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Phlebovirus diversity in ticks from livestock in arid ecologies in Kenya

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ABSTRACT

Phleboviruses are emerging pathogens of public health importance. However, their association with ticks is poorly described, particularly in Africa. Here, adult ticks infesting cattle, goats and sheep were collected in two dryland pastoralist ecosystems of Kenya (Baringo and Kajiado counties) and were screened for infection with phleboviruses. Ticks mainly belonged to the species *Rhipicephalus appendiculatus, Hyalomma impeltatum*, and *Hyalomma rufipes*. A fragment of the RNA-dependent RNA polymerase (RdRp) gene was identified in thirty of 671 tick pools, of which twenty-nine were from livestock sampled in Baringo county. Phylogenetic analyses revealed that twenty-five sequences were falling in three clades within the group of tick-associated phleboviruses. The sequences of the three clades showed nucleotide distances 8%, 19% and 22%, respectively, to previously known viruses suggesting that these sequence fragments may belong to three distinct viruses. Viruses of the group of tick-associated phleboviruses have been found in several countries and continents but so far have not been associated with disease in humans or animals. In addition, five sequences were found to group with the sandfly-associated phleboviruses Bogoria virus, Perkerra virus and Ntepes virus recently detected in the same region. Further studies are needed to investigate the transmission and maintenance cycles of these viruses, as well as to assess their potential to infect vertebrates.

1. Introduction

The genus *Phlebovirus* (family *Phenuiviridae*) comprises some of the most pathogenic viruses to humans, exemplified by Toscana virus (TOSV) and Rift Valley fever virus (RVFV) transmitted by sandflies and mosquitoes, respectively (Ayhan and Charrel, 2020; Sang et al., 2010). Several studies describe an increase in the incidence and geographic range of newly emerging tick-borne phleboviruses (TBPV) (Lefkowitz et al., 2018; Matsuno et al., 2018). This includes reports of Mukawa virus in Asia and Lihan tick virus in the Americas (Lopez et al., 2020; Matsuno et al., 2018; Torii et al., 2019). Short sequence fragments of TBPV RNA-dependent RNA polymerase (RdRp) gene are regularly detected in ticks from Europe. For example, Lesvos, AnLuc and Glabbeek/Osterholz viruses have been described from Greece, Portugal, and Belgium/Germany, respectively (Papa et al., 2017; Pereira et al., 2017; Prinz et al., 2017). The pathogenicity of these TBPV remains to be determined (Ohlendorf et al., 2019). It might be that these viruses are arthropod-specific viruses and non-pathogenic for vertebrates, including humans. It has also been suggested that TBPV sequences may have integrated into tick genomes as described for other viruses and arthropods, e.g. insect-specific flaviviruses and mosquitoes (Ballinger et al., 2014; Lequime et al., 2017; Crava et al., 2021; Spadar et al., 2021).

In Africa, infections of phleboviruses in ticks such as Odaw virus (ODWV) and Shibuyunji virus have been documented in Ghana and Zambia, respectively (Kobayashi et al., 2017; Simulundu et al., 2021). Odaw virus showed a close relationship to Antigone virus (ANTV)

Abbreviations: RdRp, RNA-dependent RNA polymerase; TOSV, Toscana virus; RVFV, Rift Valley fever virus; TBPV, tick-borne phleboviruses; BOGV, Bogoria virus; PERV, Perkerra virus; NTPV, Ntepes virus; ODWV, Odaw virus; ANTV, Antigone virus; IFTV, Iftin tick virus; MATV, Mbalambala tick virus; ADTPV, American dog tick phlebovirus; KBGV, Kiborgoch virus; cDNA, Complementary DNA; RT, Reverse transcription; CCHFV, Crimean-Congo hemorrhagic fever virus; BDTPV, Brown dog tick phlebovirus; ML, maximum likelihood; HTS, high-throughput sequencing.

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identified in *Rhipicephalus sanguineus* from Greece (Papa et al., 2016), while Shibuyunji virus is a close relative of American dog tick phlebovirus (ADTPV) (Simulundu et al., 2021). In Kenya, two novel phleboviruses including Iftin tick virus (IFTV) and Mbalambala tick virus (MATV) were recently described in ticks infesting camels (Zhang et al., 2021). Iftin tick virus was identified in *Hyalomma dromedarii* ticks while MATV was identified in *H. truncatum* and *H. rufipes* (Zhang et al., 2021). MATV antibodies have not been detected in humans but in 3% of the tested camels (Zhang et al., 2021). To date, the maintenance mechanisms of these TBPV including transmission between vertebrate hosts and tick vectors are poorly understood in the region. Further, evidence for human infection remains scarce.

Increasing reports of novel *Phlebovirus* detection in sub-Saharan Africa is thought to be driven by expansion of endemic infections, advancements in diagnostic approaches, and changing tick bionomics and ecology associated with climate change (Dinçer et al., 2017; Zhang et al., 2021). Certain environmental conditions could favor survival of specific tick species and consequently define the risk area for different tick-borne diseases (Parola and Raoult, 2001). Located in the Great Rift Valley, Baringo and Kajiado counties have a semi-arid ecology with harsh climatic and physical conditions resulting in sparse human populations. These areas are dominated by local, nomadic pastoralist communities whose economic mainstay is livestock keeping and as such these areas provide a window into the future drier and hotter climates, predicted under climate change. The periodic movement of livestock in search of pasture and water favours human-livestock-wildlife interaction and facilitates both the transmission and spread of zoonotic pathogens such as TBPV, and expansion of pathogen range. As is the case for other tick-borne pathogens, TBPV may be transmitted more efficiently by specific tick species highlighting the importance of improved knowledge of tick species distribution for evaluating the risk of exposure to these agents (Kazimírová et al., 2017; Labuda and Nuttall, 2004).

In Kenya, other than the circulation of IFTV and MATV, there is limited information on TBPV prevalence and epidemiology (Zhang et al., 2021). Following our recent description of novel sandfly-borne phleboviruses in Baringo county with potential for causing disease in humans (Marklewitz et al., 2020; Tchouassi et al., 2019), we aimed to explore the genetic diversity of phleboviruses in ticks infesting livestock

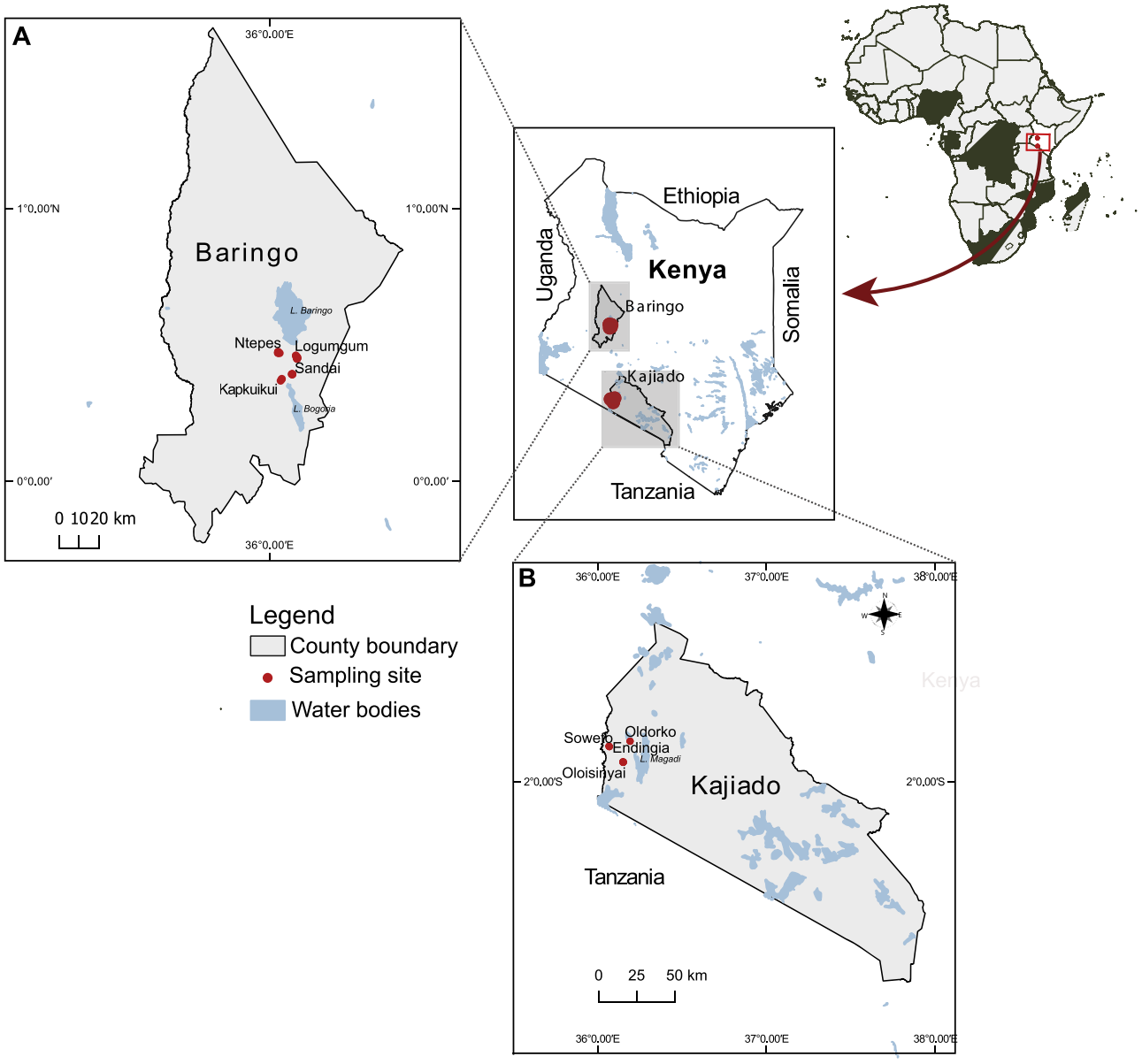

Fig. 1. Map showing tick collection sites in Kenya (red dots) within the Great Rift Valley in Baringo and Kajiado counties. Sampling sites in **A**, Baringo and **B**, Kajiado. The map was generated in the open source GIS software, QGIS 2.12 (QGIS Development Team, 2019).

(cattle, goats and sheep) in Kenya.

2. Material and methods

2.1. Study location

Ticks were collected in two counties; Baringo and Kajiado within the Great Rift Valley in Kenya (Fig. 1). Both counties have similar semi-arid ecologies consisting of heterogeneous savannah vegetation dominated by short Acacia bushes, tall grass and discontinuous woodland. The topography ranges in elevation from 500 to 3,000 m above sea level. There are seasonal river valleys with low-lying elevations and isolated hilly habitats. Annual rainfall of 200 to 700 mm with temperatures ranging from 16 to 42 °C characterize the climate. The area is sparsely populated by four local agro-pastoralist communities (Tugen, Njemps and Pokot in Baringo, and Maasai in Kajiado) living in rural rangelands where they practise nomadic pastoralism by keeping cattle, sheep, and goats. The area is also home to the unfenced Ruko (Baringo) and Olkirimatian (Kajiado) wildlife conservancies and there is frequent human-livestock-wildlife interaction as herders move within the region in search of pasture and water. The local communities also rely on limited production of crops such as maize, tomatoes and onions along the Perkerra (Ntepes) and Kajiado (Soweto) irrigation schemes. Unlike Baringo county which has a history of arboviral infections (Ajamma et al., 2018; Marklewitz et al., 2020; Tchouassi et al., 2019; Tigoi et al., 2015), there is little information on the status of Kajiado county due to limited arbovirus surveillance (Ogola et al., 2022; Nguku et al., 2007).

2.2. Ethical approval

Tick sampling was approved (SERU protocol number 3312) by the Kenya Medical Research Institute Scientific Ethics Review Unit (KEMRI-SERU). Following ethical clearance guidelines, informed oral consent was obtained from local authorities and owners and/or caretakers of domestic animals during each sampling exercise.

2.3. Ticks collection and identification

Targeting adult ticks infesting livestock, active life stages of ticks were sampled from animal hosts including cattle (*Bos taurus*), goats (*Capra hircus*) and sheep (*Ovis aries*). The sampling was undertaken at communal and/or private owned farms and animal shelters across four sites in Baringo, namely Ntepes, Sandai, Logumgum, Kapkuikui, and at three sites in Kajiado including Oloisinyai, Oldorko and Endingia (Soweto). As one of the novel sandfly-borne phleboviruses (Kiborgoch virus, KBGV) previously reported in Baringo county was detected in a sandfly that had fed on cattle, the focus of the current study was on livestock species in Baringo county (Marklewitz et al., 2020; Tchouassi et al., 2019). The sampling sites in Kajiado were informed by the area having similar semi-arid ecology and pastoralist practices as those practised in Baringo county but limited arbovirus surveillance.

Livestock estimates from 2019 indicate that Baringo county (Marigat constituency) had about 228,000 livestock comprising 138,000 goats, 52,000 sheep and 38,000 cattle and Kajiado county (Kajiado North constituency) had about 54,000 livestock comprising of 22,000 sheep, 21,000 goats and 11,000 cattle (https://knoema.com/KELP2020/livestock-population-by-type-and-district-kenya-2019). All three livestock species were sampled by restraining individual animals to allow manual removal of attached live ticks from the host body. Collected ticks from each animal type were placed in individually labelled sterile 15-mL centrifuge tubes, flash frozen in liquid nitrogen and transportated to the laboratory where they were preserved for further processing at −80 °C. To remove contaminants from the environment and host, ticks were washed with distilled water and surface sterilized with 70% ethanol before sorting and morphological identification using established morphologic keys (Matthysse and Colbo, 1987; Okello-Onen et al., 1999; Walker et al., 2003, 2000). Identification involved examination of ticks in petri-dishes on a pre-chilled ice pack under a dissecting microscope (Stemi 2000-C microscope, Zeiss). The identified ticks were pooled in groups of up to eight according to tick species and sex, host type, collection date and sampling site. Highly engorged ticks were not included in the study to reduce vertebrate host material during RNA extraction and cell culture inoculation.

2.4. Detection and identification of phleboviruses

Tick pools were homogenized for 45 s in 1.5-mL microcentrifuge tubes containing lysis matrix of zirconia beads (2.0 mm and 0.1 mm diameter) and one millilitrer of DPBS (Dulbeccos phosphate-buffered saline, pH 7.4) using a Mini-Beadbeater-16 (Biospec, Bartlesville, OK, USA). The homogenate was centrifuged for 10 mins in a bench top centrifuge (Eppendorf, USA) at 2500 rpm at 4°C. Viral RNA was extracted from an aliquot of 140 µl of the supernatant using the Viral RNA Mini kit (Qiagen, Hilden, Germany) and eluted in two steps with sixty microlitre of elution buffer. Complementary DNA (cDNA) was synthesized using the High Capacity cDNA Reverse Transcription (RT) kit (Life Technologies, CA, USA) and 600 µM non-ribosomal random hexanucleotide primers, as stipulated by the manufacturer (Endoh et al., 2005).

A previously reported three generic nested-PCR targeting 233, 253, or 501 bp of the RNA-dependent RNA polymerase (RdRp) gene was utilized to screen for the presence of a wide range of phleboviruses in cDNA templates of tick pool homogenates (Ohlendorf et al., 2019) using Mytaq DNA polymerase (Bioline, London, UK). We included Gouléako virus as a positive control and a template-free reaction as a negative control (Marklewitz et al., 2011). Thermal cycling conditions were conducted as described previously (Marklewitz et al., 2011; Ohlendorf et al., 2019). Positive PCR products of expected sizes were purified using ExoSAP-IT (USB Corporation, Cleveland, OH, USA) and submitted for bidirectional Sanger sequencing (Macrogen, Amsterdam, Netherlands).

2.5. Virus isolation

Positive tick homogenates were inoculated onto semi-confluent monolayers of 3×10^4 Vero E6 (*Ceropithecus aethiops*), 1×10^5 C6/36 (*Aedes albopictus*), 1×10^5 PP-9 (*Phlebotomus papatasi*) and 8×10^4 LL-5 (*Lutzomyia longipalpis*) cells as previously described (Junglen et al., 2009). The cells were monitored regularly for cytopathic effect (CPE) for up to seven days post-inoculation. Three blind passages on fresh cells were carried out, with transfers to fresh cells occurring every seven days. After the third blind passage (one month after inoculation), 75 µL of cell culture supernatant was aliquoted for viral RNA isolation in the MagNa Pure 96 extraction system (Roche Diagnostics, Mannheim, Germany). cDNA was synthesized using Superscript IV reverse transcriptase (Thermo Fisher Scientific GmbH, Dreieich, Germany) and random hexamer primers (Integrated DNA Technologies Germany GmbH, Munich, Germany) as directed by the manufacturer and screened for virus presence as described earlier in Section 2.4.

2.6. Multiple-sequence alignment and phylogenetic analysis

The nucleotide (nt) sequences were analysed in Biomatters's Geneious Prime (http://www.geneious.com) (Kearse et al., 2012) and queried against the GenBank nt database using the nucleotide Blast (blastN) function (Altschul et al., 1990). Nucleotide sequences were aligned with sequences of selected viruses of the same viral family using MAFFT E-INS-I plugin in Geneious prime software (Kearse et al., 2012). The maximum likelihood (ML) phylogenetic trees were infered using PhyML v. 2.2.4 and nodal support was evaluated through 1000 bootstrap replications (Guindon et al., 2010).

2.7. Sequence accession numbers

The partial RdRp gene sequences were deposited in GenBank under the accession numbers ON310555-ON310558 (unassigned tick phlebovirus strains), ON310535-ON31054 (unassigned tick phlebovirus strains), ON256357-ON256358 (Bogoria virus) and ON256359 (Ntepes virus).

2.8. Statistical analysis

We created a database summarizing field entomological data including GPS co-ordinates on a series of Excel spreadsheets and relative abundance of tick species infesting different hosts for subsequent analyses in R version 3.6.3 (R Development Team, 2016). The summary data were imported and the estimated individual-level prevalence of detected viruses in each tick pool was calculated online at https://epitools.ausvet.com.au/ppvariablepoolsize (Sergeant, 2018). The epidemiological calculator (EPITOOL) employs maximum likelihood using frequentist model (Chiuya et al., 2020; Christopher and Christine, 2001; Cowling et al., 1999).

3. Results

3.1. Relative abundance of tick species infesting livestock

Between August 2019 and July 2020 during the dry season, 4503 adult ticks representing 11 species infesting cattle, sheep and goats were collected (Table 1). Fifty-six *Hyalomma* ticks could not be identified to species due to distorted ventral plates and genital pores. A slightly greater number of ticks were collected from Kajiado (52.8%, 2379/4503) than Baringo county (47.2%, 2124/4503). The sample set comprised more males (55.9%, 2518/4503) than females (44.1%, 1985/4503). A striking difference in tick species abundance was observed between both sampling sites with *Rhipicephalus appendiculatus* almost exclusively infecting livestock in Baringo compared to various ticks collected from livestock in Kajiado county (Fig. 2). Greater species richness was observed in ticks infesting cattle (10 species) followed by sheep (9 species) and goats (7 species) (Table 1; Fig. 2). The predominant tick species found were *Rh. appendiculatus* (52.7%, $n = 2374$), collected mostly from goats (26.4%; $n = 1190$), followed by *Hyalomma impeltatum* (21.8%, $n = 984$) and *Hy. rufipes* (17.2%, $n = 773$) which were mainly found to be feeding on sheep (9.7%, $n = 435$) and goats (8.3%, $n = 373$), respectively (Table 1). Details of the tick sample composition are provided in Table 1 and Fig. 2. The tick species *Rh. appendiculatus, Rh. evertsi,* and *Hy. rufipes* were commonly identified across the three livestock hosts while *Amblyomma gemma* was only encountered on cattle and goats. Further, *Am. lepidum* and *Rh. pulchellus* were found only on cattle and *Am. variegatum* and *Hy. marginatum* on sheep. Based on sampling sites, occurrence of the most abundant tick species, *Rh. appendiculatus* was common in Ntepes and Logumgum (Baringo county) whereas higher numbers of *Hy. impeltatum* (16.8%, $n = 755$) and *Hy. rufipes* (8.7%, $n = 390$) were registered in Oldorko (Kajiado county). While tick species richness in Kajiado county (9 species) and in Baringo county (7 species) was similar, the relative

Table 1
Relative abundance of ticks sampled from different livestock hosts in Baringo and Kajiado counties, Kenya.

Tick species	County	Sampling site	Goats (%)	Sheep (%)	Cattle (%)	n (%)
Rh. appendiculatus (52.72%, $n = 2374$)	Baringo	Logumgum	276 (6.13)	240 (5.33)	0	516 (11.46)
		Ntepes	670 (14.88)	423 (9.39)	0	1093 (24.27)
		Sandai	220 (4.89)	52 (1.15)	154 (3.42)	426 (9.46)
	Kajiado	Endingia	4 (0.09)	38 (0.84)	39 (0.87)	81 (1.80)
		Oloisinyai	20 (0.44)	226 (5.02)	12 (0.27)	258 (5.73)
Hy. impeltatum (21.85%, $n = 984$)	Kajiado	Endingia	0	26 (0.58)	58 (1.29)	84 (1.87)
		Oldorko	315 (7.00)	393 (8.73)	47 (1.04)	755 (16.77)
		Oloisinyai	66 (1.47)	16 (0.36)	63 (1.40)	145 (3.22)
Hy. rufipes (17.17%, $n = 773$)	Kajiado	Endingia	1 (0.02)	17 (0.38)	160 (3.55)	178 (3.95)
		Oldorko	356 (7.91)	14 (0.31)	20 (0.44)	390 (8.66)
		Oloisinyai	16 (0.36)	0	189 (4.20)	205 (4.55)
Rh. evertsi (2.29%, $n = 103$)	Baringo	Logumgum	2 (0.04)	8 (0.18)	0	10 (0.22)
		Sandai	0	3 (0.07)	3 (0.07)	6 (0.13)
	Kajiado	Endingia	6 (0.13)	18 (0.40)	4 (0.09)	28 (0.62)
		Oldorko	1 (0.02)	4 (0.09)	4 (0.09)	9 (0.20)
		Oloisinyai	13 (0.29)	32 (0.71)	5 (0.11)	50 (1.11)
Am. gemma (1.55%, $n = 70$)	Baringo	Logumgum	1 (0.02)	0	0	1 (0.02)
		Sandai	0	0	19 (0.42)	19 (0.42)
	Kajiado	Endingia	0	3 (0.07)	15 (0.33)	18 (0.40)
		Oldorko	16 (0.36)	3 (0.07)	4 (0.09)	23 (0.51)
		Oloisinyai	5 (0.11)	3 (0.07)	1 (0.02)	9 (0.20)
Hy. albiparmatum (1.35%, $n = 61$)	Kajiado	Endingia	16 (0.36)	5 (0.11)	22 (0.49)	43 (0.95)
		Oloisinyai	11 (0.24)	6 (0.13)	1 (0.02)	18 (0.40)
Hy. truncatum (1.38%, $n = 62$)	Baringo	Logumgum	7 (0.16)	3 (0.07)	0	10 (0.22)
		Ntepes	4 (0.09)	0	0	4 (0.09)
		Sandai	5 (0.11)	4 (0.09)	15 (0.33)	24 (0.53)
	Kajiado	Endingia	6 (0.13)	5 (0.11)	2 (0.04)	13 (0.29)
		Oldorko	1 (0.02)	0	0	1 (0.02)
		Oloisinyai	3 (0.07)	7 (0.16)	0	10 (0.22)
Rh. pulchellus (0.07%, $n = 3$)	Baringo	Sandai	0	0	2 (0.04)	2 (0.04)
	Kajiado	Endingia	0	0	1 (0.02)	1 (0.02)
Am. variegatum (0.24%, $n = 11$)	Baringo	Ntepes	0	11 (0.24)	0	11 (0.24)
Am. lepidum (0.09%, $n = 4$)	Kajiado	Endingia	0	0	1 (0.02)	1 (0.02)
		Oloisinyai	0	0	3 (0.07)	3 (0.07)
Hy. marginatum (0.04%, $n = 2$)	Baringo	Ntepes	0	1 (0.02)	0	1 (0.02)
		Sandai	0	1 (0.02)	0	1 (0.02)
Hy. spp (1.24%, $n = 56$)	Kajiado	Endingia	0	8 (0.18)	15 (0.33)	23 (0.51)
		Oloisinyai	29 (0.64)	4 (0.09)	0	33 (0.73)
n (%)			2070 (45.97)	1574 (34.95)	859 (19.08)	

n: number of ticks.

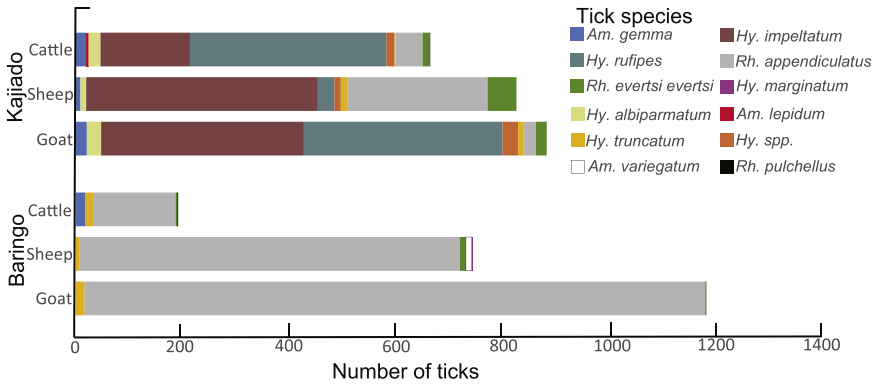

Fig. 2. Species composition of ticks found to be infesting cattle, goats and sheep from Baringo and Kajiado counties sampled between August 2019 and July 2020.

abundance of certain species showed strong difference between the counties, e.g. *Rh. appendiculatus* (Fig. 2). Tick species richness from goats was higher in Kajiado (8 species) than Baringo (4 species) (Fig. 2). A similar pattern was evident for ticks sampled on cattle and sheep in both counties.

3.2. Identification of phlebovirus sequence fragments in ticks

Thirty of the 671 pools were tested positive for phleboviruses with an estimated individual-level prevalence of 0.7% (95% CI 0.5–1.0). The positive pools included *Rh. appendiculatus* (0.7%, 17/311), *Hy. truncatum* (18.0%, 9/23), *Rh. evertsi* (2.0%, 2/35), *Rh. pulchellus* (33.3%, 1/3) and *Hy. marginatum* (50.0%, 1/2) (Table 2). PCR-positive tick pools originated from all three vertebrate hosts with ticks from sheep having the highest estimated individual-level prevalence (1.6%, $n = 12$) compared to those from cattle (1.0%, $n = 8$) and goats (0.5%, $n = 10$) (Table 2).

Upon visual inspection of sequence chromatograms, double sequencing signals were observed in three samples including MT74 and MT111 collected in Sandai, and MT29 collected in Logumgum suggesting presence of more than one phlebovirus in the pooled samples. Phylogenetic analysis of the obtained sequences excluding samples with mixed infections showed that majority of sequences established three new clades (clades I – III) within the group of tick-associated phleboviruses (Fig. 3A). Clade I consisted of four strains identified in *Rh. appendiculatus, Hy. truncatum* and *Rh. evertsi* and formed a sister-clade to Brown dog tick phlebovirus 2. Clade II consisted of nine strains identified in *Rh. appendiculatus* and *Rh. evertsi* and grouped as sister clade to the larger clade containing Odaw virus, clade I and other tick-associated phleboviruses. Clade III contained nine strains and grouped as sister clade to Bole and Iftin tick viruses. Surprisingly, we also detected variants of the recently identified sandfly-borne phleboviruses Bogoria virus in *Rh. appendiculatus* and *Hy. truncatum*, Perkerra virus and Ntepes virus in *Rh. appendiculatus* and *Hy. truncatum*, respectively (Fig. 3B). Trees of tick-associated and sandfly-associated sequences were based on two separate alignments due to short sequence length and overlapping regions.

Closely related sequences were detected in different tick species. For instance, sequences retrieved from pools of *Rh. appendiculatus* ($n = 2$), *Hy. truncatum* ($n = 4$) and *Rh. pulchellus* ($n = 1$) had 78–81% nucleotides (nt) similarity to Bole tick virus 1 (Bole lineage) recovered from *Hy. asiaticum* in China (Li et al., 2015) (Table 3). Similarly, sequences from pools of *Rh. appendiculatus* (2), *Hy. truncatum* (1) and *Rh. evertsi* (1) displayed 92% nt similarity to Brown dog tick phlebovirus 2 (BDTPV 2) sequences identified from *Rh. sanguineus* and *R. microplus* in Trinidad and Tobago (Sameroff et al., 2019) (Table 3). Further, sequences obtained from two pools of *Hy. truncatum* and three pools of *Rh. appendiculatus* had 98–99% nt identity to the sandfly-borne phleboviruses Bogoria, Perkera and Ntepes viruses (Marklewitz et al., 2020; Tchouassi et al., 2019) (Table 3).

Except a single sequence, all sequences were obtained from ticks collected at three sampling sites namely Logumgum and Sandai in Baringo county (Table 3). Sequences of clade I were only found among ticks collected in Logumgum and sequences of clade II were only detected in ticks from Sandai. Phleboviruses were identified in both male and female ticks. Attempts to isolate the detected viruses in Vero E6, C6/36, PP-9 and LL-5 cells for further characterization were not successful. Further attempts to generate more sequence information were also not successful.

Table 2
Livestock host species distribution of phleboviruses-positive tick species.

Tick species (% of the total ticks sampled)	Estimated individual-level prevalence (number of positive tick pools/total number of pools tested) Overall	Goats (n)	Sheep (n)	Cattle (n)
Rh. appendiculatus (52.72)	0.73 (17/311)	0.43 (5/152)	0.84 (8/127)	2.10 (4/32)
Hy. truncatum (1.38)	17.96 (9/23)	17.71 (4/11)	11.15 (2/8)	36.67 (3/4)
Rh. evertsi (2.29)	1.96 (2/35)	4.65 (1/8)	1.55 (1/17)	0 (0/10)
Hy. marginatum (0.04)	50.00 (1/2)	0 (0/0)	50.00 (1/2)	0 (0/0)
Rh. pulchellus (0.07)	33.33 (1/3)	0 (0/0)	0 (0/0)	33.33 (1/3)
Total	**0.68 (30/374)**	**0.49 (10/171)**	**1.61 (12/154)**	**0.95 (8/49)**

n: proportion of positive tick pools.

4. Discussion

In this study we have identified diverse *Phlebovirus* sequence fragments in ticks infesting sheep, goats and cattle in Kenya, a country where tick-borne pathogens are scarcely characterized and tick screening has historically focused largely on bacterial and protozoan pathogens, and Crimean-Congo hemorrhagic fever virus (CCHFV) (Chiuya et al., 2020; Getange et al., 2021; Oundo et al., 2020). Of the 671 tick pools screened for presence of phleboviruses, 30 tested positive corresponding to an estimated individual-level prevalence of 0.68% (95% CI 0.46–0.95). The positive tick pools originated from three of the seven sampling sites, namely Logumgum and Sandai in Baringo county, and Oloisinyai in Kajiado county. Limited reports from sub-Saharan Africa have confirmed the presence of phleboviruses in *Rhipicephalus* sp., *Amblyomma* sp., *Hy. dromedarii, Hy. truncatum* and *Hy. rufipes* tick species in Ghana, Kenya and Zambia (Amoa-Bosompem et al., 2021; Kobayashi et al., 2017; Simulundu et al., 2021; Zhang et al., 2021). We found high infection rate in tick species that were rarely detected, e.g. *Hy. marginatum* (prevalence 50.00%; abundance 0.04%), *Rh. pulchellus* (33.33%; abundance 0.07%), and *Hy. truncatum* (17.96%; abundance

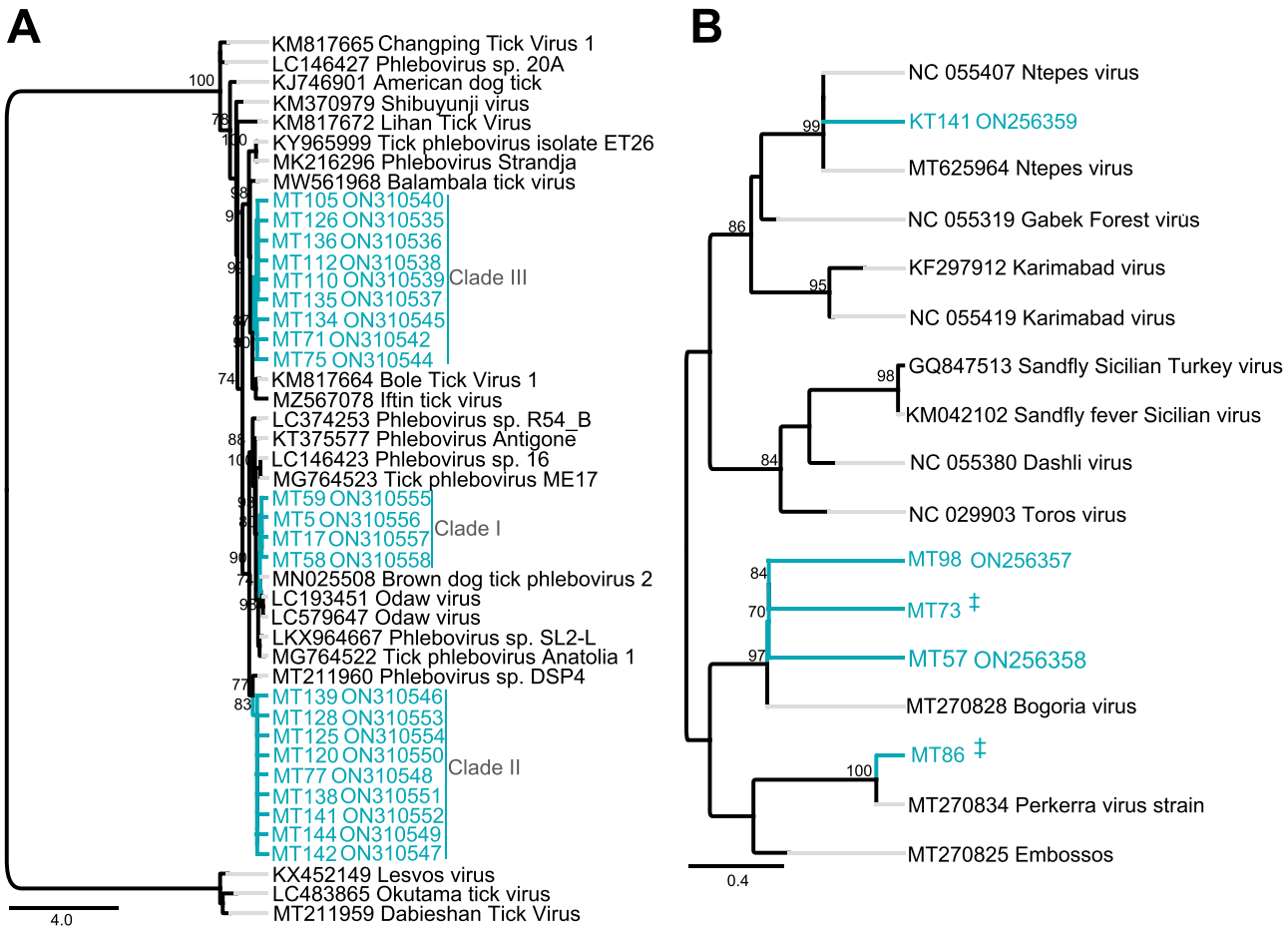

Fig. 3. Phylogenetic relationship of identified phleboviruses and related viruses. The maximum likelihood (ML) phylogenetic trees were based on RNA-dependent RNA polymerase (RdRp) gene sequence alignments of **(A)** 404 nucleotides of tick-associated phleboviruses and **(B)** 144 nucleotides of sandfly-associated phleboviruses. In total, 1000 bootstrap replicates were performed and support values ≥ 60 are indicated next to the relevant nodes. Sequences identified in this study are highlighted in turquoise. ‡: sequences not deposited to GeneBank due to short length.

1.38%). Consistent with previous studies in Kenya, this study identified eight livestock-associated tick species, namely *Rh. appendiculatus, Rh. evertsi, Rh. pulchellus, Hy. marginatum, Hy. rufipes, Hy. truncatum, Am. gemma* and *Am. variegatum* (Chiuya et al., 2020; Getange et al., 2021; Omondi et al., 2017; Oundo et al., 2020; Zhang et al., 2021). We also identified three additional tick species including *Am. lepidum, Hy. impeltatum,* and *Hy. albiparmatum* rarely associated with livestock in the region. Overall, the three dominant tick species in our sampling sites were *Rh. appendiculatus, Hy. impeltatum* and *Hy. rufipes*. The varying tick composition reported in the country can be attributed to differences in ecology of sampling points, targeted ticks (we sampled feeding rather than questing ticks), and sampling season (Esser et al., 2019). For example, the tick species identified in our study, such as *Rh. pulchellus* and *Am. variegatum*, are known to be common in semi-arid ecologies. Further, there was a higher abundance of tick species identified in Kajiado (abundance 52.8%; tick species richness 9 species) in comparison with Baringo county (abundance 47.2%; tick species richness 7 species). Also, a clear dominance of a single tick species (*Rh. appendiculatus*) was observed in Baringo county, in sheep and goats. According to livestock estimates from 2019, there appears to be more goats and sheep in Baringo county. The disparity in tick species composition and abundance could also be explained by different livestock husbandry practices including tick control interventions and grazing applied in the two counties and requires further investigation. A vector species based TBPV study could offer additional insights into the varying tick species composition and TBPV epidemiology in Kenya.

Closely-related sequences were detected in different tick species, suggesting that the respective phleboviruses might be able to infect diverse tick species. Also, we detected sequences of clade III in *Hy. marginatum* and *Rh. pulchellus* despite the low abundance of these two tick species. So far, only about 10% of existing tick species have been implicated in arbovirus transmission and species such as *Am. variegatum* has been associated with transmission of fewer arboviruses than others such as *Ixodes uriae* (Kazimírová et al., 2017; Labuda and Nuttall, 2004; Nuttall and Labuda, 2004). Most of the phleboviruses detected were from Baringo county, and primarily in *Rh. appendiculatus* from Sandai (Table 3). While the reason for the observed location-specific detections is unclear, previous reports show that microclimate and anthropogenic factors such as change in land use may contribute to host-mediated tick species dispersal (Medlock et al., 2013; Ogden et al., 2013). Further, studies on tick-borne pathogens with narrow host ranges demonstrate that spread of tick-borne pathogens depends on that of the reservoir host (Kurtenbach et al., 2002; Ogden et al., 2013). Wildlife such as small wild mammals that are locally distributed could serve as reservoirs or be involved in the virus transmission network. In the present study, the putative reservoir host of the detected phleboviruses is not known.

Analysis for infection with phleboviruses revealed presence of RdRp sequence fragments of the three sandfly-borne phleboviruses Bogoria, Perkerra and Ntepes viruses, as well as of three putative novel phleboviruses grouping with tick-associated phleboviruses. Bole tick virus 1 and *Phlebovirus* DSP4 virus were identified from *Hy. asiaticum* and tick pools in China, respectively, while BDTPV 2 was identified from *Rh. sanguineus* and *R. microplus* in Trinidad and Tobago (Li et al., 2015; Sameroff et al., 2019). BOGV and the tick *phlebovirus* closely related to

Table 3
Summary of the phlebovirus-positive ticks/tick pools sampled from livestock in Baringo and Kajiado counties, Kenya.

Code	County	Sampling site	Species	Pool size	Host	Closely related Virus	Virus ID	% ID (nt)	GenBank accession No
MT5	Baringo	Logumgum	*Rh. appendiculatus*	8♂	Sheep	BDTPV 2 MN025508	Clade I	91.9	ON310556
MT17		Logumgum	*Rh. appendiculatus*	8♂	Sheep	BDTPV 2 MN025508	Clade I	91.9	ON310557
MT58		Logumgum	*Hy. truncatum*	4♂	Goat	BDTPV 2 MN025508	Clade I	91.9	ON310558
MT59		Logumgum	*Rh. evertsi*	2♂	Goat	BDTPV 2 MN025508	Clade I	91.9	ON310555
MT77		Sandai	*Rh. appendiculatus*	8♀	Goat	*Phlebovirus* DSP4 MT211960	Clade II	78.3	ON310548
MT120		Sandai	*Rh. appendiculatus*	8♂	Cattle	*Phlebovirus* DSP4 MT211960	Clade II	78.1	ON310550
MT125		Sandai	*Rh. appendiculatus*	8♀	Cattle	*Phlebovirus* DSP4 MT211960	Clade II	78.7	ON310554
MT138		Sandai	*Rh. appendiculatus*	8♂	Sheep	*Phlebovirus* DSP4 MT211960	Clade II	80.3	ON310551
MT139		Sandai	*Rh. appendiculatus*	8♂	Sheep	*Phlebovirus* DSP4 MT211960	Clade II	78.4	ON310546
MT141		Sandai	*Rh. appendiculatus*	8♀	Sheep	*Phlebovirus* DSP4 MT211960	Clade II	78.1	ON310552
MT142		Sandai	*Rh. appendiculatus*	8♀	Sheep	*Phlebovirus* DSP4 MT211960	Clade II	77.8	ON310547
MT144		Sandai	*Rh. evertsi*	2♂	Sheep	*Phlebovirus* DSP4 MT211960	Clade II	76.9	ON310549
MT128		Sandai	*Rh. appendiculatus*	8♀	Cattle	*Phlebovirus* DSP4 MT211960	Clade II	78.2	ON310553
MT134		Sandai	*Hy. marginatum*	1♀	Sheep	Bole Tick Virus 1 KM817664	Clade III	80.7	ON310545
MT135		Sandai	*Hy. truncatum*	2♀	Sheep	Bole Tick Virus 1 KM817664	Clade III	78.9	ON310537
MT136		Sandai	*Hy. truncatum*	2♂	Sheep	Bole Tick Virus 1 KM817664	Clade III	78.9	ON310536
MT71		Logumgum	*Rh. appendiculatus*	8♂	Goat	Bole Tick Virus 1 KM817664	Clade III	81.2	ON310542
MT75		Sandai	*Hy. truncatum*	2♂	Goat	Bole Tick Virus 1 KM817664	Clade III	81.2	ON310544
MT105		Sandai	*Rh. pulchellus*	1♀	Cattle	Bole Tick Virus 1 KM817664	Clade III	79.2	ON310540
MT110		Sandai	*Hy. truncatum*	3♀	Cattle	Bole Tick Virus 1 KM817664	Clade III	79.2	ON310539
MT112		Sandai	*Hy. truncatum*	4♂	Cattle	Bole Tick Virus 1 KM817664	Clade III	79.2	ON310538
MT126		Sandai	*Rh. appendiculatus*	8♀	Cattle	Bole Tick Virus 1 KM817664	Clade III	78.9	ON310535
MT98		Sandai	*Rh. appendiculatus*	8♂	Goat	BOGV QNJ99604	–	97.4	ON256357
MT73		Logumgum	*Rh. appendiculatus*	4♂	Goat	BOGV MT270828	–	97.8	‡
MT57		Logumgum	*Hy. truncatum*	1♀	Goat	BOGV MT270828	–	96.9	ON256358
MT86		Sandai	*Rh. appendiculatus*	8♀	Goat	PERV MT270834	–	97.3	‡
KT141	Kajiado	Oloisinyai	*Hy. truncatum*	1♂	Sheep	NTPV NC_055407	–	99.5	ON256359

♀: female; ♂: male; ‡: sequence length less than 200 bp; aa: amino acids; ODWV: Odaw virus; BOGV: Bogoria virus; PERV: Perkerra virus; NTPV: Ntepes virus; ID: Identity; BDTPV: Brown dog tick phlebovirus; -: strains of established virus species.

Bole tick virus 1 were spatially the most widely distributed phleboviruses in our study, occurring in Logumgum and Sandai (Baringo county). Our results, together with previous reports of Bogoria, Perkerra and Ntepes viruses in sandflies collected in Marigat, (Baringo county, Kenya) (Marklewitz et al., 2020; Tchouassi et al., 2019), likely expand on both the geographical and invertebrate host species range of these viruses. However, arboviruses are usually associated with a certain vector taxon, for instance either mosquitoes or ticks or sandflies, but rarely with several of these taxa. Therefore, the detection of sequence fragments of sandfly-borne phleboviruses in engorged ticks needs to be interpreted with caution and needs further confirmation.

Phleboviruses of the bole lineage have been suspected to lack the M-segment that encodes the viral glycoproteins and is important for cellular entry. Some studies have postulated that M-segment deficient viruses may be endosymbionts utilizing alternate routes such as transovarial transmission (Bouquet et al., 2017; Souza et al., 2018). As these viruses have been largely described by high-throughput sequencing (HTS), it is also likely that the M-segment sequence has so far not been detected (Souza et al., 2018). Unfortunately, we were also not able to generate more sequence information of the viruses detected in this study than a fragment of the RdRp gene. Knowledge of genome variability of this group of viruses remains to be elaborated.

Our findings provide the latest update on phleboviruses detected in ticks in Kenya following identification of IFTV and MATV in ticks infesting dromedary camels (Zhang et al., 2021) and confirm that divergent TBPV may be widely distributed in sub-Saharan Africa and are not only limited to Europe, Asia and in the Americas (Matsuno et al., 2018; Papa et al., 2017; Pereira et al., 2017; Prinz et al., 2017; Torii et al., 2019). Further, the detection of phleboviruses in five engorged tick species infesting cattle, sheep and goats provide an opportunity for the viruses to get in contact with livestock. This may have consequences for the community whose economic mainstay is livestock keeping, an occupation that predisposes them to tick-borne diseases (Esser et al., 2019). Future studies should investigate the role of different tick species as possible maintenance hosts as well as identify if the detected viruses can infect livestock and humans. In addition, given that pastoralist activities increase contact opportunities between humans and domestic and wild animals, future surveillance studies should aim to assess regional TBPV infection and genetic diversity including potential wildlife hosts (Ergunay et al., 2022).

5. Conclusion

We identified diverse tick species infesting livestock and detected a wide genetic diversity of phlebovirus genome fragments in ticks sampled from Kenya. Further studies are needed to obtain full genome sequences and to investigate the maintenance and transmission cycle of the potential viruses falling within the group of tick-borne phleboviruses. The detection of sequence fragments of sandfly-borne phleboviruses in engorged ticks needs to be interpreted with caution and needs to be addressed in future studies.

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Declaration of Competing Interest

The authors declare no conflicts of interest. The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

Data availability

Data will be made available on request.

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References

Ajamma, Y.U., Onchuru, T.O., Ouso, D.O., Omondi, D., Masiga, D.K., Villinger, J., 2018. Vertical transmission of naturally occurring Bunyamwera and insect-specific flavivirus infections in mosquitoes from islands and mainland shores of Lakes Victoria and Baringo in Kenya. PLoS Negl. Trop. Dis. 12, e0006949 https://doi.org/10.1371/journal.pntd.0006949.

Altschul, S.F., Gish, W., Miller, Web, Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. https://doi.org/10.1016/S0022-2836(05)80360-2.

Amoa-Bosompem, M., Kobayashi, D., Astri, Faizah, N., 2021. Screening for tick-borne and tick-associated viruses in ticks collected in Ghana. Arch. Virol. 1, 3. https://doi.org/10.1007/s00705-021-05296-4.

Ayhan, N., Charrel, R.N., 2020. An update on Toscana virus distribution, genetics, medical and diagnostic aspects. Clin. Microbiol. Infect. 26, 1017–1023. https://doi.org/10.1016/j.cmi.2019.12.015.

Ballinger, M.J., Bruenn, J.A., Hay, J., Czechowski, D., Taylor, D.J., 2014. Discovery and evolution of bunyavirids in arctic phantom midges and ancient bunyavirid-like sequences in insect genomes. J. Virol. 88, 8783–8794. https://doi.org/10.1128/JVI.00531-14/SUPPL_FILE/ZJV999099319SO1.PDF.

Bouquet, J., Melgar, M., Swei, A., Delwart, E., Lane, R.S., Chiu, C.Y., 2017. Metagenomic-based surveillance of Pacific Coast tick *Dermacentor occidentalis* identifies two novel bunyaviruses and an emerging human ricksettsial pathogen. Sci. Rep. 7, 12234. https://doi.org/10.1038/s41598-017-12047-6.

Chiuya, T., Masiga, D.K., Falzon, L.C., Bastos, A.D.S., Fèvre, E.M., Villinger, J., 2020. Tick-borne pathogens, including Crimean-Congo haemorrhagic fever virus, at livestock markets and slaughterhouses in western Kenya. Transbound. Emerg. Dis. 68, 2429–2445. https://doi.org/10.1111/tbed.13911.

Christopher, J.W., Christine, M.M., 2001. A critique of methods of sampling and reporting pathogens in populations of fish. J. Aquat. Anim. Health 13, 300–309.

Cowling, D.W., Gardner, I.A., Johnson, W.O., 1999. Comparison of methods for estimation of individual-level prevalence based on pooled samples. Prev. Vet. Med. 39, 211–225. https://doi.org/10.1016/S0167-5877(98)00131-7.

Crava, C.M., Varghese, F.S., Pischedda, E., Halbach, R., Palatini, U., Marconcini, M., Gasmi, L., Redmond, S., Afrane, Y., Ayala, D., Paupy, C., 2021. Population genomics in the arboviral vector *Aedes aegypti* reveals the genomic architecture and evolution of endogenous viral elements. Mol. Ecol. 30, 1594–1611. https://doi.org/10.1111/mec.15798.

Dinçer, E., Brinkmann, A., Hekimoğlu, O., Hacloğlu, S., Földes, K., Karaplnar, Z., Polat, P.F., Oğuz, B., Orunç Kllnç, Ö., Hagedorn, P., Özer, N., Özkul, A., Nitsche, A., Ergünay, K., 2017. Generic amplification and next generation sequencing reveal Crimean-Congo hemorrhagic fever virus AP92-like strain and distinct tick phleboviruses in Anatolia. Turkey. Parasit. Vectors 10, 335. https://doi.org/10.1186/s13071-017-2279-1.

Endoh, D., Mizutani, T., Kirisawa, R., Maki, Y., Saito, H., Kon, Y., Morikawa, S., Hayashi, M., 2005. Species-independent detection of RNA virus by representational difference analysis using non-ribosomal hexanucleotides for reverse transcription. Nucl. Acids Res. 33, e65. https://doi.org/10.1093/nar/gni064.

Ergunay, K., Mutinda, M., Bourke, B., Justi, S.A., Caicedo-Quiroga, L., Kamau, J., Mutura, S., Akunda, I.K., Cook, E., Gakuya, F., Omondi, P., Murray, S., Zimmerman, D., Linton, Y.M., 2022. Metagenomic investigation of ticks from Kenyan wildlife reveals diverse microbial pathogens and new country pathogen records. Front. Microbiol. 13, 932224 https://doi.org/10.3389/fmicb.2022.932224.

Esser, H.J., Mögling, R., Cleton, N.B., Van Der Jeugd, H., Sprong, H., Stroo, A., Koopmans, M.P.G., De Boer, W.F., Reusken, C.B.E.M., 2019. Risk factors associated with sustained circulation of six zoonotic arboviruses: a systematic review for selection of surveillance sites in non-endemic areas. Parasit. Vectors 12, 265. https://doi.org/10.1186/S13071-019-3515-7.

Getange, D., Bargul, J.L., Kanduma, E., Collins, M., Bodha, B., Denge, D., Chiuya, T., Githaka, N., Younan, M., Fèvre, E.M., Bell-Sakyi, L., Villinger, J., 2021. Ticks and tick-borne pathogens associated with dromedary camels (*Camelus dromedarius*) in northern Kenya. Microorg. 9, 1414. https://doi.org/10.3390/MICROORGANISMS9071414.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. https://doi.org/10.1093/sysbio/syq010.

Junglen, S., Kopp, A., Kurth, A., Pauli, G., Ellerbrok, H., Leendertz, F.H., 2009. A new flavivirus and a new vector: characterization of a novel flavivirus isolated from *Uranotaenia* mosquitoes from a tropical rain forest. J. Virol. 83, 4462–4468. https://doi.org/10.1128/JVI.00014-09.

Kazimírová, M., Thangamani, S., Bartíková, P., Hermance, M., Holíková, V., Štibrániová, I., Nuttall, P.A., 2017. Tick-borne viruses and biological processes at the tick-host-virus interface. Front. Cell. Infect. Microbiol. 7, 339. https://doi.org/10.3389/FCIMB.2017.00339/BIBTEX.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Kobayashi, D., Ohashi, M., Osei, J.H.N., Agbosu, E., Opoku, M., Agbekudzi, A., Joannides, J., Fujita, R., Sasaki, T., Bonney, J.H.K., Dadzie, S., Isawa, H., Sawabe, K., Ohta, N., 2017. Detection of a novel putative phlebovirus and first isolation of Dugbe virus from ticks in Accra, Ghana. Ticks Tick. Borne. Dis. 8, 640–645. https://doi.org/10.1016/j.ttbdis.2017.04.010.

Kurtenbach, K., De Michelis, S., Etti, S., Schäfer, S.M., Sewell, H.S., Brade, V., Kraiczy, P., 2002. Host association of Borrelia burgdorferi sensu lato–the key role of host complement. Trends Microbiol. 10, 74–79. https://doi.org/10.1016/S0966-842X(01)02298-3.

Labuda, M., Nuttall, P.A., 2004. Tick-borne viruses. Parasitology 129 (Suppl). https://doi.org/10.1017/S0031182004005220.

Lefkowitz, E.J., Dempsey, D.M., Hendrickson, R.C., Orton, R.J., Siddell, S.G., Smith, D.B., 2018. Virus taxonomy: the database of the international committee on taxonomy of viruses (ICTV). Nucleic Acids Res. 46, D708–D717. https://doi.org/10.1093/nar/gkx932.

Lequime, S., Lambrechts, L., 2017. Discovery of flavivirus-derived endogenous viral elements in *Anopheles* mosquito genomes supports the existence of *Anopheles*-associated insect-specific flaviviruses. Virus Evol. 1, 3. https://doi.org/10.1093/ve/vew035.

Li, C.X., Shi, M., Tian, J.H., Lin, X.D., Kang, Y.J., Chen, L.J., Qin, X.C., Xu, J., Holmes, E. C., Zhang, Y.Z., 2015. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. Elife 4, e05378. https://doi.org/10.7554/eLife.05378.013.

Lopez, Y., Miranda, J., Mattar, S., Gonzalez, M., Rovnak, J., 2020. First report of Lihan Tick virus (*Phlebovirus*, Phenuiviridae) in ticks, ColomJubia. Virol. J. 17, 63. https://doi.org/10.21203/rs.2.22974/v1.

Marklewitz, M., Handrick, S., Grasse, W., Kurth, A., Lukashev, A., Drosten, C., Ellerbrok, H., Leendertz, F.H., Pauli, G., Junglen, S., 2011. Gouleako virus isolated from West African mosquitoes constitutes a proposed novel genus in the family Bunyaviridae. J. Virol. 85, 9227–9234. https://doi.org/10.1128/JVI.00230-11.

Marklewitz, M., Tchouassi, D.P., Hieke, C., Heyde, V., Torto, B., Sang, R., Junglen, S., 2020. Insights into the evolutionary origin of Mediterranean sandfly fever viruses. mSphere 5, e00598. https://doi.org/10.1128/mSphere.00598-20.

Matsuno, K., Kajihara, M., Nakao, R., Nao, N., Mori-Kajihara, A., Muramatsu, M., Qiu, Y., Torii, S., Igarashi, M., Kasajima, N., Mizuma, K., Yoshii, K., Sawa, H., Sugimoto, C., Takada, A., Ebihara, H., 2018. The unique phylogenetic position of a novel tick-borne phlebovirus ensures an ixodid origin of the genus *Phlebovirus*. mSphere 3, e00239. https://doi.org/10.1128/msphere.00239-18.

Matthysse, J.G., Colbo, M.H., 1987. The Ixodid Ticks of Uganda together with Species Pertinent to Uganda because of Their Present Known Distribution. Entomological Society of America, p. 426.

Medlock, J.M., Hansford, K.M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J. C., Golovljova, I., Jaenson, T.G.T., Jensen, J.K., Jensen, P.M., Kazimirova, M., Oteo, J.A., Papa, A., Pfister, K., Plantard, O., Randolph, S.E., Rizzoli, A., Santos-Silva, M.M., Sprong, H., Vial, L., Hendrickx, G., Zeller, H., Van Bortel, W., 2013. Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasites Vectors 6, 1. https://doi.org/10.1186/1756-3305-6-1/FIGURES/1.

Nguku, S., Sharif, A., Omar, C., 2007. Rift Valley fever outbreak-Kenya, November 2006-January 2007. Morb. Mortal. Wkly. Rep. 56, 73–74.

Nuttall, P.A., Labuda, M., 2004. Tick-host interactions: saliva-activated transmission. Parasitology 129 (Suppl), S177–S189. https://doi.org/10.1017/S0031182004005633.

Ogden, N.H., Mechai, S., Margos, G., 2013. Changing geographic ranges of ticks and tick-borne pathogens: drivers, mechanisms and consequences for pathogen diversity. Front. Cell. Infect. Microbiol. 4, 46. https://doi.org/10.3389/FCIMB.2013.00046/BIBTEX.

Ogola, E.O., Kopp, Anne, Bastos, A.D.S., Slothouwer, I., Marklewitz, M., Omoga, D., Rotich, G., Getugi, C., Sang, R., Torto, B., Junglen, S., Tchouassi, D.P., 2022. Jingmen tick virus in ticks from Kenya. Viruses 14, 1041. https://doi.org/10.3390/V14051041.

Ohlendorf, V., Marklewitz, M., Kopp, A., Yordanov, S., Drosten, C., Junglen, S., 2019. Huge diversity of phleboviruses in ticks from Strandja Nature Park. Bulgaria. Ticks Tick. Borne. Dis. 10, 697–703. https://doi.org/10.1016/j.ttbdis.2019.03.001.

Okello-Onen, J., Hassan, S.M., Essuman, S., 1999. Taxonomy of African ticks: an Identification Manual, 1st ed. International Centre of Insect Physiology and Ecology ;African Postgraduate Programme in Insect Science, Nairobi Kenya, p. 124.

Omondi, D., Masiga, D.K., Fielding, B.C., Kariuki, E., Ajamma, Y.U., Mwamuye, M.M., Ouso, D.O., Villinger, J., 2017. Molecular detection of tick-borne pathogen diversities in ticks from livestock and reptiles along the shores and adjacent Islands of Lake Victoria and Lake Baringo, Kenya. Front. Vet. Sci. 4, 1–15. https://doi.org/10.3389/fvets.2017.00073.

Oundo, W., Villinger, J., Jeneby, M., Ong'amo, G., Otiende, M.Y., Makhulu, E.E., Musa, A.A., Ouso, D.O., Wambua, L., 2020. Pathogens, endosymbionts, and blood-meal sources of host-seeking ticks in the fast-changing Maasai Mara wildlife ecosystem. PLoS ONE 15, e0228366. https://doi.org/10.1371/journal.pone.0228366.

Papa, A., Kontana, A., Tsioka, K., Chaligiannis, I., Sotiraki, S., 2016. Novel phleboviruses detected in ticks, Greece. Ticks Tick. Borne. Dis. 7, 690–693. https://doi.org/10.1016/j.ttbdis.2016.02.017.

Papa, A., Kontana, A., Tsioka, K., Saratsis, A., Sotiraki, S., 2017. Novel phlebovirus detected in Haemaphysalis parva ticks in a Greek island. Ticks Tick. Borne. Dis. 8, 157–160. https://doi.org/10.1016/j.ttbdis.2016.10.012.

Parola, P., Raoult, D., 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin. Infect. Dis. 32, 897–928. https://doi.org/10.1086/319347.

Pereira, A., Figueira, L., Nunes, M., Esteves, A., Cotão, A.J., Vieira, M.L., Maia, C., Campino, L., Parreira, R., 2017. Multiple phlebovirus (Bunyaviridae) genetic groups detected in *Rhipicephalus, Hyalomma* and *Dermacentor* ticks from southern Portugal. Ticks Tick. Borne. Dis. 8, 45–52. https://doi.org/10.1016/j.ttbdis.2016.09.015.

Prinz, M., Fuchs, J., Ehrmann, S., Scherer-Lorenzen, M., Kochs, G., Panning, M., 2017. Molecular identification of novel phlebovirus sequences in European ticks. Ticks Tick. Borne. Dis. 8, 795–798. https://doi.org/10.1016/j.ttbdis.2017.06.005.

QGIS Development Team, 2019. Welcome to the QGIS project! [WWW Document]. URL https://qgis.org/en/site/(accessed 6.11.19).

R Development Team, 2016. R: a language and environment for statistical computing.

Sameroff, S., Tokarz, R., Charles, R.A., Jain, K., Oleynik, A., Che, X., Georges, K., Carrington, C.V., Lipkin, W.I., Oura, C., 2019. Viral diversity of tick species parasitizing cattle and dogs in Trinidad and Tobago. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-46914-1.

Sang, R., Kioko, E., Lutomiah, J., Warigia, M., Ochieng, C., O'Guinn, M., Lee, J.S., Koka, H., Godsey, M., Hoel, D., Hanafi, H., Miller, B., Schnabel, D., Breiman, R.F., Richardson, J., 2010. Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations. Am. J. Trop. Med. Hyg. 83, 28–37. https://doi.org/10.4269/ajtmh.2010.09-0319.

Sergeant, E.S.G., 2018. Epitools Epidemiological Calculators. Ausvet. https://epitools.ausvet.com.au/. accessed 11.4.21.

Simulundu, E., Mbambara, S., Chambaro, H.M., Sichibalo, K., Kajihara, M., Nalubamba, K.S., Sawa, H., Takada, A., Changula, K., Chitanga, S., 2021. Prevalence and genetic diversity of Shibuyunji virus, a novel tick-borne phlebovirus identified in Zambia. Arch. Virol. 166, 915–919. https://doi.org/10.1007/s00705-020-04924-9.

Souza, W.M.de, Fumagalli, M.J., Torres Carrasco, A., de, O., Romeiro, M.F., Modha, S., Seki, M.C., Gheller, J.M., Daffre, S., Nunes, M.R.T., Murcia, P.R., Acrani, G.O., Figueiredo, L.T.M., 2018. Viral diversity of *Rhipicephalus microplus* parasitizing cattle in southern Brazil. Sci. Rep. 8, 16315. https://doi.org/10.1038/s41598-018-34630-1.

Spadar, A., Phelan, J.E., Benavente, E.D., Campos, M., Gomez, L.F., Mohareb, F., Clark, T. G., Campino, S., 2021. Flavivirus integrations in *Aedes aegypti* are limited and highly conserved across samples from different geographic regions unlike integrations in *Aedes albopictus*. Parasit. Vectors. 14, 1–14. https://doi.org/10.1186/s13071-021-04828-w.

Tchouassi, D.P., Marklewitz, M., Chepkorir, E., Zirkel, F., Agha, S.B., Tigoi, C.C., Koskei, E., Drosten, C., Borgemeister, C., Torto, B., Junglen, S., Sang, R., 2019. Sand fly-associated phlebovirus with evidence of neutralizing antibodies in humans, Kenya. Emerg. Infect. Dis. 25, 681–690. https://doi.org/10.3201/eid2504.180750.

Tigoi, C., Lwande, O., Orindi, B., Irura, Z., Ongus, J., Sang, R., 2015. Seroepidemiology of selected arboviruses in febrile patients visiting selected health facilities in the lake/river basin areas of lake baringo, lake naivasha, and tana river, Kenya. Vector-Borne Zoonotic Dis. 15, 124–132. https://doi.org/10.1089/vbz.2014.1686.

Torii, S., Matsuno, K., Qiu, Y., Mori-Kajihara, A., Kajihara, M., Nakao, R., Nao, N., Okazaki, K., Sashika, M., Hiono, T., Okamatsu, M., Sakoda, Y., Ebihara, H., Takada, A., Sawa, H., 2019. Infection of newly identified phleboviruses in ticks and wild animals in Hokkaido, Japan indicating tick-borne life cycles. Ticks Tick. Borne. Dis. 10, 328–335. https://doi.org/10.1016/j.ttbdis.2018.11.012.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M., 2003. Ticks of domestic animals in municipal abattoir for their technical support. Africa: a guide to identification of tick species. Biosci. Rep., Edinburgh, Scotland, U.K.

Walker, J.B., Keirans, J.E., Horak, I.G., 2000. The Genus *Rhipicephalus* (Acari, Ixodidae) - A Guide to Brown Ticks of the World. Cambridge University Press.

Zhang, Y., Hu, B., Agwanda, B., Fang, Y., Wang, J., Kuria, S., Yang, J., Masika, M., Tang, S., Lichoti, J., Fan, Z., Shi, Z., Ommeh, S., Wang, H., Deng, F., Shen, S., 2021. Viromes and surveys of RNA viruses in camel-derived ticks revealing transmission patterns of novel tick-borne viral pathogens in Kenya. Emerg. Microbes. Infect. 10, 1975–1987. https://doi.org/10.1080/22221751.2021.1986428/SUPPL_FILE/TEMI_A_1986428_SM4975.DOCX.