley Fever Virus in Ruminants in Nyandarua County, Kenya. *East African Agricultural and Forestry Journal*, 86(1-2), 11-11.
- Watters, D., & Battaglia, A. (2019). The summertime diurnal cycle of precipitation derived from IMERG. *Remote Sensing*, 11(15), 1781.