Clinical features, immunological interactions and household determinants of visceral leishmaniasis and malaria coinfections in West Pokot, Kenya: protocol for an observational study

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ABSTRACT

Introduction Visceral leishmaniasis (VL) and malaria are two deadly parasitic diseases that coexist in West Pokot County, Kenya. The local population is at considerable risk of coinfection with VL and malaria; however, few studies have described the clinical implications of this comorbidity. Questions remain regarding the immune responses responsible for possible predisposing or protective effects. Moreover, characterisation of environmental and household risk factors for co-acquiring VL and malaria is warranted to increase awareness and guide implementation of targeted control strategies. This protocol intends to address these knowledge gaps concerning VL–malaria coinfections.

Methods and analysis This observational research project will have a multimethod approach, starting with a cross-sectional study at Kacheliba Sub-County Hospital in West Pokot, Kenya. Patients with laboratory confirmation of a VL and/or malaria infection will be clinically assessed and symptomatology of monoinfections and coinfections will be compared. Second, a questionnaire will be addressed to all included patients and to healthy controls in local communities. This case–control study will aim to describe household and environmental determinants associated with VL–malaria coinfection. Lastly, blood samples will be collected from a small cohort of VL and malaria monoinfected and coinfected patients during treatment of their infection(s), and from healthy controls and asymptomatic VL and malaria cases recruited in local communities. These specimens will be used for serum cytokine measurements and molecular quantification of Plasmodium and Leishmania. In this way, the immune response and parasite dynamics during VL–malaria coinfection will be characterised longitudinally and compared with those observed in clinical and asymptomatic monoinfections.

Ethics and dissemination Ethical approval was obtained from the Ethics and Scientific Research Committee of Amref Health Africa. The study findings will be presented at international conferences and published in open-access, peer-reviewed journals.

Trial registration number ISRCTN Registry (ISRCTN15023306).

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Through a tripartite design, this research project will address clinical, immunological and epidemiological knowledge gaps concerning visceral leishmaniasis (VL)–malaria coinfections.
⇒ This will be the first study to investigate individual and household risk factors for VL–malaria coinfections in West Pokot, Kenya.
⇒ Longitudinal characterisation of cytokine profiles in VL–malaria coinfections and comparison with both symptomatic and asymptomatic monoinfections will offer the opportunity to study associations between the immune response, parasite densities and clinical presentation.
⇒ Given the lack of recent data on VL–malaria coinfection rates in West Pokot, the number of coinfected cases recruited in this study could potentially be low.
⇒ Measuring cytokine levels will not reflect the full extent of the immune response induced by a VL–malaria coinfection.

INTRODUCTION

Visceral leishmaniasis (VL) and malaria (caused by Leishmania and Plasmodium spp, respectively) are two vectorborne protozoan parasite infections that cause high morbidity and mortality, particularly in remote regions of low-income countries. In East Africa, Kenya is one of the countries most affected by VL.3 Here, an important focus of endemic VL transmission is located in West Pokot County, which is part of the Pokot territory situated at the border region between Kenya and Uganda.2,4 Between 2018 and 2021, annual numbers of reported VL cases in West Pokot varied from 250 to 450, which is likely to be an underestimation of the actual incidence of this neglected disease (unpublished data, patient records from Kacheliba Sub-County Hospital, West Pokot County, Kenya). Apart
from being endemic for VL, this area is also characterised by recurrent outbreaks of seasonal malaria, with 40,000 confirmed Plasmodium falciparum cases in 2020 (data from Kenya Ministry of Health (MoH), 2023).\(^5\) Due to the overlapping epidemiology of VL and malaria in the Pokot region, the local population is at risk of being infected with both diseases concurrently. Indeed, it appears that co-infections with *Leishmania donovani* and *P. falciparum* are not uncommon: studies among patients with VL attending the regional VL treatment hospitals of Kacheliba (Kenya) and Amudat (Uganda) have reported rates of concomitant malaria ranging from 3.8% to 34.4%.\(^3\)\(^4\)\(^6\) Despite these apparently high numbers of VL–malaria co-infections, the condition is still understudied in terms of risk factors, clinical presentation and immunology.

The overlap of VL and malaria transmission in West Pokot relies on the presence of favourable environmental conditions for their insect vectors, and subsequently, human exposure to these vectors. The local malaria mosquito vectors, *Anopheles arabiensis* and *An. funestus*, have a preference for dry savannah habitats where they lay eggs in small, temporary freshwater pools.\(^7\)\(^8\) As such, malaria incidence in West Pokot often peaks during and after seasonal rainfall. The individual malaria risk may vary from person to person due to household factors: house structure aspects have been associated with indoor *Anopheles* abundance in neighbouring Baringo County.\(^9\) It is unknown whether these results are also applicable in the context of West Pokot. Like malaria, the endemicity of VL in West Pokot is partly attributable to its semi-arid climate. However, the ecology of the local VL vector is substantially different, as the sandfly *Phlebotomus martini* is believed to lay its eggs in the ventilation shafts of termite mounds.\(^10\)\(^-\)\(^13\) Some studies have therefore found living close to these mounds to be associated with VL infection risk.\(^14\) Considering the differences in VL and malaria vector ecology in West Pokot, a very specific combination of human behavioural, environmental and household conditions may predispose for concomitant infections with both parasites. Better understanding of this VL–malaria co-infection risk profile is crucial for increasing awareness among exposed populations, and could also guide policymakers in drafting more focused VL and malaria vector control strategies.

Despite the potentially deadly outcome of VL and malaria mono-infections, much remains unknown about the clinical consequences when both these infections occur in one individual. Only a handful of case reports have described the symptomatology of VL–malaria co-infections, and larger-scale studies have shown contradictory results.\(^6\)\(^15\)\(^-\)\(^25\) A case–control analysis of hospitalised patients with VL in Amudat Hospital found that co-occurring malaria did not clearly exacerbate the clinical picture of VL, and correlated with a lower frequency of anaemia.\(^6\) On the other hand, a study in Sudan in patients with VL–malaria coinfection revealed an increased frequency of anaemia, emaciation and jaundice, compared with their VL monoinfected counterparts.\(^22\) As neither of these studies included a control group of malaria monoinfected patients, it was not studied how malaria symptomatology is affected by a co-occurring VL infection. Hence, additional research into the clinical interactions seen in VL–malaria co-infections is warranted to improve recognition and management of this condition.

Beneath the clinical features of a VL–malaria co-infection lie the pathophysiological processes and immune responses elicited by the infecting *Leishmania* and *Plasmodium* parasites, which have both developed mechanisms to evade host immunity and alter it to their advantage.\(^26\)\(^-\)\(^28\) During a VL–malaria coinfection, *Leishmania* and *Plasmodium* parasites will simultaneously modulate the host immune response, which may have an effect on the control or progression of the concomitant disease. Such mechanisms are well known for people living with HIV, but have also been described for conditions of poly-parasitism, such as helminth co-infections in malaria and *Leishmania* patients.\(^29\)\(^-\)\(^34\) So far, there has been limited research into the parasitological and immunological dynamics during VL–malaria coinfections. Results from animal models have shown both aggravating and mitigating effects of the two diseases upon each other.\(^35\)\(^-\)\(^39\)

To date, there has only been a single study looking at the immunology of VL–malaria coinfections in humans: van den Bogaart *et al* compared the cytokine profiles of VL and malaria mono-infected and coinfected patients in Sudan and found that the immune response during a coinfection was mainly characterised by the release of pro-inflammatory cytokines, and reflected features of the responses seen in both mono-infections.\(^40\) Interestingly, high levels of the pro-inflammatory cytokine interleukin (IL)-17A distinguished co-infected patients from both mono-infected groups, suggesting a synergistic interaction of the two diseases. The same study also found a significantly lower *Plasmodium* parasitaemia in VL–malaria coinfected patients compared with malaria mono-infections. As the interpretation of these study results is limited by their cross-sectional nature, longitudinal assessment of patients with VL–malaria coinfections and comparison with mono-infected patients (both clinical and asymptomatic) are required to unravel the associations between the immune response, parasite loads and clinical features.

To address the knowledge gaps in our understanding of VL–malaria coinfections, this paper describes the protocol of an observational research project aimed at characterising VL–malaria coinfections in West Pokot on three different levels: symptomatology, epidemiology and immunology. These aspects will be studied, respectively, by means of a cross-sectional study, a case–control study and a cohort study. The research project will be conducted at Kacheliba Sub-County Hospital in West Pokot through a collaboration between the Amsterdam University Medical Centres (UMC), Amref Health Africa and the Kenya MoH.
Study objectives
The following study objectives have been formulated for this research project:

► To determine the prevalence of VL and malaria coinfections among patients suspected with either infection attending Kacheliba Sub-County Hospital, West Pokot, Kenya.

► To identify individual and household-level determinants of VL–malaria coinfections in West Pokot, Kenya.

► To examine and compare the cytokine response in patients with VL and malaria monoinfections and coinfections (both clinical and asymptomatic), before, during and after treatment, and determine whether these cytokine responses can be related to the (sub) clinical presentation of the infection(s).

METHODS
Study design
To address the different study objectives, this research project will consist of three components: a prospective, hospital-based cross-sectional study among patients; a case–control study among hospital patients and healthy volunteers in local communities; and a prospective cohort study among hospital patients and healthy and asymptomatic infected household members of these patients.

For the prospective cross-sectional study, patients attending Kacheliba Sub-County Hospital in West Pokot, Kenya, with clinical suspicion of malaria and/or VL infection, will be asked to participate. Laboratory diagnostic tests will be performed for both malaria and VL in consenting patients. A patient will remain included in the study if positive for one or both infections. Clinical and parasitological data will be collected from these study subjects and compared between VL–malaria coinfected cases and patients with VL and malaria monoinfections.

Participants of the cross-sectional study will also serve as cases in the case–control study, to whom a structured household questionnaire will be administered. Exposure to certain individual and household factors will be compared between monoinfected and coinfected patients. Additionally, per VL-infected case, two age-matched and sex-matched healthy controls living in the same village as the case will be recruited and administered the questionnaire as well.

Lastly, a small cohort of subjects of the cross-sectional study with confirmed VL and/or malaria infection will be followed up during standard treatment. This cohort study will entail repeated collection of venous blood samples from participating patients, to characterise their immunological profiles over time. Additionally, blood samples will also be collected from healthy individuals and asymptomatic VL/malaria cases, who will be actively recruited in the households of the patient cohort. The healthy individuals will provide immunological baseline data, whereas the immunological profiles of asymptomatic VL/malaria cases will be compared with those of patients with active clinical disease. Healthy and asymptomatic subjects will be sampled once upon inclusion into the study. In case asymptomatic cases require treatment for their VL and/or malaria infection, they will also be sampled several times during this treatment.

Study site and timing
The research will be performed in West Pokot County in Kenya (figure 1), an area that is endemic for VL all year round and has seasonal transmission of malaria. Previous studies have reported that VL–malaria coinfections occur in the Pokot region. Participants will be recruited from the catchment area of the Kacheliba Sub-County Hospital, which is a government hospital located about 30 km northwest of West Pokot’s county capital, Kapenguria. It is an important regional reference centre for VL diagnosis and treatment, supported by Drugs for Neglected Diseases Initiative. The study will be conducted in October and November 2022. This 2-month period coincides with the short rainy season (October–December) during which malaria incidence often peaks.

Study population
The population of the cross-sectional study will comprise individuals who attend the Kacheliba Sub-County Hospital and are clinically suspected of an infection with VL and/or malaria. The study participants will be grouped according to their VL and malaria diagnosis, as determined by routine diagnostic procedures:

► Newly diagnosed patients with active primary VL, defined as patients with clinical symptoms such as prolonged fever (>2 weeks), splenomegaly, weakness or wasting, with either a positive rk39 rapid diagnostic test (RDT), positive direct agglutination test (DAT, titre ≥1:3200) and/or microscopy-positive spleen aspirate.

► Patients with uncomplicated malaria, defined as patients with fever or history of fever within the last 48 hours (with or without other symptoms) and a positive thick and thin blood film for Plasmodium, with a parasite count <250 000/µL of blood.

► Patients coinfected with malaria and primary VL (actively for one or both infections) defined as patients with symptoms of VL and/or malaria, with a positive Plasmodium blood film (parasite count <250 000/µL of blood) and positive VL diagnostic test (rk39 RDT, DAT, spleen aspirate).

All subjects of the cross-sectional study with laboratory-confirmed VL and/or malaria infection will also be included as cases in the case–control study. Two age-matched and sex-matched healthy controls per VL-infected case (including those coinfected with malaria) will be recruited in the case’s village of residence and will be compared between monoinfected and coinfected cases in the case–control study, to whom a structured household questionnaire will be administered. Exposure to certain individual and household factors will be compared between monoinfected and coinfected patients. Additionally, per VL-infected case, two age-matched and sex-matched healthy controls living in the same village as the case will be recruited and administered the questionnaire as well.

Lastly, a small cohort of subjects of the cross-sectional study with confirmed VL and/or malaria infection will be followed up during standard treatment. This cohort study will entail repeated collection of venous blood samples from participating patients, to characterise their immunological profiles over time. Additionally, blood samples will also be collected from healthy individuals and asymptomatic VL/malaria cases, who will be actively recruited in the households of the patient cohort. The healthy individuals will provide immunological baseline data, whereas the immunological profiles of asymptomatic VL/malaria cases will be compared with those of patients with active clinical disease. Healthy and asymptomatic subjects will be sampled once upon inclusion into the study. In case asymptomatic cases require treatment for their VL and/or malaria infection, they will also be sampled several times during this treatment.
be defined as individuals without current signs or symptoms of VL or malaria, no history of VL, no malaria in the preceding 2 weeks, and with a negative rk39 RDT and negative malaria RDT. The individual should have lived in their current house for at least 1 year.

Clinical subjects of the cohort study will be recruited among the monoinfected and coinfected participants of the cross-sectional study. Only malaria infections with \textit{P. falciparum} will be eligible, and the patient must be aged between 6 and 30 years old. These age limits are set to exclude infants and children whose immune system has not yet fully developed, and patients above 30 years who are more likely to have developed a significant level of acquired immunity to malaria.\textsuperscript{6} 43 The cohort study will also recruit healthy controls and asymptomatic cases in the households of the clinically ill participants. This recruitment strategy will minimise the variability of environmental confounders between the different study groups. Moreover, the likelihood of finding asymptomatic VL and malaria infections will be higher in households of symptomatic patients.\textsuperscript{44–48} The healthy and asymptomatic cohorts are defined as follows:

- Healthy endemic controls, defined as individuals above the age of 6 years, without current signs or symptoms of VL or malaria, with no self-reported history of VL, no malaria in the preceding 2 weeks, with a negative DAT test (DAT titre ≤1:200) and negative malaria blood films.

- Patients with asymptomatic VL, defined as individuals above the age of 6 years, without VL-associated symptoms for at least 15 days before study inclusion and no self-reported history of active VL, with a positive DAT (DAT titre ≥1:3200).

- Patients with asymptomatic malaria, defined as individuals above the age of 6 years, with no symptoms suggestive of malaria at the time of inclusion and with no history of malaria in the preceding 2 weeks, with a positive thick and thin blood film for \textit{P. falciparum}.

A complete overview of all eligibility criteria for the different study components can be found in online supplemental table 1.

**Sample size**

The cross-sectional study aims to include 244 malaria-infected patients, allowing to detect an OR of 1.8 at a confidence level of 95% (two sided), with an expected 20% of exposure among VL-infected cases and a power of 80%.\textsuperscript{49} Within the 2-month time frame of the study, all patients at the study hospital with a confirmed VL infection will be included. Considering that 350 VL cases were reported at Kacheliba Sub-County Hospital in the first 5 months of 2022 (personal communication with David Kiptanui, clinical officer at Kacheliba Sub-County Hospital, 2022), this pragmatic approach is expected to recruit approximately 140 patients with VL. Among these subjects, approximately 5–30 cases are expected to be
coinfected with malaria, based on previously reported coinfection rates in Kacheliba Hospital ranging from 3.8% to 21.4%.

All participants of the cross-sectional study will also be included in the case–control study. Additionally, two healthy controls will be recruited per VL-infected patient, meaning that the study will aim to include approximately 280 healthy controls.

Given the explorative nature of the immunological cohort study, the population size per study group will be set at 20–30 subjects, depending on their availability. Based on the results of van den Bogaart et al about cytokine levels in VL–malaria coinfected patients, this group size should be sufficient to detect significant differences in immunological parameters with 80% power at 5% level of statistical significance.

Clinical sample and data collection
Cross-sectional study
Patients presenting at the study hospital with clinical signs and/or symptoms indicating a potential malaria and/or VL infection will be asked to participate in the study if they meet the inclusion criteria (online supplemental table 1). Patients not willing to participate in the research will be excluded from the study and will be referred to the clinician for usual diagnosis and treatment. Patients who give their informed consent will be included and tested at the hospital for both malaria and VL according to routine procedures: a finger-prick blood sample will be collected to prepare a thick and thin blood film for microscopic detection of malaria. VL diagnosis will be done by means of an rk39 RDT for detection of VL antibodies in finger-prick blood. In case of a negative rk39 test, a DAT will be performed to confirm or rule out VL. If the DAT result is borderline, a spleen aspirate will be taken for parasitological diagnosis by microscopy. All test outcomes, data on malaria parasitaemia (as determined by microscopy) and DAT titre will be recorded on a case report form (CRF).

Blood from the diagnostic finger prick of each VL and/or malaria-suspected participant will also be used to measure the haemoglobin level and to prepare dried blood spots (DBS) on a filter paper card. These will later be sent to the Amref Health Laboratories in Nairobi, where they will be used for DNA extraction with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA isolates will be tested with realtime quantitative PCR (qPCR) for malaria and VL, using an 18s rRNA gene target for *P. falciparum* and kinetoplast DNA (kDNA) target for *Leishmania*, respectively. This will allow for detection of low-density malaria/VL infections that might be missed by the point-of-care diagnostics, and for quantitation of parasite densities.

For patients with a confirmed VL and/or malaria infection, according to the diagnostic testing at the recruitment hospital in Kacheliba, clinical features and medical history will be recorded on their CRF, while those who test negative for both malaria and VL will be excluded from further study procedures.

Following the diagnosis outcome at the clinic, participants will be treated for their infection(s) through the Kenyan national treatment programme for VL and malaria. Before treatment is initiated, some patients will be asked to participate in the cohort study as well.

Case–control study
Directly after the participants of the cross-sectional study have received the first treatment for their infection, a trained interviewer will administer a structured household questionnaire. Information collected will include place of residence, housing conditions, house environment, occupation, sleeping habits, night-time activities and travel history. Participants below the age of 15 years may be assisted in answering questions by their parent or legal guardian.

For each VL-infected patient case, two healthy controls will be recruited at the case’s village of residence. At the central point of the village, a household will be randomly selected by spinning a pen. In the selected household, an individual, age-matched and sex-matched with the VL-infected case and meeting the eligibility criteria (online supplemental table 1), will be asked to participate. If multiple household members are eligible, one will be selected by rolling a die. After providing informed consent, finger-prick blood from the household member will be tested with a malaria RDT and a VL rk39 RDT to exclude both infections. In case both tests are negative, the structured household questionnaire will be administered to the healthy control, or parent/legal guardian in case of children <15 years. Afterwards, a pen will be spun at the doorstep of the house to select the next household where the second matched control will be recruited. All procedures will be repeated until two healthy controls per VL-infected case have been recruited.

Cohort study
Subjects in the cross-sectional study with a laboratory-confirmed infection with VL, *P. falciparum* malaria or both, and meeting all eligibility criteria (online supplemental table 1), will be asked to participate in the cohort study as well. After giving informed consent, they will be monitored during the treatment of their infection(s). Treatment will be according to the national treatment guidelines for both infections: for VL, this is sodium stibogluconate injections (20mg/kg body weight/day) and paromomycin injections (15mg/kg body weight/day) for 17 days; for uncomplicated *P. falciparum* malaria, oral doses of 20mg artemether and 120mg lumefantrine tablets, two times per day for 3 days (dosing adjusted by weight and age). In case of VL–malaria coinfection, malaria is treated first before initiation of VL treatment. From each participant, 10mL of peripheral venous blood (5mL in a serum isolation tube, 5mL in an EDTA anticoagulation tube) will be collected prior to treatment initiation (day 0) and on the following time points during their treatment:
In VL monoinfected patients, on day 7 of VL treatment and day 17 (end of VL treatment).

In malaria monoinfected patients, on day 1 of malaria treatment and day 3 (end of malaria treatment).

In VL-malaria coinfected patients, on day 1 and day 3 of malaria treatment, and day 7 and day 17 of VL treatment.

At each follow-up time point, the patients’ clinical features will be recorded on their CRF.

Healthy individuals and cases with asymptomatic VL or malaria will be recruited by a study team visiting the households of the clinically ill participants of the cohort study. When a household member has no history of VL or recent malaria and shows no symptoms of either disease (see eligibility criteria in online supplemental table 1), a finger-prick blood sample will be taken for microscopic detection of malaria and for VL testing with DAT at Kacheliba Sub-County Hospital. Based on the results of these tests, participants will be grouped either in the healthy control cohort, the asymptomatic malaria cohort or the asymptomatic VL cohort. The study team will return to the local communities to share the results with the respective participants. Participants who complain of symptoms suggestive of VL and/or malaria at this stage will be referred for further management and excluded from the study. If still without symptoms, participants will be physically examined and 10 mL of venous blood (5 mL in a silicone-coated tube for separating the serum, 5 mL in EDTA anticoagulation tube) will be collected. Healthy controls will only be sampled at this time. Asymptomatically infected patients will be referred to Kacheliba Hospital for further management. If placed on treatment, the asymptomatic patients will be sampled during their treatment, following the same scheme as the clinically ill patients of the cohort study.

All collected venous blood samples will be processed at the Kacheliba Sub-County Hospital for isolation of serum, white cell counting using an Ac-T diff Hematology Analyzer (Beckman Coulter, Brea, California, USA) and preparation of DBS. DBS cards will be shipped to the Amref Laboratories, where they will be used for nucleic acid isolation and subsequent Leishmania and P. falciparum detection and quantitation, using qPCR for Leishmania kDNA and real-time quantitative nucleic acid sequence-based amplification for P. falciparum 18s rRNA. Isolated serum samples will be sent to the Amsterdam UMC and used in a Luminex-based assay, to measure levels of pro-inflammatory and anti-inflammatory cytokines that have been shown to play an important role in the immune response against VL and/or malaria: tumour necrosis factor-α, interferon-γ, IL-1β, IL-2, IL-4, IL-10, IL-12p70, IL-13, IL-17A and IL-22.

Statistical analysis

Cross-sectional study

All data collected from the cross-sectional study will be compared between VL monoinfected cases, malaria monoinfected cases and VL–malaria coinfected cases. In a univariate analysis, the association between a VL–malaria coinfection and measured characteristics will be explored using the Pearson’s $X^2$ test or the Fisher’s exact probability test. Continuous variables will be categorised into predefined groups. Found associations will be quantified as prevalence ORs with 95% CIs, determined at the 5% level. To identify independent characteristics associated with VL–malaria coinfection and adjust for confounding, a multivariate logistic regression model will be made in a backward stepwise manner with variables that have a p value of $<0.10$ in the univariate analyses. Only variables with a p value of $<0.05$ will be retained in the final model.

Case–control study

Data collected with the structured questionnaire will be used to identify household and environmental risk factors associated with VL and malaria (co)infections in West Pokot. VL and malaria infections will be considered as two separate response variables, for which individual univariate logistic regression analyses will be applied to evaluate associations (expressed as ORs) with the questionnaire variables. Per predefined thematic section of the household questionnaire, variables with a p value of $<0.2$ in the univariate analysis will be included in a multivariate regression model. The same variables will also be used as input for multivariate multiple response regression models, which will identify predictors that jointly contribute to both VL and malaria infections and as such, coinfections. Both the separate disease models and multiple response models of each section will be optimised through stepwise backward elimination of variables with p$>0.2$. The retained significant variables of each thematic section will then be merged into final multivariate regression models for VL, malaria and VL–malaria coinfections, in which only significant (p$<0.05$) variables will be kept.

Cohort study

Cytokine levels and clinical characteristics measured at baseline (day 0) in VL–malaria coinfected patients will be compared with those of VL or malaria monoinfected patients, either actively or asymptotically, and of healthy controls, who will provide immunological reference data. Longitudinal comparison of cytokine levels will be performed within the separate groups who are followed up during treatment. For both baseline and longitudinal comparisons, standard parametric statistical tests will be used for normally distributed numerical data. Non-normal data will be analysed using non-parametric tests. Comparison of nominal data will be done with the $X^2$ test or Fisher’s exact probability test. For all statistical analyses, significance will be determined at the 5% level (p$<0.05$). Correlations between the levels of individual cytokines will be investigated with Spearman’s rank correlation analysis.

Ethics and dissemination

The protocol of this study received ethical approval from the Amref Health Africa Ethics and Scientific Review
Committee (ESRC) (ref. ESRC P1196/2022). The ESRC is accredited by the Kenyan National Commission for Science, Technology and Innovation (NACOSTI). A NACOSTI research licence was obtained before study initiation (ref. 791964).

Written informed consent will be collected from all participants, or their parents/legal guardians, for study participation, export of clinical samples for analysis at the Amsterdam UMC and future use of study data and samples. All collected data and clinical specimens will be anonymised and stored at the Amsterdam UMC for at least 5 years after completion of the study. Dataset will be available upon reasonable request to the corresponding author. None of the results of the study will be published with individual name identification or with identifiers of patients.

All study findings will be communicated to the national health authorities of Kenya. The research team will write scientific papers on the study results, which will be submitted to open-access, peer-reviewed international scientific journals and presented at national and international scientific meetings.

Patient and public involvement

Due to the remote setting in which this study will be conducted, it was not possible to involve the local public of West Pokot in the design phase of the study. However, during study implementation, awareness among local communities will be achieved by involvement of community leaders and the patients recruited at the hospital. Community health workers will be approached to assist with the recruitment of asymptomatic patients and healthy controls in local villages. Patients, local healthcare staff and the public will be consulted to select an appropriate method for dissemination of the study findings among the community.

DISCUSSION

With a cross-sectional study, a case–control study and a cohort study, this observational research project will apply a multifaceted approach to address important knowledge gaps concerning the clinical implications, environmental risk factors and immunology of VL–malaria coinfections in West Pokot. The significance of these findings is underlined by the fact that concomitant VL and malaria infections are still largely neglected, despite the apparently high rates of this condition in West Pokot.316 This research will contribute to increased awareness among the local population of West Pokot, its healthcare workers and disease control policymakers. This may lead to more timely detection and treatment of VL–malaria coinfections, thereby reducing associated morbidity and mortality.

This will be the first study to describe the parasite dynamics and cytokine responses of VL–malaria coinfection in a longitudinal fashion. This approach will allow investigating associations of the immunological profile of a VL–malaria coinfection with its clinical picture. It should be mentioned that the design of the immunological cohort study is restricted by the limited available resources in the remote setting of the study hospital. For example, participants will not be screened for other underlying (infectious) conditions, such as HIV or helminthiasis, which are known to significantly impact the host’s immune response. Furthermore, this study will not isolate patient peripheral blood mononuclear cells to investigate leucocyte dynamics underlying the observed cytokine responses. Nevertheless, findings from this exploratory study will generate an evidential basis to direct future research into coinfection immunology. Eventually, better understanding of the immunology of VL–malaria coinfections will help improve clinical management and support the development of official treatment guidelines.

The questionnaire study will generate critical data on individual, household and environmental factors that may increase the risk of co-acquiring VL and malaria. In this way, the results of the case–control study can guide a more targeted approach to control and elimination of both infections in the Pokot area. This translational step will be facilitated by the involvement of the Kenyan MoH in the research project. Although this study is focusing on VL–malaria coinfections in West Pokot, its results may provide valuable insights for other co-endemic areas as well.

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Contributors NVd was responsible for the instigation of this research project, developed the protocol and drafted the manuscript. JC, PM and HS contributed to the study design and protocol development, and critically read the manuscript. WD provided national surveillance and healthcare data on VL and malaria in Kenya and critically read the protocol and manuscript. All authors approved the final version of the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not required.

Ethics approval The protocol of this study received ethical approval before initiation. Refer to the Ethics and dissemination section for further details.


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Supplemental table 1: Eligibility criteria for the different study components

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<th>Cross-sectional study</th>
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<td><strong>Inclusion criteria</strong></td>
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<tr>
<td>- Showing symptoms suggestive of malaria and/or VL</td>
</tr>
<tr>
<td>- Living in the catchment area of the study hospital</td>
</tr>
<tr>
<td><strong>Exclusion criteria</strong></td>
</tr>
<tr>
<td>- Already under drug treatment for malaria and/or VL</td>
</tr>
<tr>
<td>- Having a positive VL diagnosis in their medical history</td>
</tr>
<tr>
<td>- Pregnant</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case-control study: patient cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inclusion criteria</strong></td>
</tr>
<tr>
<td>- Included in the cross-sectional study</td>
</tr>
<tr>
<td>- Having a laboratory-confirmed VL diagnosis and/or malaria diagnosis</td>
</tr>
<tr>
<td><strong>Exclusion criteria</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case-control study: healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inclusion criteria</strong></td>
</tr>
<tr>
<td>- Living in the village of residence of a VL-infected participant of the cross-sectional study</td>
</tr>
<tr>
<td>- Living in the current house for at least 1 year</td>
</tr>
<tr>
<td>- Not showing symptoms suggestive of malaria and/or VL</td>
</tr>
<tr>
<td>- Negative for malaria with malaria RDT</td>
</tr>
<tr>
<td>- Negative for VL with rk39 RDT</td>
</tr>
<tr>
<td><strong>Exclusion criteria</strong></td>
</tr>
<tr>
<td>- A positive VL diagnosis in their medical history</td>
</tr>
<tr>
<td>- A history of clinical malaria in the preceding 2 weeks</td>
</tr>
<tr>
<td>- Already under drug treatment for malaria and/or VL</td>
</tr>
<tr>
<td>- Pregnant</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cohort study: clinically ill patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inclusion criteria</strong></td>
</tr>
<tr>
<td>- Included in the cross-sectional study</td>
</tr>
<tr>
<td>- Between 6 and 30 years of age</td>
</tr>
<tr>
<td>- Having a laboratory-confirmed VL diagnosis and/or malaria diagnosis with <em>P. falciparum</em> (parasite counts between 1000 and 250,000 /μL of blood only)</td>
</tr>
<tr>
<td>- Eligible for first-line treatment for VL and/or malaria as stated in the national treatment guidelines</td>
</tr>
<tr>
<td><strong>Exclusion criteria</strong></td>
</tr>
<tr>
<td>- Having a haemoglobin level of ≤5 g/dL</td>
</tr>
<tr>
<td>- Being diagnosed with malaria caused by a <em>Plasmodium</em> species different than <em>P. falciparum</em></td>
</tr>
<tr>
<td>- Suffering from any other infectious disease, acute or chronic, different from malaria and/or VL, of which the patient has knowledge</td>
</tr>
<tr>
<td>- Suffering from any immune system disorder, acute or chronic, of which the patient has knowledge</td>
</tr>
</tbody>
</table>
Cohort study: healthy and asymptomatic household members

**Inclusion criteria**
- Living in the household of one of the clinically ill participants of the cohort study
- 6 years old and above
- Not showing any major symptoms suggestive of malaria and VL
- Having a body temperature below 37.5°C

**Exclusion criteria**
- A positive VL diagnosis in their medical history
- A history of clinical malaria in the preceding 2 weeks
- Already under drug treatment for malaria and/or VL
- Pregnant
- Being diagnosed with malaria caused by a *Plasmodium* species different than *P. falciparum*
- Suffering from any other infectious disease, acute or chronic, different from malaria and/or VL, of which the patient has knowledge
- Suffering from any immune system disorder, acute or chronic, of which the patient has knowledge
- Being under antimicrobial and/or anti-inflammatory treatment
- Being under immune-suppressive or immune-stimulatory treatment