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Resurgence of visceral and cutaneous leishmaniasis in Kajiado County, Kenya: A coordinated response and entomological survey

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Abstract:	Background Leishmaniasis is of major global health concern with global elimination targets set for 2030. East Africa accounts for ~75% of global visceral leishmaniasis (VL) cases. Hence, attention to endemic, recurrent or emerging foci is critical to meeting global elimination targets. In 2021, Kajiado County, on the southern border of Kenya with Tanzania, emerged with cases of local transmission that necessitated this

investigation.

Methods

This was a mixed methods coordinated response. We conducted a training for clinicians, sensitised communities, and set up medical camps in four villages. Here we screened the community for VL and other common ailments and undertook an entomological survey for sandfly vector species in the same villages. We screened 100 individuals (males: 56; females: 44) for leishmaniasis using the rK39 rapid antibody test and by microscopic examination. Dry blood spots (DBS) were collected from suspected cases. The samples were further analyzed using PCR targeting the internal transcribed spacer 1 (ITS1) region, followed by sequencing for Leishmania species identification. For entomological surveys, sandfly sampling was done in the four villages where medical camps were implemented, and another four adjacent villages using CDC miniature or Silverbullet 2.0 light traps. Sandflies were identified morphologically using taxonomic keys and the speciation confirmed by PCR analysis of the cytochrome c oxidase subunit 1 (Cox1) gene and sequencing. We characterized sandfly blood feeding preference by PCR analysis of the vertebrate cytochrome-b (Cyt-b) gene, followed by HRM analysis and sequencing.

Results

Ten people (10/100; 10%) tested positive for VL (eight below 15 years old - six males and two females). Of the 22 suspected cases with lesions suspected of cutaneous leishmaniasis (CL), eight were confirmed as being infected with Leishmania tropica. We trapped 4,781 sandflies and identified 1,624 specimens, consisting of 422 males and 1202 females, to species. They represented four Phlebotomus spp. and eight Sergentomyia spp. The Phlebotomus spp. included Ph. martini (8.4%; n = 136), Ph. saevus (2.2%; n = 35), Ph. orientalis (1.2%; n = 19), and Ph. guggisbergi (0.1%; n = 1). Overall, Sergentomyia species (62.7%, 1019 specimens) were abundant. Of the 450 sandflies examined for presence of Leishmania parasites by PCR, we detected DNA of Leishmania donovani in Sergentomyia clydei (n = 13), Sergentomyia adleri (n = 4), S. antennatus (n = 2), and Ph. saevus (n = 1), while L. tropica DNA was detected in Ph. saevus (n = 1). Analysis of the blood meal sources of the 68 engorged sand flies showed that most fed on humans followed by goats. We also detected blood from donkeys, mongoose and rock hyraxes.

Conclusion

We confirmed L. donovani and L. tropica as the main Leishmania species responsible for VL and CL, respectively, in Kajiado. The presence of the parasites in humans and the high human blood indices in sandflies indicate active transmission and high vector-human contact in the area. The co-occurrence of VL and CL is unusual and complicates case management as the two forms are managed differently, and raises the possibilities of genetic recombination in Leishmania parasites. Since access to diagnostic and treatment facilities are at the core of infectious diseases management, and elimination efforts, the establishment of two treatment Centres in Kajiado, for the first time, constitutes an important step in enhancing community access to treatment and stemming the spread of leishmaniasis in the region. This study thus contributes to global and national VL elimination targets, and demonstrates the value of coordinated outbreak preparedness.

Resurgence of visceral and cutaneous leishmaniasis in Kajiado County, Kenya: A coordinated response and entomological survey

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Abstract

Background: Leishmaniasis is of major global health concern with global elimination targets set for 2030. East Africa accounts for ~75% of global visceral leishmaniasis (VL) cases. Hence, attention to endemic, recurrent or emerging foci is critical to meeting global elimination targets. In 2021, Kajiado County, on the southern border of Kenya with Tanzania, emerged with cases of local transmission that necessitated this investigation.

Methods: This was a mixed methods coordinated response. We conducted a training for clinicians, sensitised communities, and set up medical camps in four villages. Here we screened the community for VL and other common ailments and undertook an entomological survey for sandfly vector species in the same villages. We screened 100 individuals (males: 56; females: 44) for leishmaniasis using the rK39 rapid antibody test and by microscopic examination. Dry blood spots (DBS) were collected from suspected cases. The samples were further analyzed using PCR targeting the internal transcribed spacer 1 (ITS1) region, followed by sequencing for *Leishmania* species identification. For entomological surveys, sandfly sampling was done in the four villages where medical camps were implemented, and another four adjacent villages using CDC miniature or Silverbullet 2.0 light traps. Sandflies were identified morphologically using taxonomic keys and the speciation confirmed by PCR analysis of the cytochrome c oxidase subunit 1 (*Cox1*) gene and sequencing. We characterized sandfly blood feeding preference by PCR analysis of the vertebrate cytochrome-b (*Cyt-b*) gene, followed by HRM analysis and sequencing.

Results: Ten people (10/100; 10%) tested positive for VL (eight below 15 years old - six males and two females). Of the 22 suspected cases with lesions suspected of cutaneous leishmaniasis (CL), eight were confirmed as being infected with *Leishmania tropica*. We trapped 4,781 sandflies and identified 1,624 specimens, consisting of 422 males and 1202 females, to species. They represented four *Phlebotomus* spp. and eight *Sergentomyia* spp. The *Phlebotomus* spp. included *Ph. martini* (8.4%; n = 136), *Ph. saevus* (2.2%; n = 35), *Ph. orientalis* (1.2%; n = 19), and *Ph. guggisbergi* (0.1%; n = 1). Overall, *Sergentomyia* species (62.7%, 1019 specimens) were abundant. Of the 450 sandflies examined for presence of *Leishmania* parasites by PCR, we detected DNA of *Leishmania donovani* in *Sergentomyia clydei* (n = 13), *Sergentomyia adleri* (n = 4), *S. antennatus* (n = 2), and *Ph. saevus* (n = 1), while *L. tropica* DNA was detected in *Ph. saevus* (n = 1). Analysis of the blood meal sources of the 68 engorged sand flies showed that most fed on humans followed by goats. We also detected blood from donkeys, mongoose and rock hyraxes.

Conclusion: We confirmed *L. donovani* and *L. tropica* as the main *Leishmania* species responsible for VL and CL, respectively, in Kajiado. The presence of the parasites in humans and the high human blood indices in sandflies indicate active transmission and high vector-human contact in the area. The co-occurrence of VL and CL is unusual and complicates case management as the two forms are managed differently, and raises the possibilities of genetic recombination in *Leishmania* parasites. Since access to diagnostic and treatment facilities are at the core of infectious diseases management, and elimination efforts, the establishment of two treatment Centres in Kajiado, for the first time, constitutes an important step in enhancing community access to treatment and stemming the spread of leishmaniasis in the region. This study thus contributes to global and national VL elimination targets, and demonstrates the value of coordinated outbreak preparedness.

Introduction

The leishmaniases are among the world's most neglected tropical diseases (NTDs), occurring mainly in remote areas of the tropics, sub-tropics, and Mediterranean region.[1,2] The diseases are caused by protozoan parasites of the *Leishmania* genus and transmitted through the bites of infected phlebotomine sandflies, afflicting about 1 million people annually, with an estimated 350 million people exposed to infections.[3] Three clinical forms occur in East Africa: Visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL).[4,5] Visceral leishmaniasis is fatal if not treated and is endemic in 78 countries in Southeast Asia, Brazil, and East Africa, where the most socioeconomically disadvantaged populations are the most affected.[5] In addition, even though access to tests and treatment is offered free of charge, leishmaniasis disproportionally affects the most vulnerable in endemic localities.[2]

Unlike Southeast Asia, efforts for VL in eastern Africa have been limited to controlling the disease, partly due to significant knowledge gaps on transmission hotspots and dynamics. Recently a strategy for elimination of VL as a public health problem, defined by WHO as a case fatality of 1% by 2030 has been championed for East Africa.[2] An estimated 5 million people in Kenya are at risk of VL infections, with about 4000 new cases occurring annually.[6] There is little data on CL in Kenya, but active case transmission in the Rift Valley and Mount Elgon areas is known.[7] In Kenya, VL is caused by *Leishmania donovani,* while infections with *L. major*, *L. tropica*, and *L. aethiopica* result in CL.[8] The sandflies *Phlebotomus martini* and *Ph. orientalis* are considered the main vectors of VL,[9,10] whereas *Phlebotomus duboscqi, Phl. guggisbergi* and *Ph. pedifer* are known vectors of CL in Kenya.[11,12] The distribution of these sandfly species in endemic areas is poorly understood, challenging the effective implementation of vector control as a contribution to disease management. The sandfly species have different micro-ecological preferences, which likely influence VL and CL epidemiology. *Phlebotomus martini* breeds and rests predominantly in termite mounds,[13,14] while the breeding sites for *Ph. orientalis* include dry riverbanks and cracked vertisols.[14] In contrast, *Ph. duboscqi* predominates in rodent burrows, whereas *Ph. guggisbergi, Ph. pedifer, Phlebotomus longipes, Ph. saevus* and *Phlebotomus aculeatus* have been associated with rocky areas with numerous crevices and caves.[9]

Prior to this outbreak, the only report of VL in Kajiado County that is known was three decades ago when three children from the Keekonyokie South location (Kajiado West sub-county) were diagnosed.[15] Thus, the recent outbreak necessitated a coordinated investigation and intervention. Epidemiological data is important in defining disease endemic areas, transmission and where VL could potentially spread. Although vector control is an effective approach to managing arthropod-transmitted diseases, as demonstrated with malaria,[16] this approach to sandflies is often limited to outbreak response without proper knowledge of the local disease epidemiology, vector ecology and behaviour, and disease surveillance. The increasing public health impacts of leishmaniasis warrant developing effective case management, vector control and outbreak response.

Here we report the coordinated approach of leishmaniasis outbreak response in Kajiado County, strategic case management, capacity building, community sensitization and entomological response.

Methods

Study area

We conducted an outbreak response in four villages in Kajiado West sub-county at Kenya's southern border with Tanzania, and cross-sectional sandfly survey in the same area (Fig. 1). The sub-county covers approximately 7,910.80 km^2, with a population of about 183,000 people,[17] most of whom are semi-nomadic pastoralists. The area is mainly semi-arid, dominated by acacia trees and dry grasslands, with a complex landscape consisting of fault scarp ranges, caves, seasonal rivers, and termite mounds. It has a bimodal rainfall pattern (long rains: March-May; short rains: October-December), with an annual average rainfall of 450.5 mm.[18]

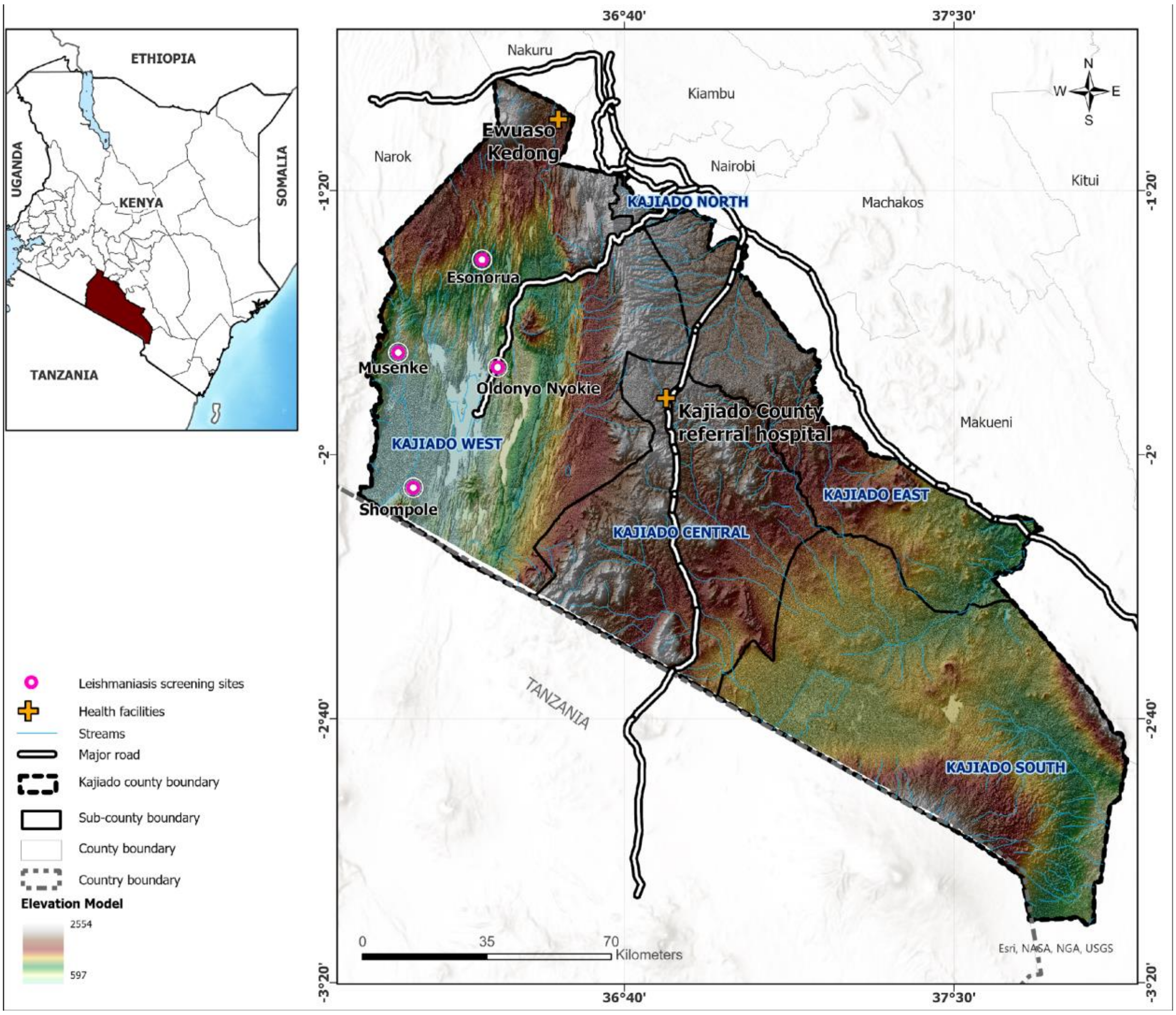

Fig. 1. An elevation model of Kajiado County showing the location of Kajiado West sub-county and the local health facilities used for VL and CL screening.

Training of Health Care workers

The sub-county clinicians and Community Health Volunteers (CHVs) were trained at a central location on clinical presentation of VL such as irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia, and for CL signs such as skin lesions, scaring mainly ulcers on exposed parts of the body.[19] As clinicians are responsible for patient management, additional training was conducted to enhance their knowledge of the disease considering it was the first time it was being reported, after the initial diagnosis of three children in 1993.[15] The training included case definition, differential diagnosis and the approved national algorithm for VL diagnosis; first and second-line treatment, drug pharmacology and response to adverse treatment-related events.[19] They were also trained on the management of special groups including paediatrics, pregnancy, co-morbidities and cases of existing malnutrition. A total of four clinicians, four nurses, three laboratory technicians, and 10 CHVs were trained.

Community mobilization and facility equipping.

Community sensitization was done through the local leadership, CHVs, churches, schools and businesses through announcements at gatherings or meetings. The mobilization was conducted a week before the medical camps. Medical camps were undertaken in four villages, where diagnosis and treatment were also done for leishmaniasis and common ailments. The treatment centres were also provided with materials and reagents including test kits, hemacytometers, and treatment guidelines for case management.

Leishmaniasis screening, sample collection and medical camps

Following community mobilisation, we screened all symptomatic patients who visited the four health facilities where medical camps were undertaken for VL or CL. Testing was done by clinicians using rk39 rapid test kits and microscopy; DBS were collected for further analysis. For CL infections, skin scraping samples from the edges of an active lesion were collected by clinicians and smears were prepared for Giemsa-staining as described.[20] Giemsa-stained lesion smears were examined using an Olympus CX31 microscope with a PlanC Achromatic 100x/1.25NA oil objective. *Leishmania* parasites were grown at 25°C in Schneider's drosophila insect medium supplemented with 20% foetal bovine serum and the stationary phase promastigotes used for molecular analyses. Anyone testing positive was referred for treatment at the leishmaniasis treatment facility in the sub-county.

Sandfly sampling

We conducted entomological surveillance by trapping sandflies in November 2021 and February 2022 in eight villages where VL cases were reported during the medical campaigns in the Kajiado West sub-county: Musenke, Shompole, Kirine, Enchanipus, Nkonyoro, Empaleki, Oloisinyei and Birika (see Fig 1). We deployed 10 CDC miniature (John W. Hock Co., Gainesville, FL, USA) and five solar-powered Silverbullet 2.0 (https://www.lumin8.co.za/) light traps from 1800 to 0630 hours for six consecutive trapping nights each month in domestic, peri-domestic, and extra-domestic sites likely to harbour sandflies (15 traps x 8 villages x 6 nights/month x 2 months = 1,440 trap nights). The trapping sites included termite mounds, rock crevices, inside houses, animal burrows and livestock sheds.

Morphological and molecular identification of sandfly species

After each trapping night, the sandflies were sorted and cleaned using 2% detergent, antifungal and antibiotic solutions.[12,21] For morphological identification of sandflies to species, we cleared each specimen's dissected head and third last abdominal segment in gum chloral hydrate, which also acted as the mountant.[22] Identification was based on relevant taxonomic keys regarding features of the external genitalia, the spermatheca, the pharynx and antennae as described.[21,23,24] After the dissections, we preserved the remaining parts of each specimen (abdomen and thorax) in nucleic acid preservation (NAP) buffer[25] for molecular analysis.

To verify the morphological species identifications, we isolated DNA from each specimen using the QIAGEN's DNeasy Blood and Tissue Kit (Hannover, Germany) according to the manufacturer's instructions, followed by PCR analysis of the *Cox1* gene according to Kumar and colleagues.[26] The resulting 700 bp *Cox1* PCR products were purified using the USB® ExoSAP-IT® (ThermoFisher Scientific) and sequenced (Macrogen, The Netherlands). We performed multiple sequence alignment using the MUSCLE algorithm (v3.8)[27] in Geneious Prime (v2022.2.2) (Biomatters; www.geneious.com), alongside reference sequences of other sandfly species retrieved from GenBank. We generated a dendrogram using the PhyML (V3.3) algorithm[28] in agreement with the General-time-reversible (GTR) substitution model. The phylogram was evaluated using 1000 bootstraps and *Lutzomyia maranonensis* as an outgroup.

Identification of sandfly blood meal sources

We identified the blood meal sources of blood-fed sandflies using PCR and high-resolution melt (HRM) analysis of the vertebrate cytochrome-b *(Cyt-b*) gene, according to Omondi and colleagues*.*[29] All the PCR reactions were performed in the Biomolecular System magnetic induction cycler (MIC) (https://biomolecularsystems.com/mic-qpcr/) using DNA extracted from the blood of known vertebrate species as the positive control and nuclease-free water as the negative control. The positive controls included livestock (goat, sheep and cow), rodents (rat and mouse) and small mammals commonly found in the study area, such as rock hyrax and rabbit. We received the blood from goats, sheep and cows from a local abattoir, while blood from rabbits, Swiss White mice and rats were obtained from *icipe's* animal-rearing unit. Sources of sandfly blood meals were identified by comparing the melt profiles of the samples to those of positive controls. Samples with unidentified melt profiles were purified using the USB® ExoSAP-IT® (Affymetrix, Santa Clara, CA, USA) and sequenced (Macrogen Europe, The Netherlands). We queried the sequences against the GenBank database using the BLASTn algorithm[30] and selected the top-hit organism with the lowest *e*-value and homology cut-off of 95-100% as the most probable vertebrate host.

***Leishmania* detection and species identification in sandflies and lesion samples**

We tested 450 individual sandflies (fed: 61; unfed: 389) and six of the eight positive lesion samples for the presence of *Leishmania* DNA by PCR analysis of the *Leishmania* internal transcribed spacer1 (ITS1) region followed by restriction fragment length polymorphism (RFLP) as previously described.[12,20] To enhance the sensitivity of *Leishmania* detections, we analysed the samples further by real-time PCR, according to Owino *et al.*[12] We performed the real-time PCR reactions in the MIC thermocycler, using a 15 min initial denaturation at 95°C, followed by 40 cycles of denaturation for 20 s at 95°C and annealing and extension for 30 s at 60°C. For the HRM analyses, we gradually increased the temperature of the PCR productsfrom 55°C to 95°C,and recorded changes in fluorescence at 0.1°C increments and plotted these with temperature changes (dF/dT). We purified positive samples, as aforementioned, and submitted them for sequencing at Macrogen (The Netherlands).

Statistical analyses

We used descriptive statistics to determine sandfly species frequencies, abundance, distribution patterns per village or habitat, and percentage positivity of *Leishmania* parasites using SPSS v26. Differences in sandfly species distribution across the sampling locations and habitats were determined using the Kruskal-Wallis test with $\alpha = 0.05$ in SPSS v26, while the bipartite feeding network was plotted in R v4.2.

Ethics statement

The study was approved by the Kenyatta National Hospital-University of Nairobi ethics review committee (KNH-UON ERC) under protocol number P422/10/2011 as well as Northeastern University (IRB # 10-11-18). We collected clinical samples after obtaining informed consent from the study participants and guardians of participants below 18 years old. We received approval from home-owners to conduct sandfly trapping in homes and private lands. All procedures involving blood sampling from rabbits, Swiss White mice and rats were performed by trained personnel according to the guidelines of the *icipe's* Institutional Animal Care and Use Committee (IACUC).

Results

Establishing new treatment centres. To enhance access to treatment, two health facilities were designated as treatment centres for leishmaniasis in Kajiado County: Kajiado County

Referral Hospital and Ewuaso Kedong Health Centre (Fig 1.). This followed clinician training on case management, using approved guidelines.

Medical outreach and leishmaniasis screening

We screened 100 patients (55 male; 42 female; 3 undetermined) for visceral leishmaniasis, based on initial clinical assessments, with 10 positive cases diagnosed (Table 1). Esonorua had the highest positivity rate with 21.7% (5/23) followed by Oldonyo Nyoike (14.3%; 2/14), Musenke (5.0%; 2/40), and Shompole (4.2%; 1/24). The majority of the infected VL patients (80%; n = 8) were below 15 years old, with more infections in males (75%; n = 6) than in females (25%; n = 2). For CL screening, we examined lesion smears from 22 suspected CL patients for the presence of *Leishmania* amastigotes. Amastigotes, characterised by a spherical cell body and prominent nucleus and kinetoplast (S2_Fig), were seen in eight of these samples, representing an overall positivity rate of 36.4% (8/22) (Table 2). Oldonyo Nyokie was the most affected area, with a positivity rate of 42.9% (6/14), followed by Esonorua with 28.6% (2/7) while we did not observe *Leishmania* amastigotes in samples from Musenke. Failure to observe a positive lesion sample from Musenke could be attributed to the smaller number of samples collected from this site. We identified a case of VL and CL co-infection from Oldonyo Nyokie, which was positive by rK39 and microscopy. Patients were referred to Ewuaso Health Centre, which was established as a VL treatment Centre as a result of this coordinated intervention.

Table 1: Summary of sample collection from suspected VL patients by sampling site, gender and positive cases

	VL rK39 screening						
Sampling site	Total		< 15 years		≥15 years		Positivity rate (%)
	Male	Female	Male	Female	Male	Female	
Esonorua	16	7	13 (3)	3 (2)	3	4	21.7 (5)
Oldonyo Nyokie	8	6	2	3	3	3 (2)	14.3 (2)
Shompole	13	8	8 (1)	3	5	5	4.2 (1)
Musenke	18	21	13 (2)	9	5	12	5.1 (2)
Total	55	42	36 (6)	18 (2)	16	24 (2)	10 (10)

The numbers in parentheses indicate patients with positive outcomes. The gender of three (3) additional patients with negative rK39 results from Shompole were not specified, while age data were unknown for three rK39 negative male patients from Oldonyo Nyokie.

Table 2. Summary of sample collection from suspected CL patients by sampling site, gender and positive cases.

	CL lesion smear microscopy						
Sampling site	Total		< 15 years		≥15 years		Positivity rate (%)
	Male	Female	Male	Female	Male	Female	
Esonorua	5	2	2	0	3 (1)	2 (1)	28.6 (2)
Oldonyo Nyokie	5 (1*)	9	1 (1)	3 (1)	3	6 (3)	42.9 (6)
Shompole	ND	ND	ND	ND	ND	ND	ND
Musenke	0	1	0	0	0	1	0 (0)
Total	10 (1*)	12	3 (1)	3 (1)	6 (1)	9 (4)	36.4 (8)

Age data for one male patient from Oldonyo Nyokie with a positive lesion smear was not known. We have shown data from this sample with an asterisk (*). ND: not determined.

Sandfly species composition, density and distribution

We sampled 4781 sandflies and identified 1624 specimens (males: 422; females: 1202) belonging to four *Phlebotomus* and eight *Sergentomyia* species. The *Phlebotomus* species included *Ph. martini* (8.4%), *Ph. saevus* (2.2%), *Ph. orientalis* (1.2%), and *Ph. guggisbergi* (0.1%). *Sergentomyia* species consisted of *S. adleri* (12.4%), *S. africanus* (1.5%), *S. antennatus* (4.9%), *S. bedfordi* (4.2%), *S. clydei* (39.2%), *S. ingrami* (0.6%), *S. schwetzi* (1.8%) and *S. squamipleuris* (23.5%) (Table 3). Overall, *Ph. martini* was the predominant species among the *Phlebotomus* species. Using the Kruskal- Wallis test, we observed a statistically significant difference in sandfly distribution across the eight sampling locations ($\chi2 = 184.7$, df = 7, $p < 0.001$). Olosinyei and Birika had the highest and lowest sandfly densities, respectively (Table 3), whereas, Nkonyoro had the highest sandfly diversity (S1_Fig).

Table 3: Sandfly composition, density, abundance and the engorged species per sampling site in Kajiado West sub-county, Kajiado County. The numbers in parentheses indicate the amount of blood-fed sandflies per location. Additionally, we have shown the percentage abundance for each sandfly species in the study area. N: sandfly counts; D: species density measured as counts/trap/night.

Sandfly species	**Trapping locations**															
	Musenke		**Shompole**		**Kirine**		**Enchanipus**		**Nkonyoro**		**Empaleki**		**Olosinyei**		**Birika**	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
Ph. guggisbergi	0	0	0	0	0	0	0	0	1	0.01	0	0	0	0	0	0
Ph. martini	1	0.13	5	0.09	54 (2)	0.75	15	0.31	6	0.05	9	0.9	46	1.92	0	0
Ph. orientalis	0	0	0	0	3	0.04	2	0.04	0	0	0	0	14	0.58	0	0
Ph. saevus	0	0	0	0	0	0	0	0	35 (5)	0.28	0	0	0	0	0	0
S. adleri	10	1.25	78	1.44	16	0.22	28 (1)	0.58	33	0.26	7	0.7	30	1.25	0	0
S. africanus	0	0	3	0.06	13	0.18	4	0.08	2	0.02	0	0	2	0.08	1	0.2
S. antennatus	0	0	7	0.13	11 (2)	0.15	2	0.04	44 (4)	0.35	0	0	11	0.46	5	1
S. bedfordi	0	0	35	0.65	15	0.21	5	0.1	7	0.06	0	0	5	0.21	1	0.2
S. clydei	84 (17)	10.5	10 (1)	0.19	101 (8)	1.4	128 (9)	2.67	60 (6)	0.48	39	3.9	214 (1)	8.92	1	0.2
S. ingrami	0	0	9	0.17	0	0	0	0	0	0	0	0	0	0	0	0
S. schwetzi	0	0	0	0	25 (5)	0.35	5	0.1	0	0	0	0	0	0	0	0
S. squamipleuris	3	0.38	13	0.24	32 (1)	0.44	67	1.4	40 (2)	0.32	6	0.6	221 (4)	9.21	0	0
Total	**98 (17)**	**12.26**	**160 (1)**	**2.97**	**270 (18)**	**3.74**	**256 (10)**	**5.35**	**228 (17)**	**1.83**	**61**	**6.1**	**543 (5)**	**22.63**	**8**	**1.6**

Most blood-fed sandflies were trapped in peridomestic sites (91.2%; 62/68), especially where these sites were near the termite hills (Table 4). Among the *Phlebotomus* species, *Ph. martini* was the most widespread, occurring in almost all the habitats. We found a significantly higher proportion of *Ph. martini* in peridomestic habitats (n=132) than in domestic sites ($\chi 2 = 88.97$, df = 1, $p < 0.001$). Similarly, *Ph. orientalis*, *Ph. saevus* and *Ph. guggisbergi* were sampled exclusively in peri-domestic habitats. Most sandflies trapped indoors were mainly of the *Sergentomyia* genus. Overall, traps placed near termite hills had the highest abundance of all sandflies caught.

Table 4: Sandfly composition, density, abundance and the engorged species per habitat in Kajiado West sub-county, Kajiado County

Sandfly species	**Trapping habitats**									
	Domestic				**Peri-domestic**					
	Indoors		**Livestock sheds**		**Termite hills**		**Rock crevices**		**Animal burrows**	
	N	D	N	D	N	D	N	D	N	D
Ph. guggisbergi	0	0	0	0	0	0	1	0.01	0	0
Ph. martini	0	0	4	0.33	123 (2)	0.18	8	0.05	1	1
Ph. orientalis	0	0	0	0	19	0.03	0	0	0	0
Ph. saevus	0	0	0	0	5 (1)	0.01	30 (4)	0.2	0	0
S. adleri	7	1.4	8	0.67	155 (1)	0.23	31	0.21	1	1
S. africanus	0	0	0	0	22	0.03	2	0.01	1	1
S. antennatus	2	0.4	0	0	28	0.04	43 (4)	0.29	7 (2)	7
S. bedfordi	20	4	0	0	41	0.06	7	0.05	0	0
S. clydei	0	0	16 (5)	1.33	571 (32)	0.85	49 (4)	0.33	1 (1)	1
S. ingrami	1	0.2	0	0	8	0.01	0	0	0	0
S. schwetzi	0	0	1 (1)	0.08	28 (3)	0.04	0	0	1 (1)	1
S. squamipleuris	2	0.4	3	0.25	341 (5)	0.51	36 (2)	0.24	0	0

Total	**32**	**6.4**	**32 (6)**	**2.66**	**1341 (44)**	**1.9 9**	**207 (14)**	**1.3 9**	**12 (4)**	**12**

The numbers in parentheses indicate blood-fed sandflies per habitat. N: sandfly counts; D: species density measured as counts/trap/night.

Molecular sandfly species identification

We amplified the *Cox1* gene of 60 specimens representing all the *Phlebotomus* and blood-fed *Sergentomyia* species identified in the study locations to verify the morphological species identifications. BLAST analysis of the *Cox1* sequence from *Ph. guggisbergi* revealed a high percentage identity (99.04%) with those of *Ph. guggisbergi* in GenBank (MK169221). Likewise, *Ph. martini, Ph. orientalis*, *Ph. saevus* and the *Sergentomyia* species identified in this study showed 98-100% sequence identities with their respective species in GenBank (JX105040, KC204967, KF483673). The species were further separated into genus-specific and sub-genus-specific clusters by phylogenetic analysis (Fig. 2). All the specimens from this study clustered into a similar sub-branch with sequences of their respective species retrieved from GenBank, confirming the morphological identifications. GenBank accession numbers for sandfly *Cox1* sequences generated from this study are OP824959-OP824975.

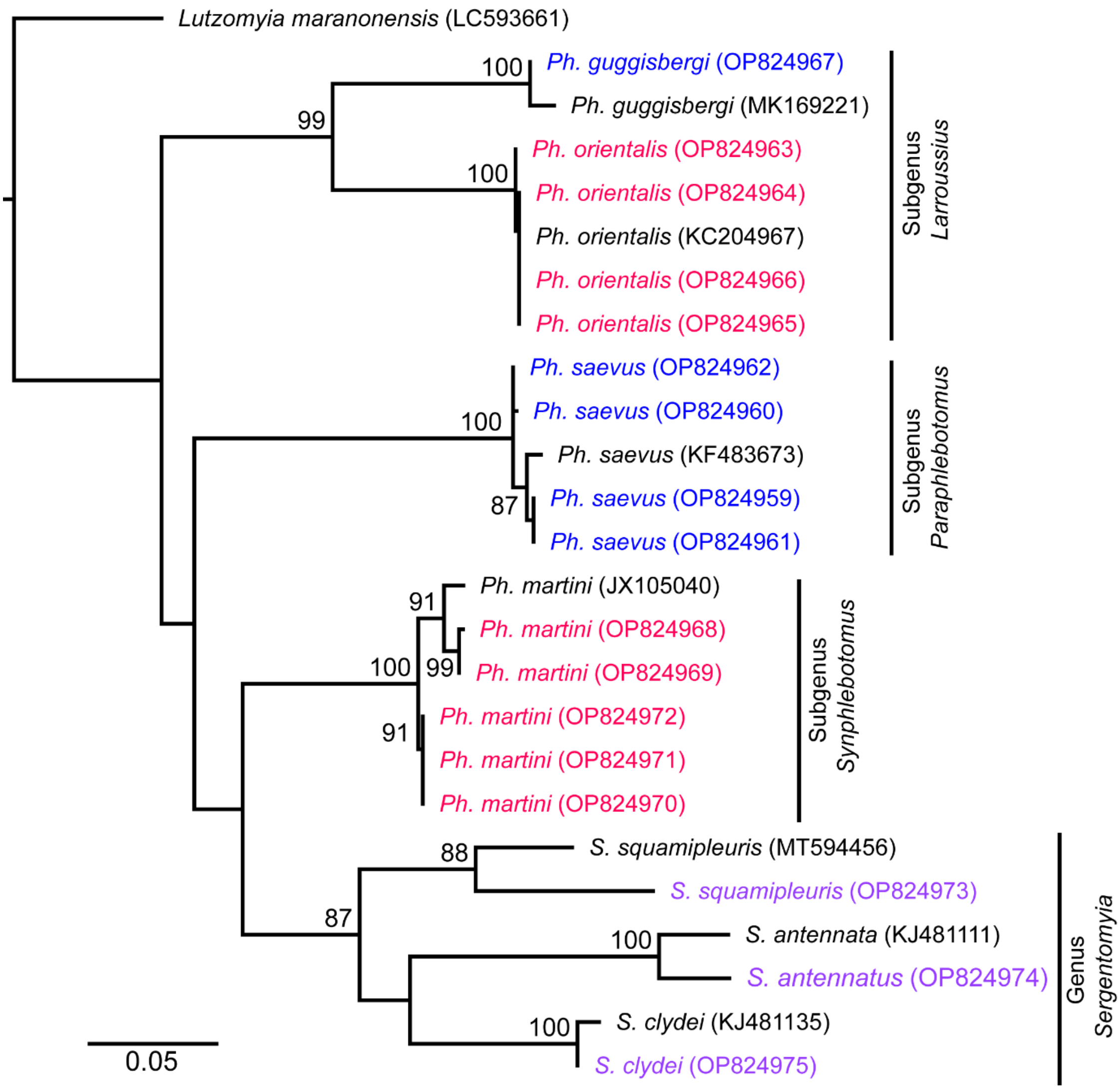

Fig. 2. Phylogenetic tree of the morphologically identified sandfly specimens using the Maximum Likelihood method. The phylogeny was inferred from 608-687 bp *Cox1*

sequences using the GTR model in Geneious prime (v2022.2.2). The values above the branches indicate bootstrap support values >85% after 1000 replications. We included *Lutzomyia maranonensis* as an outgroup. Sequences of sandfly specimens identified in this study are highlighted in red for VL vectors, blue for CL vectors, and purple for *Sergentomyia* spp. The scale bar represents substitutions per site.

Determination of sandfly bloodmeal sources in the Kajiado West sub-county

We determined the bloodmeal sources of 42 blood-fed sandflies, representing 61.8% (42/68) of all the engorged specimens (Table 5). Thirty-four (80.9%) of the blood-fed sandflies derived their blood meals from humans (*Homo sapiens*), with the crude human blood index (HBI) ranging from 36.6-100% among the different sandfly species. However, when we excluded the specimens with failed cytochrome-b (*Cyt-b)* amplifications and were thus unidentified, the adjusted HBIs increased dramatically, ranging from 71.4-100% among the sandfly species.

Table 5: Bloodmeal sources and human blood indices (HBIs) of sandflies in the Kajiado West sub-county

Sandfly species	Bloodmeal sources							%HBI	
	Human	Goat	White-tailed mongoose	Rock hyrax	Donkey	N/A	Total	Crude HBI	Adjusted HBI
Ph. martini	2	0	0	0	0	0	2	100	100
Ph. saevus	2	0	1	0	0	2	5	40	66.7
S. antennatus	6	0	0	0	0	0	6	100	100
S. clydei	15	4	1	0	1	20	41	36.6	71.4
S. schwetzi	6	0	0	0	0	0	6	100	100
S. squamipleuris	3	0	0	1	0	3	7	42.9	75
S. adleri	0	0	0	0	0	1	1	-	-
Total	**34**	**4**	**2**	**1**	**1**	**26**	**68**	**-**	**-**

We calculated the crude HBI using all the collected specimens for each species as the denominator. For the adjusted HBI calculations, we excluded the samples in which the *Cyt-b* amplification failed. HBI: human blood index; N/A: failed amplification. We rounded all the HBI values to 1 decimal place.

Among the *Phlebotomus* specimens, the only two specimens of *Ph. martini* analysed had fed exclusively on humans (Fig. 3). These specimens were sampled in peridomestic areas from traps that were set near termite hills. *Ph. saevus* obtained its blood meal from humans (n= 2) and white-tailed mongoose (*Ichneumia albicauda*) (n= 1). *Sergentomyia schwetzi* and *S. antennatus* fed exclusively on humans. On the other hand, *S. clydei* and *S. squamipleuris* obtained their bloodmeals from a broad host range, including domestic animals (donkeys and goats) and wildlife (rock hyrax and white-tailed mongoose).

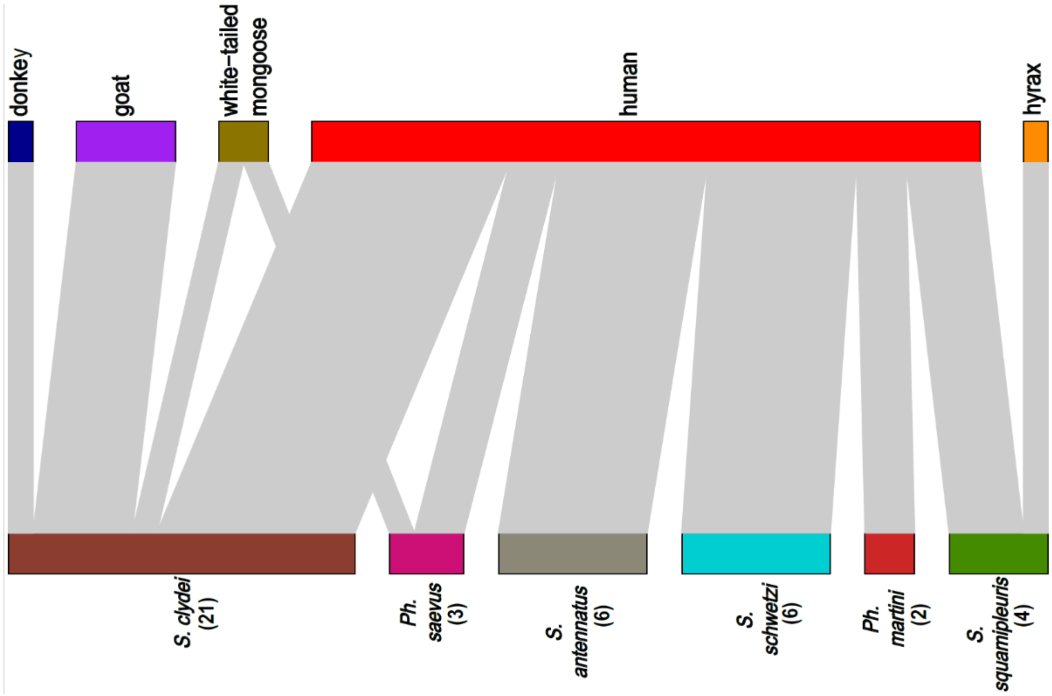

Fig. 3. Sandfly bloodmeal sources in Kajiado West sub-county. A bipartite graph showing the interactions between the blood-fed sandfly species and vertebrate hosts in the study area. The values in parenthesis indicate the number of blood-fed sandflies per species.

Leishmania parasite species screening and identification in sandflies and clinical samples

We screened a total of 450 sandflies (fed (F): 61; unfed (U): 389) for the presence of *Leishmania* DNA by PCR analysis of the *Leishmania* ITS1 region, followed by HRM analysis and sequencing. The samples comprised *Ph. martini* (F: 2; U: 42), *Ph. orientalis* (U: 15), *Ph. saevus* (F: 5; U: 20), *S. adleri* (F: 1; U: 81), *S. africanus* (U: 18), *S. antennatus* (F: 6; U: 26), *S. bedfordi* (U: 58), *S. clydei* (F: 41; U: 124), *S. ingrami* (U: 2), and *S. schwetzi* (F: 6; U: 3). We detected *Leishmania* DNA in 4.7% (21/450) of the specimens. *Leishmania donovani* was the predominant parasite species in 4.4% (20/450) of the sandflies tested. We detected *L. donovani* DNA in *S. clydei* (n= 13), *S. adleri* (n= 4), *S. antennatus* (n= 2), and *Ph. saevus* (n= 1). *Leishmania tropica* was detected only in one specimen of *Ph. saevus* (1/450).

To confirm the *Leishmania* species responsible for CL in the sub-county, we analysed six lesion samples by PCR and sequencing. A BLAST search of GenBank[30] using the ITS1 sequences from the lesion samples revealed a 100% identity with *L. tropica* sequences. The sequences and those from the sandfly specimens were further separated into either *L. tropica* or *L. donovani* clusters by phylogenetic analysis (Fig. 4), thus confirming both species in circulation. GenBank accession numbers for *Leishmania* ITS1 partial sequences generated from this study are OP811443-OP811454.

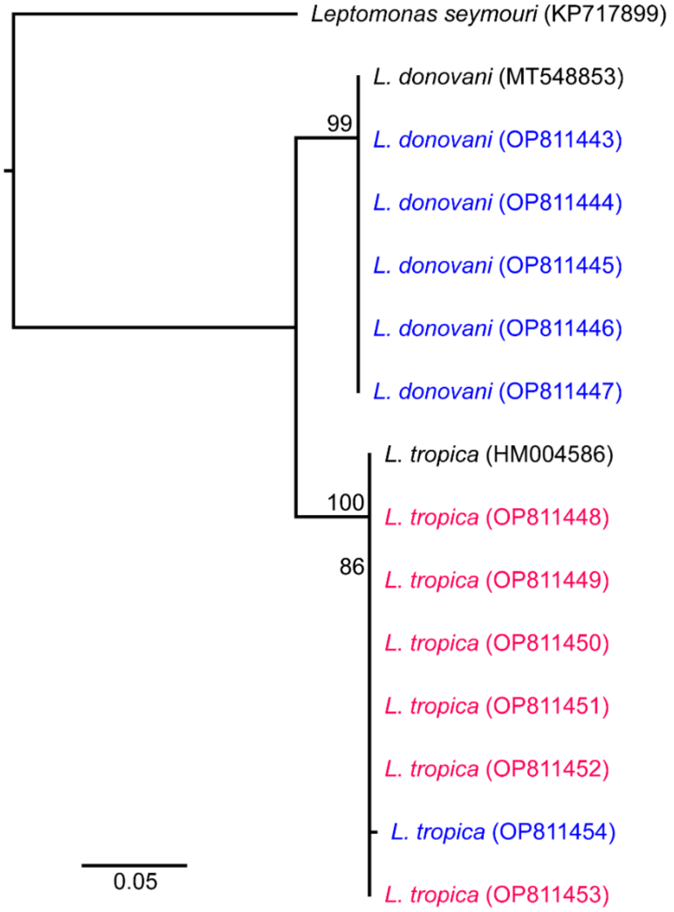

Fig. 4. Maximum Likelihood phylogenetic tree of representative *Leishmania* ITS1 sequences from sandfly and lesion samples collected from the Kajiado West sub-county. We constructed the phylogeny in Geneious prime (v2022.2.2) using the GTR model and *Leptomonas seymouri* as an outgroup. Bootstrap support values >85% are indicated next to the branches and are of agreement among 1000 replicates; the blue and red highlight parasite sequences isolated from sandfly specimens and CL lesions, respectively. The scale bar represents substitutions per site.

Discussion

Based on a recent population survey, this study aimed to investigate the resurgence of leishmaniasis in Kajiado County, Kenya. To our knowledge, only one prior report exists in the public domain from 1993, when three children were diagnosed from one village.[15]

We implemented a coordinated approach in response to the outbreak that included capacity building, where we trained ten CHVs, four clinicians, seven healthcare workers and established two treatment centres in the area. Success of the training was evaluated based on the trainees' ability to accurately identify VL and CL cases, and effectively manage the cases using the approved guidelines. We also undertook medical screening and diagnosis of leishmaniasis and other medical conditions and conducted epidemiological and entomological surveys. Among those screened and tested using rK39, we found 10 patients to be positive for VL caused by *L. donovani,* potentially indicating an increased transmission of the parasite in the area. However, given that we initially mobilised the cases to the local health facilities for screening and focused only on the symptomatic cases, the true burden of VL in the sub-county remains obscure.

VL predominantly affected children (below 15 years), with more infections in males than females, corroborating reports from other endemic areas in Kenya[31] and elsewhere.[4,32] We found a high CL positivity rate in the sub-county, with all the cases originating from Esonorua and Musenke locations. Unlike VL, CL predominantly affected people aged ≥15 years and was more frequent in females than males. This finding contrasts previous reports in the Eastern Mediterranean region, which showed a relatively well-balanced distribution of CL by gender,[32] and those from the region of the Americas where the distribution was more biased towards males.[32] Nevertheless, further studies employing a larger sample size are needed to precisely ascertain the status of VL and CL distribution by age/gender in Kenya's endemic areas, which could inform the implementation of control interventions. We know that since the establishment of the two treatment Centres, more than 200 patients have been treated for both VL and CL (MoH data, unpublished), highlighting the significant impact of this coordinated response, and contribution to elimination goals.

We examined sandfly species distribution, abundance, bloodmeal sources, and evidence of *Leishmania* parasites. Our results indicate the occurrence of at least eight *Sergentomyia* and four *Phlebotomus* sandfly species in Kajiado County. We detected *L. donovani* DNA in several sandfly species, including *Sergentomyia* species: *S. clydei*, *S. adleri* and *S. antennatus,* which are considered non-vectors of human leishmaniasis in the Old World.[33,34] However, there are increasing reports of *Leishmania* DNA detection in *Sergentomyia* species from several leishmaniasis endemic areas, including Kenya,[22,34,35] which call for further studies on the vector competence of these sandfly species in the context of global VL elimination targets.

The *Phlebotomus* sandfly species identified in this study included *Ph. martini, Ph. orientalis, Ph. guggisbergi* and *Ph. saevus.* The four species represent three of the five *Phlebotomus* subgenera described in Kenya,[9] highlighting the enormous richness of sandfly fauna in Kajiado County. *Ph. martini* and *Ph. orientalis* are the primary and secondary vectors of VL in Kenya, respectively.[10] Although we did not detect *L. donovani* infections in the two sandfly species, their occurrence in Kajiado County poses a transmission risk, as previously suggested.[15]

Phlebotomus guggisbergi is the primary vector of *L. tropica* in Kenya.[8,12] However, the sandfly species occurred in relatively low numbers in Kajiado County, and we did not sample females of this species. We could attribute the low numbers of *Ph. guggisbergi* in this study to the period of sandfly sampling, which may not have been appropriate for the species: we conducted sandfly sampling during the hot and dry seasons at <1000 m above sea level. However, studies in Kenya have reported that *Ph. guggisbergi* prefers relatively cool and humid habitats, including caves, cliffs and rock crevices, and occurs in abundance at altitudes of about 2000 m above sea level.[9,12] We detected *L. tropica* DNA in *Ph. saevus,* consistent with previous reports in Kenya[12] and Ethiopia.[36] Identifying *L. tropica* in *Ph. saevus* suggests that they are likely vectors of CL in Kajiado County and possibly *L. tropica* in Kenya. We also detected *L. donovani* DNA in one *Ph. saevus* specimen, but the vector competence of this species for this VL parasite requires further investigation.

We sampled more sandflies in peri-domestic habitats where most ant hills were than in domestic areas. The finding of more blood-fed sandflies in peri-domestic areas suggests that sandfly biting in Kajiado County occurs mainly outdoors. Termite hills had the highest sandfly abundance, most of which were blood-fed, suggesting that they are the primary breeding habitats for sandflies in the County. Notably, we found a significantly higher proportion of *Ph. martini* in termite hills, corroborating other studies in Kenya, which identified termite hills as the most preferred resting/breeding sites for the species.[10,37] However, we also sampled *Ph. martini* from livestock sheds, animal burrows and near rock crevices, highlighting the complex ecology of *the species* in this area. In contrast, *Ph. saevus* were sampled mainly from rock crevices and a few around termite hills. The occurrence of *Ph. saevus* in rock crevices in Kajiado County corroborates other reports from a CL endemic area in Kenya, which characterised rock crevices as the breeding/ resting sites for the species, however, further studies are needed to affirm this.[8,12]

The *Phlebotomus* species identified in this study were sampled almost exclusively outside houses, indicating that they are highly exophilic. We found sandfly species distribution to be significantly different across the study locations. These likely influence transmission dynamics, in addition to other variables such as human mobility. Most of Kajiado County's population are nomadic pastoralists and can move between endemic and non-endemic locations in search of fresh pastures and water, predisposing them to sandfly bites, which could facilitate the spread of *Leishmania* parasites. According to several authors,[1,38–41] the combination of favourable climatic conditions, abundant and diverse sandfly vectors, migration and conflict, and deficient health systems create a conducive environment for the emergence or re-emergence of leishmaniasis. Favourable climatic conditions, rich sandfly fauna, limited access to leishmaniasis treatment, and migration of these nomadic pastoralist populations could explain the re-emergence of leishmaniasis in the County.

Accurate identification of sandfly bloodmeal sources is crucial in determining the disease transmission patterns and developing effective disease control strategies. Our results show that humans are the primary sources of sandfly bloodmeals in the Kajiado West sub-county, with a high percentage of human blood indices (HBI) in sandfly bloodmeals. HBI measures the proportion of vector bloodmeals obtained from humans and, thus, can be used to assess the effectiveness of vector control programmes.[42,43] We however found several unidentified bloodmeal sources, likely from a range of mammals, indicating a high diversity of sandfly hosts. The high HBI in *Ph. martini* and *Ph. saevus* sandflies may indicate an increased risk of VL and CL transmissions in Kajiado County and could further explain the occurrence of VL and probable CL cases in the area. The exclusive feeding of *Ph. martini* on humans suggests that the species is anthropophilic, however, this reflects bloodmeals obtained from sandflies that were caught in the peri-domestic environment. *Ph. saevus,* on the other hand, exhibited a broader host range. Although the transmission of CL caused by *L. tropica* is regarded chiefly as anthroponotic,[43] our findings suggest the possibility of zoonotic transmission of the parasite in Kajiado County since *Ph. saevus* does not feed exclusively on humans.

Most VL and CL endemic areas in Kenya exist allopatrically[6] except Baringo County which is a well-established focus for both forms of leishmaniasis.[44,45] Our results show that both VL and CL occur in Kajiado County. As observed in Sudan,[46] the possible hybridization is of concern from a public health and patient management perspective and should be further investigated from a larger sample size of vectors and patients.

Conclusion

Our study demonstrates the value of a coordinated response to an outbreak, resulting in access to treatment Centres that did not exist before the intervention. We thus have increased awareness of the disease in the area, leading to better response times for case management. This study adds to our understanding of the extent of VL spread in Kenya, considering the new focus of East Africa as a transmission hotspot, and a targeted region for elimination. We demonstrate the occurrence of both CL and VL in Kajiado County, with an overlap in the geographical areas for the two diseases. We found both *L. donovani* and *L. tropica* in circulation. Analysis of sandfly blood meal sources revealed that humans were the main sandfly hosts. The high human blood index (HBI) in sandfly bloodmeals could indicate increased VL and CL transmissions in the County. The detection of *L. donovani* in *S. clydei* and *S. antennatus,* sandflies with anthropophagic behaviour, requires further investigations to determine their role, if any, in transmitting VL in Kajiado County. Detecting *L. tropica* and *L. donovani* in *Ph. saevus,* which may implicate the sandfly as a permissive vector of the *Leishmania* species, also requires further investigations. Moreover, additional studies are needed to determine the reservoir hosts of *L. donovani* and *L. tropica* in the County and the factors behind the recent outbreak after a long dormancy period. This study crucially adds to a small number of leishmaniases vector studies in Kenya and fills gaps in geographic understanding of the disease in the country, knowledge that will contribute to national and global elimination goals.

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Conflict of interest

The authors declare that they have no competing interests.

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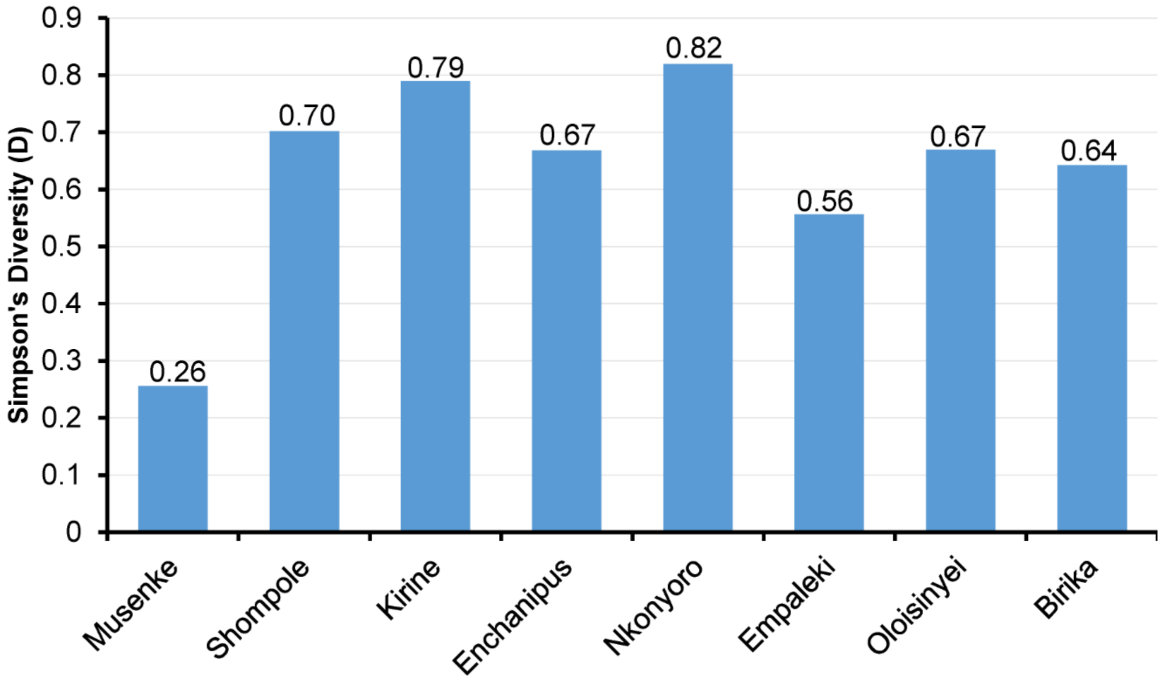
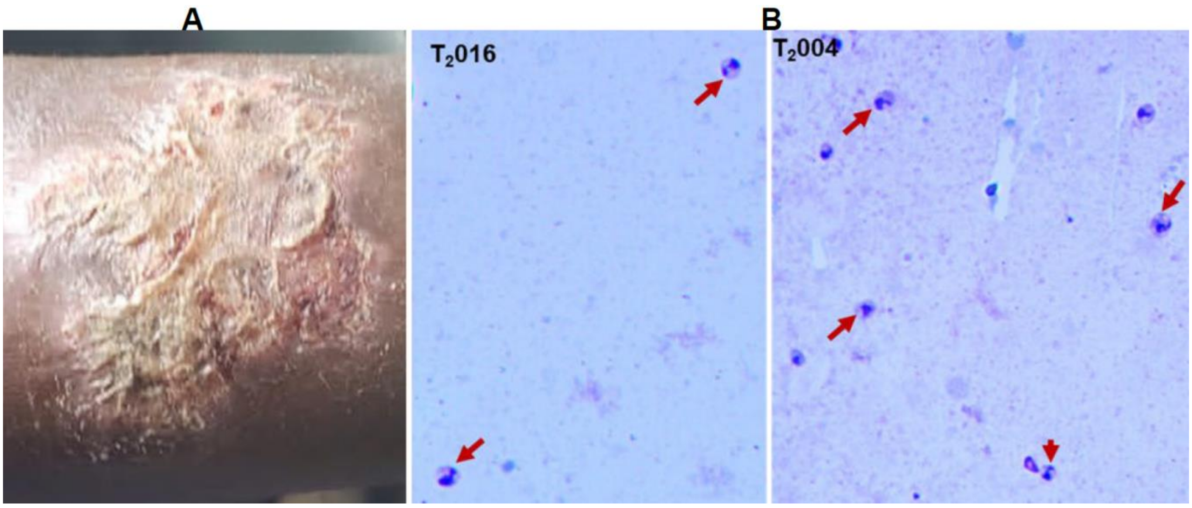
Supporting information



S1_Fig. Sandfly diversity among the sampling locations in the Kajiado West sub-county.



S2_Fig. Cutaneous leishmaniasis screening in Kajiado West sub-county. A: CL patient, C: Giemsa-stained CL lesion smears showing *Leishmania* amastigotes (red arrows) at x1000 magnification using Olympus CX31 compound microscope.