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RESEARCH ARTICLE

A case–control study on risk factors for visceral leishmaniasis in West Pokot County, Kenya

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Abstract

Background: Visceral leishmaniasis (VL) is a severe parasitic disease transmitted by phlebotomine sandflies. VL is endemic in West Pokot County, Kenya, where effective strategies to interrupt transmission are impeded by the limited understanding of VL risk factors. Therefore, this case–control study aimed to explore environmental, behavioural and household determinants of VL in West Pokot.

Methods: From November 2022 to January 2023, a structured questionnaire was administered to 36 symptomatic primary VL cases attending Kacheliba Sub-County Hospital in West Pokot and to 50 healthy controls from local villages. The VL status of all participants was confirmed using an rK39 rapid diagnostic test. Associations between questioned determinants and VL were investigated by means of age-corrected univariate logistic regression analysis.

Results: Significant associations were found between VL and housing characteristics, such as window presence and floor type. VL cases more frequently reported the presence of cattle, dogs and sheep in their house yards. VL was also associated with cutting down trees in the house yard and house proximity to several *Acacia* tree species. Furthermore, outdoor activities, including travelling outside the residence for more than 2 weeks, activities near termite mounds, and forest activities during the rainy season, increased the risk of VL.

Conclusions: This work reports a number of previously undescribed risk factors for VL in the understudied West Pokot focus. The results suggest VL transmission occurs both peri-domestically at night and outdoors during the day, particularly when sandfly resting sites are disturbed. Our findings warrant further research into sandfly ecology and potential zoonotic parasite reservoirs in West Pokot.

KEYWORDS
case–control study, Kenya, risk factors, sandfly, visceral leishmaniasis

INTRODUCTION

Visceral leishmaniasis (VL), also called kala-azar, is a serious parasitic infection caused by the protozoa *Leishmania donovani* or *Leishmania infantum*. These parasites are transmitted from host to host by blood-feeding phlebotomine female sandflies (*Diptera*: *Psychodidae*). Patients with VL present with chronic fever, abdominal swelling and weight loss, and progressively deteriorate. If left untreated, VL will eventually be fatal in 95% of cases [1]. The eastern African region, where several countries face endemic transmission of *L. donovani*, has the highest VL case load in the world [2]. VL is mainly found in rural areas with impoverished populations suffering from poor health and high rates of malnutrition [3]. Consequently, the burden of VL causes a vicious cycle of disease and poverty in these communities [4].

Sustainable Development Goal: Good Health and Wellbeing

In Kenya, a major VL focus is located in West Pokot County, situated in the northwest on the border with Uganda [5]. Inhabitants of West Pokot are mainly pastoralists of the Pokot tribe, and VL (locally referred to as *termes*) mostly affects children and adolescents below the age of 15 years [6]. VL in West Pokot is generally considered to be anthroponotic, but due to a paucity of research into *L. donovani* infections in wild or domestic animals, it is unknown whether a zoonotic reservoir exists. The semi-arid climate in this lowland area, with seasonal rains and sparse vegetation with *Acacia* trees, creates a favourable environment for *Phlebotomus martini*, the local sandfly vector transmitting *L. donovani* [7, 8]. *P. martini* is known to breed in the ventilation shafts of termite mounds [9, 10]. Besides this breeding site preference, many characteristics of *P. martini*, such as habitat aspects and blood feeding behaviour, are still poorly defined, complicating the design of effective vector control strategies. The current passive case detection is insufficient to reduce the VL burden in West Pokot, as demonstrated by the significant increase in annual incidence in recent years: in 2022, the VL treatment centre at Kacheliba Sub-County Hospital registered 600 confirmed VL cases, compared to 245 cases in 2019 (unpublished data, patient records from Kacheliba Sub-County Hospital, West Pokot County, Kenya).

Effective interventions to combat VL transmission warrant better characterisation of associated risk factors. Apart from the nutritional, immunological and genetic status of the human host, the risk of acquiring VL is dependent on the presence of, and exposure to, its sandfly vector [6, 11, 12]. Although multiple studies have investigated behavioural and environmental VL determinants in endemic regions in Ethiopia and Sudan, where *Phlebotomus orientalis* is responsible for disease transmission, these results may not be translatable to the context of West Pokot due to different vector biology [13, 14]. Reliable data on VL determinants in West Pokot are scarce. A study from Kolaczinski *et al.* [15] described a number of VL-associated factors, including increased risk of infection due to low socio-economic status, and protective effects of mosquito net ownership and sleeping near livestock. To date, these findings have not been confirmed by other studies, nor have they been addressed in detail. Therefore, this case–control study aimed to further explore potential behavioural, environmental and household factors that could increase the risk of developing VL in West Pokot, Kenya.

METHODS

Ethics

Ethics clearance was obtained from the Amref Health Africa Ethics and Scientific Review Committee (ESRC—ref. ESRC P1196/2022), and a research licence was obtained from the National Commission for Science, Technology and Innovation (NACOSTI—ref. 791964). To all potential participants, the study and its procedures were orally explained in local Pokot language by a clinical officer and/or community health worker. All participants, or the parents or legal guardians of minors, provided written informed consent before enrolment into the study. Illiterate individuals gave their consent through fingerprint, and a literate witness who was not part of the study team would sign to confirm that the informed consent form was accurately read to the participant, with the opportunity to ask questions. All collected data were anonymised.

Study setting

This study was performed in West Pokot County, Kenya, which lies on a plateau at 1000–1500 m above sea level, bordered by the Eastern Rift Valley in the east (see Figure 1). The area experiences a long rainy season from April until June, and short rains in November and December. West Pokot has a population of 621,000 (census 2019), of which 94.9% live in rural areas with limited infrastructure or healthcare facilities [16]. About half the population is below 15 years of age, and 36% of the population has not received any formal education [16]. Pastoralism is the most frequent occupation, practiced mostly by adolescent and adult males, whereas females are generally responsible for taking care of the household and children. Pastoralist villages consist of several separated compounds of houses, called *manyattas*, surrounded by a fence of thorny *Acacia* branches; each *manyatta* is inhabited by several family households.

Participant enrolment

This study had a matched case–control design. Following a pragmatic sampling approach, all participants were enroled from November 8, 2022, to January 8, 2023, during the short rains period and the start of the subsequent dry season. Cases were untreated VL patients who were recruited at Kacheliba Sub-County Hospital. This is the reference centre for VL diagnosis and treatment in West Pokot, run by the Ministry of Health with support from Drugs for Neglected Diseases initiative [6]. Patients with clinical symptoms of VL, including splenomegaly, chronic fever and weight loss, were tested with the IT LEISH (Bio-Rad, Hercules, USA) rK39 rapid diagnostic test (RDT) for the presence of anti-*Leishmania* antibodies in finger prick blood, following the hospital's standard diagnostic practice. The sensitivity and specificity of IT LEISH in Kenya are approximately 89% and 90%, respectively [17]. In case of a positive rK39 RDT, the patient was asked to participate in the study through an informed consent procedure, followed by administration of a structured questionnaire (see below). In addition, results of standard diagnostic testing for malaria through blood film microscopy were also collected.

To ensure sufficient analytical power in case of a potentially low recruitment number of VL cases, this study aimed

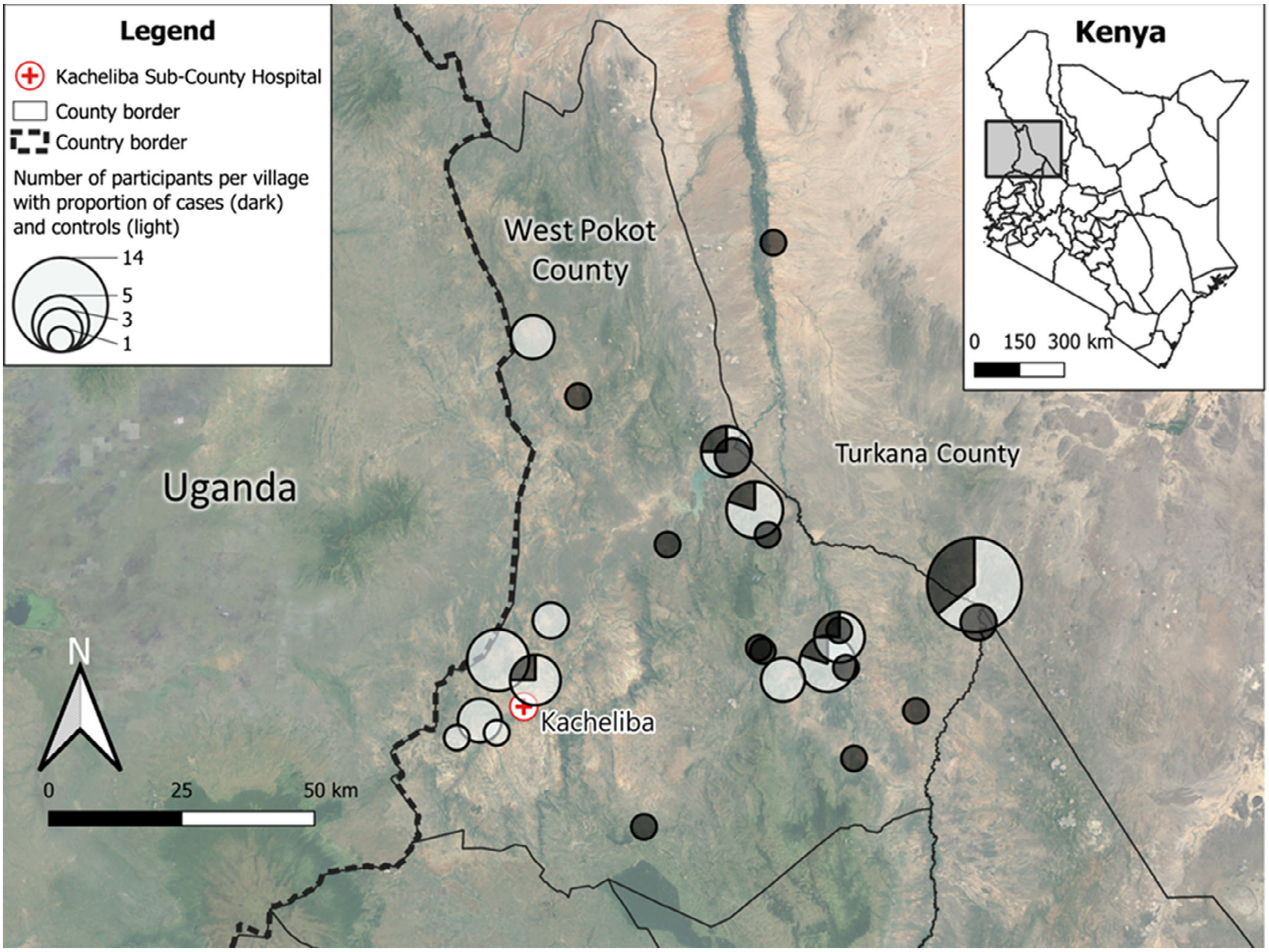

FIGURE 1 Map of the study area in West Pokot County, Kenya, with the location and number of participants for 26 of the 38 participant villages for which GPS location data were available. Map was created using QGIS Desktop software (version 3.16). Source satellite imagery: Google Earth Engine.

to recruit two to four healthy controls per enroled VL case [18]. Where possible, controls were matched by sex, age and village. A study team travelled to villages in West Pokot from which a VL case originated, where a house was randomly selected by spinning a pen at a central point in the village. At the selected house, an eligible household member was chosen to match the sex and age (within a range of ±2 years) of the VL case from that village. A matched candidate control participant had to be free of the VL-associated symptoms described above, and have no self-reported history of VL. After providing informed consent, the participant was tested with rK39 RDT. In case of a negative test result, the healthy control was interviewed using a structured questionnaire (see below), and malaria status was determined on finger prick blood with SD Bioline Malaria Differential P.f/Pan rapid test (Standard Diagnostics Inc., Suwon, South Korea). If the rK39 RDT was positive, the participant was excluded and referred for care at Kacheliba Hospital, and another household member was randomly selected for participation in the study. After the interview, a pen was spun in front of the house to select the next house for recruiting a healthy volunteer. These household visits were repeated until at least two healthy controls per VL case from each selected village were recruited.

Questionnaire

A structured questionnaire was administered to each study participant by a trained interviewer (File S1). The survey had an exploratory design and included potential determinants for VL based on available literature. Questions related to housing characteristics, day- and night-time activities, sleeping habits, and the house yard and its immediate surroundings, including the presence of domestic animals. When the answer to a question was expected to vary depending on the season, separate answers were obtained for the dry and rainy season. For questions about the presence of tree species in or surrounding the house yard, the vernacular Pokot tree names reported by the participant were recorded. During data analysis, the scientific tree species names corresponding to these Pokot names were retrieved from a previous publication [19].

All interviewers were experienced in administering health and socioeconomic surveys, Chara and received instructions for each individual question in the study's questionnaire before the start of recruitment. The questionnaire was in English and was piloted by the interviewers on VL patients at Kacheliba Hospital before the study start. The interviews were conducted in Swahili or the local Pokot language through the assistance of a local interpreter. For participants below 15 years of age, the parent or legal guardian was allowed to assist or answer on behalf of the child. Answers provided by the participants were entered directly into the smartphone application KoboCollect (version v2022.2.3, KoboToolBox, Cambridge, MA, USA).

Data analysis

Questionnaire data were exported from the KoboCollect online database into Microsoft Excel 2016. The Excel file was imported into the statistical software IBM SPSS Statistics (version: 28.0, Armonk, NY, USA) for data processing and analysis. Associations between determinants and VL were studied using univariate logistic regression and expressed as odds ratio (OR). In case a statistically significant difference in age and/or sex was found between cases and controls, adjustment for these variables was applied. p-values of the found associations were obtained using the Wald test. Associations with a p-value of <0.05 were considered statistically significant. In case of missing data for a particular variable, the participant was excluded from the analysis of this variable. To explore the relationships between the independent variables that were significantly associated with VL, χ^2 testing was performed for each possible combination of these variables. In case the expected frequency in one of the contingency table cells was lower than five, Fisher's exact test was used instead. A significance level of $p < 0.05$ was used.

RESULTS

Study participant demographics

Thirty-six rK39 RDT-confirmed VL patients were recruited at Kacheliba Sub-County Hospital and 50 healthy controls were recruited from villages in West Pokot (see Figure 1). The majority of enroled VL cases were from the northern and eastern parts of West Pokot, which were difficult to reach due to the distance from Kacheliba Hospital, as well as security concerns. As a result, a number of healthy controls were not village-matched with cases, but recruited in villages less than half a day's travel from Kacheliba. All recruited cases and controls ($N = 86$) were included in the analysis.

Participants came from 38 different villages, of which 36 were situated in West Pokot County, one in Turkana County and one in Trans-Nzoia County (see Table 1). Cases had a median age of 11 years (interquartile range: 5–20 years), whereas controls (median 6 years, interquartile range: 2–9 years) were significantly younger ($p = 0.001$). The proportion of females was similar in controls and VL cases (50% and 47%, respectively, see Table 1).

TABLE 1 Demographics of visceral leishmaniasis (VL) cases and controls enroled in the study.

		Controls		VL cases	
Characteristics	**Categories**	***N***	**%**	***N***	**%**
Sex	Female	25	50	17	47
Age group	0–9 years	39	78	16	44
	10–19 years	8	16	9	25
	20–29 years	3	6	8	22
	30 years and above	0	0	3	8
Village of residence	Akulo	9	18	5	14
	Asilong	6	12	0	0
	Dungdung	4	8	1	3
	Nasolot	4	8	1	3
	Katuperot	3	6	1	3
	Lonyangalem	3	6	1	3
	Marich	3	6	1	3
	Ausikion	3	6	0	0
	Kapeta	3	6	0	0
	Kases	3	6	0	0
	Pokatusa	3	6	0	0
	Akorikwang	0	0	2	6
	Amoler	0	0	2	6
	Kongelai	2	4	0	0
	Lokatukoi	2	4	0	0
	Turkwel	0	0	2	6
	22 other villages	2	4	20	56

Note: Names of villages with two or more participants (cases and/or controls) are listed.

VL risk factors

Due to the different age distribution of cases and controls, associations of all other variables with VL were studied after adjustment for age. All variables with a statistically significant association ($p < 0.05$) with VL are shown in Table 2. A complete overview of the univariate analysis with age correction is shown in Table S1.

There were two VL cases with a concurrent *Plasmodium falciparum* infection, based on malaria microscopy done at the time of hospital admission. All healthy controls had a negative result for malaria RDT, and malaria status could thus not be associated with VL risk. The household size of cases and controls was similar (mean 7.27 and 7.64 household members, respectively). Among those of school-going age and above, cases were more frequently illiterate than controls (41% vs. 16%, respectively), of which the majority

TABLE 2 Determinants associated with visceral leishmaniasis (VL) ($p < 0.05$) after univariate logistic regression analysis with age correction.

		Controls		VL cases			95% CI		
Variable	**Categories**	***N***	**%**	***N***	**%**	**Adjusted OR**	**Lower**	**Upper**	***p*-value**
Travel outside village >2 weeks	Yes (vs. no)	1	2	10	28	13.98	1.62	120.9	0.017
House floor type	Sandy soil	31	62	7	19	1.00			0.002
	Mixture based from cattle dung	4	8	18	50	12.98	3.12	54.01	<0.001
	Earthen covered with gravel	14	28	2	6	0.38	0.06	2.33	0.294
	Cement/concrete	1	2	3	8	7.09	0.54	92.64	0.135
	Clay	0	0	3	8	NA			
	Black cotton soil	0	0	2	6	NA			
	Wood	0	0	1	3	NA			
Windows present in house	Yes (vs. no)	1	2	8	22	9.99	1.09	91.37	0.042
Open water bodies in yard during rainy season	No	24	48	20	56	1.00			0.008
	Yes, stagnant rain water	9	18	15	42	2.31	0.76	6.99	0.139
	Yes, bucket/open container	17	34	1	3	0.06	0.01	0.59	0.015
River within visual range of the house	No	26	52	20	56	1.00			0.013
	Yes, at 10–100 m	2	4	10	28	4.70	0.85	26.10	0.077
	Yes, at >100 m	22	44	6	17	0.33	0.11	1.02	0.055
Tree species around the house									
Acacia seyal	Yes (vs. no)	3	6	17	47	21.11	4.94	90.20	<0.001
Terminalia brownii	Yes (vs. no)	9	18	18	50	4.91	1.70	14.14	0.003
Acacia senegal	Yes (vs. no)	6	12	24	67	19.75	5.74	67.88	<0.001
Acacia mellifera	Yes (vs. no)	20	40	26	72	6.16	2.03	18.70	0.001
"Panyirit" tree (*Acacia etbaica*, *Acacia reficiens*, *Acacia mellifera*)	Yes (vs. no)	10	20	17	47	4.61	1.61	13.26	0.005
Trees cut down in yard in past year	Yes (vs. no)	5	10	9	25	4.50	1.25	16.15	0.021
Animals in yard (during day or night)									
Dogs	Yes (vs. no)	1	2	10	28	26.78	3.00	239.3	0.003
Cattle	Yes (vs. no)	14	28	27	75	15.39	4.12	57.42	<0.001
Sheep	Yes (vs. no)	19	38	24	67	6.08	1.94	19.10	0.002
Activities in forest during day (rainy season)	Never	38	76	11	31	1.00			0.007
	Rarely	11	22	14	39	3.18	1.06	9.51	0.039
	Frequently	1	2	11	31	23.22	2.56	210.9	0.005
Activities close to termite mounds (dry season)	Never	44	88	15	42	1.00			<0.001
	Rarely	4	8	13	36	13.79	2.28	83.34	0.004
	Frequently	2	4	8	22	11.09	2.83	43.49	<0.001
Activities close to termite mounds (rainy season)	Never	45	90	15	42	1.00			<0.001
	Rarely	3	6	16	44	20.69	4.74	90.31	<0.001
	Frequently	2	4	5	14	8.15	1.15	57.64	0.036

Abbreviations: 95% CI, 95% confidence interval; NA, not applicable; OR, odds ratio.

(76%) were still attending primary school. After excluding children that were reported to be too young to have an occupation, cases were more frequently involved in animal herding than controls, for whom taking care of the house was most often reported. Neither education nor occupation significantly affected VL risk.

Travelling outside the village of residence for more than 2 weeks in the past year was significantly associated with increased VL odds. Subjects mainly travelled to other villages in West Pokot, except for one case that travelled to neighbouring Baringo County (data not shown).

When reviewing nighttime and sleeping habits, there was no significant association between VL risk and bedtime, location after sunset, sleeping location within own house (yard), number of people sleeping in the same room as the participant, nighttime toilet location or outdoor activities around sunrise (6.30 a.m.). While all interviewed controls normally slept in their own house or yard, 31% (dry season) and 19% (rainy season) of the VL cases frequently slept somewhere else in the village, in a neighbouring village or in a field. An OR could not be calculated for this variable as there were no observations of controls sleeping away from their house yard.

Houses of both cases and controls were most often constructed with mud walls without outer sheeting and roofs made of grass and wood. Cracked walls were equally reported by both groups (8%). House floor type was significantly associated with increased VL risk ($p = 0.002$): controls most frequently had floors of sandy soil, while VL cases more often used a mixture based on cattle dung as flooring. Windows were significantly more common ($p = 0.042$) in the houses of VL cases. One healthy control and one VL case reported having windows with shutters, while there were three VL cases with a window screen or curtains, and four VL cases with uncovered windows. Indoor residual spraying with insecticides in the last year had been performed in the houses of only one control and three cases. House yard soil type and having compost or decaying material in the house yard were not significantly associated with VL, while having water in an open bucket or container in the yard during the rainy season was found to be associated with decreased VL odds ($p = 0.008$). VL cases more frequently reported to have cut down trees in the house yard in the past year ($p = 0.021$).

No associations with VL were found for house proximity to a pond, well, irrigated agriculture or termite mounds. However, distance of the house to a river was significantly associated with VL risk ($p = 0.013$), with cases more frequently living within 100 m from a river than controls. *Acacia seyal*, *Terminalia brownii*, *Acacia senegal*, *Acacia mellifera* and *panyirit* (Pokot vernacular name that can refer to *Acacia mellifera*, *Acacia recifiens* or *Acacia etbaica* [19]) trees close to the house also increased the risk of VL.

Having domestic animals inside the house at night was uncommon, but the majority of participants had animals in their house yard. Goats were most frequently reported by both VL cases and healthy controls, whereas cattle, sheep and dogs were all significantly associated with having VL ($p < 0.001$, $p = 0.002$ and $p = 0.003$, respectively). Other reported domestic animals included poultry, camels and donkeys, but these were not significantly associated with VL.

When considering outdoor activities during the day, activities close to termite mounds significantly increased the risk of VL infection in both dry and rainy seasons ($p < 0.001$). Additionally, activities in forests or woodlands during the rainy season were significantly associated with VL ($p = 0.007$). The most commonly reported forest activities included herding animals and collecting firewood (data not shown). Activities close to water bodies were not associated with VL.

Mosquito net use was equally reported by 64% of the cases and controls, with no difference with regard to the season. No statistically significant difference was found between VL cases and controls in the frequency of net use, net impregnation with insecticides, the period of net usage, the net condition or washing of the net. Use of skin insect repellents was only reported by one (VL case) participant.

Relationships between VL-associated independent variables

Several significant associations were found among the independent variables that were significantly associated with VL. The *p*-values of the χ^2 and Fisher's exact tests are shown in Table. *p*-values were significant between activities in forests and activities near termite mounds (in both dry and rainy seasons). These three independent variables were also significantly associated with travelling outside the village for more than 2 weeks, cutting down trees in the house yard, *Acacia* trees in its surroundings, and cattle presence in the house yard. House floor type was significantly associated with most of the other independent variables, although the high number of categories for this variable may have inflated these results.

DISCUSSION

This explorative study identified several household, behavioural and environmental factors that could contribute to increased risk of VL infection in West Pokot, including some that have not been described before for this region. VL risk was found to be associated with the presence of dogs, cattle and sheep in the house yard, housing aspects, and multiple tree-related factors, such as forest activities, felling trees in the house yard and house proximity to various tree species.

VL patients in this study were mostly children and teenagers, with similar numbers of both sexes. This sex distribution differs from the general epidemiology of the disease in West Pokot, which affects males more commonly than females [6, 15]. Potential bias of this unrepresentative sex distribution in the study sample was minimised by sex-matching of controls. Although the association with VL risk was not statistically significant for main occupation, the results suggest that the disease mostly affects animal-herding pastoralists, as has been reported before [15]. Their outdoor and migratory lifestyle is likely to increase contact with *L. donovani*-infected sandflies. This was also reflected by the risk-increasing behaviour of travelling outside the village of residence for more than 2 weeks and the association of this variable with activities near termite mounds. High rates of illiteracy among cases may have negatively affected their knowledge of VL disease and transmission, although this was not formally assessed here.

This study investigated participants' nighttime and sleeping behaviour, as *P. martini* sandflies are considered to be most active between dusk and midnight [9, 20, 21]. Whether these VL vectors are generally endophagous or exophagous in this period was not clear from our results, as staying or sleeping outdoors after sunset was not more frequently reported by VL cases compared with controls. However, sleeping outside their own house (yard) was only observed for VL cases. This behaviour could be associated with more outdoor activities at night, thereby increased likelihood of encountering sandflies. For those sleeping inside, using a mosquito net was not associated with decreased risk of VL. This is in contrast with previous studies in the Pokot territory, Sudan and Ethiopia, which found that owning a mosquito net was protective for VL, suggesting indoor biting by sandflies [15, 22–24]. This hypothesis was supported by our observation that having windows increases the risk of acquiring VL. The absence of an association between VL and mosquito net use in the current study could be the result of the small sample size; however, it cannot be excluded that other factors are also involved, such as net mesh size or indoor sandfly biting before bedtime.

The role of animals in *L. donovani* transmission in eastern Africa, either through attraction of sandflies or as parasite reservoir (zoonotic transmission), is still a matter of debate. A study in Sudan found increased VL risk due to the presence of cattle around the house, whereas the same was associated with decreased odds of the disease in the Pokot territory [15, 25]. The latter study also found that applying insecticide to cattle reversely led to increased VL risk, all in all suggesting that *P. martini* is mainly zoophilic but will shift to feeding on humans when its preferred source of blood meal is inaccessible. The findings of the current study are in contradiction with the previous report from the Pokot area, as we found that having cattle and sheep around the house increased the risk of VL. As such, it seems likely that *P. martini* feeding is opportunistic. Indeed, both humans and goats have previously been identified as most common blood meal source for *P. martini* in Kenya [26–28]. Possibly, domestic animals attract sandflies towards human residencies, thereby exposing the livestock owners to increased sandfly biting as well.

Besides livestock, the association between dogs in the yard and increased VL risk was particularly noteworthy. Canines are an important reservoir for *L. infantum* in the Mediterranean region and South America. *L. donovani* in eastern Africa is mostly believed to be anthroponotic, but earlier studies in Sudan and Ethiopia found evidence of *L. donovani* infection in dogs, and sleeping near dogs has been associated with increased risk of VL in Ethiopia as well [29–34]. In Kenya, studies from the 1970s and 1980s identified one *L. donovani*-infected dog in West Pokot and two more in Machakos District [35, 36]. Given these low numbers, the authors assumed that dogs were an accidental parasite host. Nevertheless, the current study demonstrates for the first time an association between dog ownership and VL risk in West Pokot. The role of dogs in VL transmission in this area should therefore be investigated further, ideally by screening domestic dogs for *L. donovani* infection using highly sensitive serological and molecular diagnostic methods. Furthermore, xenodiagnosis to infected animals should be performed to assess their parasite transmission potential.

Multiple studies have demonstrated termite mounds to be one of the major resting and breeding sites of *P. martini* [9, 10]. Paradoxically, no association was found between VL and the proximity of termite mounds to the household. This was also reported by Kolaczinski et al. [15] and is possibly due to omnipresence of these structures throughout West Pokot. However, the current study demonstrated that having activities close to these mounds, which is likely to disturb resting and breeding sandflies inside, did result in increased VL risk. Outdoor daytime transmission of *L. donovani* parasites by sandflies was also implied by the risk-increasing activities in forests during the rainy season, when *P. martini* is believed to be most abundant [10, 13]. Here, trees may play a particular role in the attraction of sandflies, since cutting down trees in the house yard and household proximity to a number of different *Acacia* tree species were associated with increased VL risk as well. *A. seyal* and *Balanites aegyptiaca* trees are known to be an essential habitat component for *P. orientalis*, and in Baringo County, Kenya, *P. martini* was demonstrated to forage on *Acacia* trees [14, 37]. Our results advocate for further investigations into *P. martini* ecology in this area, where tree barks or root systems may provide an alternative breeding site beside termite mounds.

Considering the risk-increasing effects of outdoor activities, personal protection against sandfly bites, such as applying skin repellent, may be an effective way to prevent VL infection and disrupt its transmission [38–40]. However, virtually none of the participants in this study reported use of insect skin repellents. There could be various reasons for this behaviour: the local population may not be accustomed to it, and chemical repellents may not be affordable.

Due to the low number of study participants, the findings of this research should be interpreted with reservations. For some variables, it was not possible to make a precise estimation of the OR, and there might have been insufficient power to identify more subtle differences between cases and controls. The biological and behavioural significance of the found associations should therefore be confirmed by future studies. These should also attempt to further investigate associations between these risk factors and assess potential confounding. The current explorative study did not undertake multivariate regression and confounding correction, but cross-testing of VL-associated independent variables suggested that several interrelationships exist among them. Another limitation of this study was that *Leishmania*-exposed controls with undetectable or no antibody titres may have been incorrectly classified by rK39 RDT. Nevertheless, this diagnostic tool was considered the best option due to its feasibility in the field and its potential to detect both ongoing *Leishmania* infection and past exposure. Finally, it is possible that differences in health-related factors (like malnutrition) and residential villages between the groups may have confounded some of the study findings.

The recruitment of uninfected volunteers in the villages and their matching to cases was complicated by limited transport options, poor road networks, large distances between households and villages, and security issues. Despite these considerable challenges, the study team was able to recruit controls in several remote areas, including the border region with Turkana County.

CONCLUSION

This study has provided more insight into possible environmental and behavioural risk factors for VL in the understudied focus in West Pokot. We have confirmed the association between VL and termite mounds, the major resting and breeding site for the *P. martini* sandfly vector. Additionally, the described increase in VL risk due to daytime activities in forests, house proximity to *Acacia* tree species and cutting down trees in the house yard may suggest that sandflies dwell around certain trees as well. As some housing characteristics were also found to be associated with VL, more in-depth investigations into *P. martini* biting behaviour is needed to understand the balance between indoor and outdoor VL transmission. Moreover, the finding that presence of dogs in the house yard increases the risk of VL calls for further research into the potential of canines to harbour *L. donovani* and transmit this parasite to sandflies. Outcomes of these follow-up studies can help to design targeted measures for VL prevention and vector control in West Pokot. Finally, relevant social factors beyond parasite and vector biology should also be addressed, such as the knowledge, attitude and practices of the local population towards VL.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical restrictions.

INFORMED CONSENT STATEMENT

Written informed consent was obtained from all subjects involved in the study, or their parent/legal guardian in case of minors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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