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Profiling vivax malaria incidence, residual transmission, and risk factors using reactive case detection in low transmission settings of Ethiopia

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Abstract

Background Identification of local *Plasmodium vivax* transmission foci and its hidden reservoirs are crucial to eliminating residual vivax malaria transmission. This study assessed whether reactive case detection (RCD) could better identify *P. vivax* cases and infection incidences in Arjo-Didessa, Southwestern Ethiopia.

Methods A RCD survey was conducted from November 2019 to October 2021 in Arjo-Didessa and the surrounding vicinity in southwestern Ethiopia. RCD was performed at 0, 30, and 60 days following reports of *P. vivax* infections by health facilities to detect further cases and potential transmission networks. Household members of the index case and neighbours living within 200 m of the index household were screened for *P. vivax*. Households 200–500 m away are considered controls and were also screened for *P. vivax*. *Plasmodium vivax* was detected by microscopy, rapid diagnostic testing (RDT), and quantitative polymerase chain reaction (qPCR). Risk factors associated with vivax malaria were analysed using generalized estimating equations (GEE).

Results A total of 3303 blood samples were collected from the index (n = 427), neighbouring (n = 1626), and control (n = 1240) household in the three rounds of follow-up visits for malaria infection, the overall positivity rate of *P. vivax* malaria was 1.6% (95% CI 1.2–2.2%), 1.9% (95% CI 1.5–2.4), and 3.9% (95% CI 3.2–4.6%) by microscopy, RDT, and qPCR, respectively. Microscopy and RDT detected 41.5% (54 of 130) and 49.1% (64 of 130) of the qPCR-confirmed *P. vivax* cases, respectively. Of qPCR-positive samples, 77.7% of the total *P. vivax* infections circulated in the index and neighbouring households, while control households accounted for 23.3% of the infections. Of the *P. vivax* infections detected 81.0% (95% CI 72.9–87.1%) were asymptomatic. In this study, *P. vivax* infection incidence was higher in index case households (53.8 cases per 1000 person-months) and (44.0 cases per 1000 person-months) in neighbouring households compared to the control households (25.1 cases per 1000 person-months) with statistical difference (p = 0.02). In index case households, children < 5 years and school-age children were at higher risk of *P. vivax* infection (AOR: 6.3, 95% CI: 2.24–18.02, p = 0.001 and AOR: 2.7, 95% CI: 1.10–6.64, p = 0.029).

†Delenasaw Yewhalaw and Guiyun Yan are senior authors with equal contribution.

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Conclusions This study found clustering of asymptomatic and sub-microscopic *P. vivax* infections in the index case household and their neighbours using RCD and molecular methods. Children under 5 years and of school age were more likely to have *P. vivax* infection in index households. Thus, tailored RCD approaches and targeted interventions for interrupting residual *P. vivax* transmission networks are needed to eliminate *P. vivax* malaria in low transmission settings.

Keywords Vivax malaria, Residual malaria transmission, Clustering, Reactive case detection, Ethiopia

Background

Plasmodium vivax is the most widespread malaria parasite worldwide and mainly affects Asia, Central, and South America [1, 2]. More than 200 million clinical malaria cases are reported each year due to *P. vivax*. India, Indonesia, Pakistan, and Ethiopia account for more than 80% of the global *P. vivax* burden [3]. In *Plasmodium falciparum* and *P. vivax* co-endemic regions, *P. vivax* poses challenges of elimination, due to the dormant liver stage, hypnozoites complicates vivax malaria elimination efforts [4]. However, *P. falciparum* has greatly declined in elimination-targeted areas [2, 5].

The main challenges to the elimination of *P. vivax* are its distinct biological features and epidemiological characteristics [6], which include low parasitaemia that can be difficult to diagnose and treat [7], persistent hypnozoite that can cause relapse [8], and treatment complexity [9]. In addition, *P. vivax* presents with asexual and sexual parasite stages at the early stage of clinical illness [10]. Its gametocytes are transmitted more efficiently to Anopheline mosquitoes [11]. They are potentially important sources of transmission to mosquitoes over several weeks or months [12]. Also, compared to other *Plasmodium* species, *P. vivax* has a shorter development cycle in the vector and increased transmissibility because of the early development of gametocytes during blood-stage infection [10, 13, 14]. Furthermore, the majority of *P. vivax* infections are asymptomatic, and studies have predicted that asymptomatic carriers contribute to approximately 30–80% of *P. vivax* malaria transmission [15, 16].

Plasmodium falciparum has shown a significant reduction in many endemic areas targeted for malaria elimination. However, this has not been achieved in *P. vivax* malaria due to the ability of the parasite to form hypnozoites, low-density infections, and the risk of the silent reservoirs as well as heterogeneous patterns of parasite transmission [6]. Thus, it is challenging to control *P. vivax* due to few or the absence of symptomatic patients and submicroscopic infections carrying the disease that can remain undetected by conventional diagnostic methods in areas where approaching elimination [17]. Moreover, submicroscopic carriers are estimated to be the source of 20–50% of all human-to-mosquito transmissions [18].

To overcome this challenge, reactive case detection (RCD) based strategy is more likely to identify additional *P. vivax* infections, symptomatic and asymptomatic infections, particularly among individuals living in the same household and neighbours near a detected malaria infection or index cases [19, 20]. Detection of the *P. vivax* hypnozoites stage remain challenging with currently available diagnostic methods [21].

The Federal Ministry of Health of Ethiopia has set a strategic plan to achieve malaria elimination by 2030 [22]. *Plasmodium falciparum* and *P. vivax* species are co-endemic species in many parts of the country. *Plasmodium falciparum* accounts for 65% of all cases and; *P. vivax* accounts for most of the remaining cases. *Plasmodium vivax* elimination requires more species-specific tools considering the biological features of the parasite and low parasite density, which is undetectable by routine diagnostic tests [23], and the fact that gametocytes appear at an early stage of infection, which is responsible for transmission to mosquitoes before the administration of anti-malarial drugs. Despite chloroquine combined with a 14-day course of low-dose primaquine treatment regimen having been used to treat *P. vivax* malaria in low-transmission areas, it has been difficult to eliminate due to poor drug adherence, and latent stages of the parasite.

This study considered the relapsing nature of *P. vivax* by examining the hidden reservoirs with additional follow-up visits. The study also assessed the spatial clustering of *P. vivax* infection within the 200 m and 500 m radius of the index case household. Therefore, the study aimed to determine RCD effectiveness in the identification of additional *P. vivax* cases and infection incidence of *P. vivax*. In addition, the study sought to evaluate individual and household-level risk factors associated with *P. vivax* infections.

Methods

Study area and population

This study was conducted from November 2019 to October 2021 in Arjo-Didessa, Southwestern Ethiopia.

The study area is located 395 km from Addis Ababa. in the Didessa Valley in Jimma Arjo and Buno Bedele districts, with a population size of 86,329 people living in the study districts (CSA, 2007). About half (48.7%, n = 42,093) of the population were females. The latitude of the study area is 8°41′35.5″N and the longitude is 36°25′54.9″E (Fig. 1). It is also located at an altitude of 1300 to 2280 m above sea level (a.s.l.). The districts have two malaria transmission seasons: peak malaria incidence occurs between September and December following the main rainy season of June to September, and low transmission occurs from April to May during and after a short rainy season from February to March. The remaining months of the year are dry. The mean annual rainfall is approximately 1477 mm. The temperature ranges from 20 to 30°C. This study was conducted in health facilities located in 9 clusters/kebeles (small administrative units) using passive case detection (PCD) clinics. Nine clusters/kebeles include: Command 2 (CO2), Command 5 (CO5), and Command 11 (CO11) clusters, Abote Didessa 1 and 2, Soyoma and Hundie Gudina kebeles from Jimma Arjo district and Kerka, and Sefera Tabiya kebeles were from Dabo Hana district included in this study. Residents are predominantly farmers who depend on crop farming and cattle/goat herding for subsistence. The remaining are Arjo-Didessa sugar factory and plantation workers.

Arjo Didessa is among a low malaria transmission areas with annual parasite incidence (API) of 5–10 and in one of the districts targeted for elimination by the Ministry of Health (MOH), Ethiopia since 2017 [22]. In this study areas, malaria transmission occurs mainly between September to December and April to May. *Plasmodium falciparum* and *P. vivax* are the most predominant malaria species. According to a 10-year retrospective study, *P. vivax* had the highest number of cases compared to *P. falciparum* [24]. The prevalence of overall malaria species was 2.0% in the study area in 2019 [25]. The primary malaria vector is *Anopheles arabiensis*, which peaks during the rainy season [26].

In the country, the national malaria control and elimination programme (NMEP) has benefited from the large number of health extension workers (HEWs) nationwide in the reduction of malaria mortality and morbidity through massive anti-malarial interventions, such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) for all at-risk communities. In addition, all public health facilities, including health posts, use confirmatory RDT testing and treat confirmed malaria cases with appropriate anti-malarial drugs (ACT, chloroquine), including primaquine treatment [22]. In this study area, community-based cross-sectional repeated surveys were conducted, and all positive cases received treatment with ACT/CQ + PQ

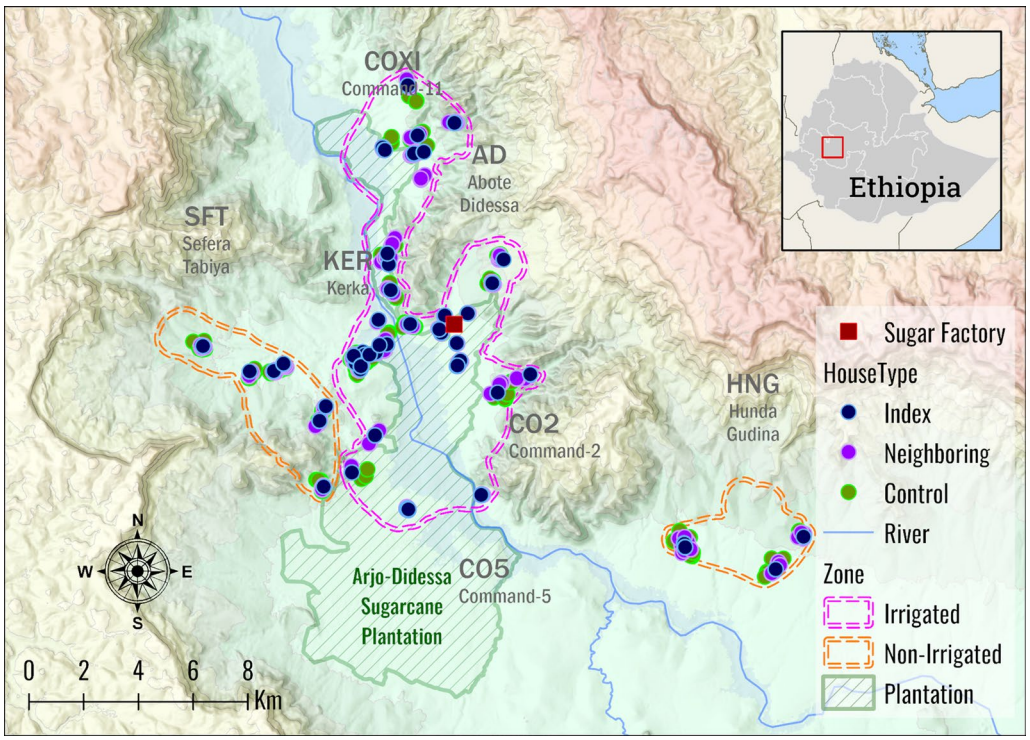

Fig. 1 Map of RCD households in clusters in Arjo Didessa and the surrounding vicinity, Ethiopia

during the survey. Additionally, the local district malaria control unit has worked in treatment, including PQ administration, as well as the distribution of ITN and IRS, with the goal of malaria control and elimination according to malaria stratification using passive or active case detection strategy.

Sample size calculation

Following the detection of passive cases from PCD clinics (index case/s), a prospective cohort study was used to identify additional *vivax* malaria cases with repeated visits. All household members of the index cases were screened. In addition, randomly selected neighbouring household members were screened for *P. vivax* malaria. The sample size was determined based on previous RCD studies in Brazil, Ethiopia, India, Cambodia, and many parts of South African countries [19, 27, 28]. In this RCD study period, based on the previous studies elsewhere [21, 29–32], a total of 52 vivax malaria index cases were detected, and members of index cases, neighbouring households, and control households were recruited for three follow-up visits. Accordingly, neighbour and control households were chosen within a radius of 200 m and 500 m, respectively.

Study design and sampling procedure

Plasmodium vivax index cases were identified from nine passive case detection (PCD) clinics using both microscopy and RDT in Arjo Didessa and the surroundings. Five neighbouring households (within a 1–200 m radius) and five control households located further away (within a 201–500 m radius) from the index households were randomly selected. A total of three visits were made for each recruited household member. The first visit was done after the time of index case diagnosis between 1 and 7 days (day 0), the second following 30 (± 10) days, and the third round 60 (± 10) days later (Fig. 2). The distance from the index case households to neighbouring and control households was measured by a laser rangefinder HLR600 (HUEPAR; Guangdong Province, China).

Inclusion criteria

All residents living in the recruited HHs, children and adults whose ages ranged from 6 months to 70 years, were willing to participate and planned to remain in the study area for three months from the day they were included. Chronically ill subjects were excluded from the study.

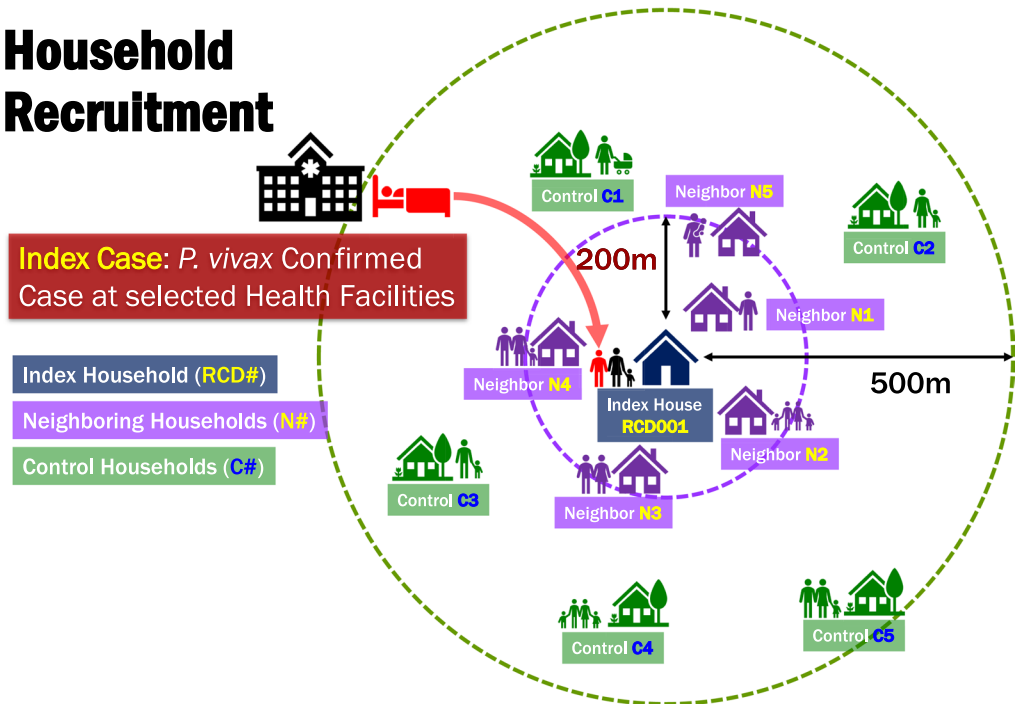

Fig. 2 Illustrates the schematic representation of the RCD households. Index households are depicted in dark blue; Five neighbouring households (N) were randomly selected within a radius of 1–200 m from the index case households, represented in violent; Five control households (C) were randomly chosen within a radius of 201–500 m away from the index case households, represented in green

Data collection

In this study, reactive case detection study team at Arjo was notified when an eligible *P. vivax* index case was detected by ICEMR PCD clinic staff who carried out routine passive case detection (PCD) surveillance. The RCD study team members discussed with the head of household and visited the index case home. Residents were advised with the same message that after finding an index case, the study team would return in a week to conduct screening for malaria parasite detection and handle any positive cases. The geographic coordinates of all households were measured with a hand-held Global Positioning System (GPS) to a horizontal accuracy of less than 10. The Open Data Kit (ODK) recorded all household and resident information. A questionnaire in the local language was used to gather data on demographic characteristics, health-seeking behaviors, vector control coverage (number of ITNs, the number of people sleeping under ITNs, and IRS status), household condition and structures, recent travel history, clinical history such as fever (T^0), chills, and headache, recent malaria treatment and number of people sleeping in the house.

Blood sample collection

Blood samples were collected from household members of the index case, neighbours, and control households for RDT testing, microscopy, and dried blood spot (DBS). Finger prick blood samples were taken for the detection of the *Plasmodium* parasites using RDT and DBS on filter paper for molecular (PCR) analysis and blood slides for microscopy examination. During follow-up visits, recruited household residents who missed the previous visit were invited to participate and enrolled at the next scheduled follow-up visit. Displaced households were replaced in the 2nd and 3rd visits.

Parasitological examinations

Microscopy

Parasitic identification and density quantification of *Plasmodium* parasites were performed on thick and thin blood films according to a standard protocol [33]. The blood smear was fixed with methanol and stained with 10% Giemsa for 10 min using the standard procedure for malaria. Blood films were examined microscopically using 100X (oil immersion) objectives. Two experienced certified malaria microscopists independently examined slides and parasite density was calculated per 200 white blood cells (WBC), assuming 8000 WBC/µl of blood stage. Inter-observer discordances were solved with a reading by a third microscopist.

Rapid diagnostic testing (RDT)

Finger prick blood (300 µl) was used for the detection of malaria parasites. CareStart™ Malaria *Pf/Pv* (HRP2/pLDH) Ag combo RDT test kit (Access Bio Ethiopia, INC.) was used for the differential diagnosis of *P. vivax* and *P. falciparum* infections. CareStart™ products target two specific antigens: namely histidine-rich protein 2 (HRP-2), lactate dehydrogenase (LDH), or *Plasmodium* aldolase (malaria *Pf/Pv* (HRP2/pLDH). If *P. falciparum* presents, the HRP-2 (Pf) line turns positive and if only the pLDH (Pv) line is positive, it suggests *P. vivax* species. If both HRP2 and pLDH lines are positive, it indicates mixed infections (e.g., P. falciparum and P. vivax). Only the presence of a line next to control indicates a negative result. For confirmation, microscopy and PCR were performed on all samples.

Testing and treatment

In each round of follow-up, all positive cases (in the index, neighbouring or control households) by RDT and /or microscopy during RCD visits were treated free of charge by anti-malarial drugs. P. vivax-positive cases were treated with oral chloroquine (total dose, 25 mg of base/kg) over 3 days plus with oral primaquine (PQ) (0.25 mg / kg) daily for 14 days. In addition, Coartem® (artemisinin-based combination therapy (ACT)/ artemether plus lumefantrine, Novartis, Basel, Switzerland) plus a single low dose of PQ (0.25 mg /kg) day 0 was given for *P. falciparum* infection at each PCD centre (health facility) according to malaria treatment guidelines of the MOH. Furthermore, all index case were treated and followed at PCD clinics. All positive cases (*P. vivax* or *P. falciparum*) received treatment.

Molecular analysis

DNA extraction

DNA was extracted from a DBS filter paper using an extraction protocol as described in [34]. Parasitic genomic DNA (gDNA) was extracted from 5×3 mm hole punches (discs) of dried blood blots from the filter paper using a Chelex-100 resin-based protocol. The discs for each sample were incubated in 1000 µl of a 10% saponin/phosphate-buffered saline (PBS) solution overnight (>4 h) in the refrigerator. After incubation, the discs were washed twice with 1 ml of PBS, followed by centrifugation at 14,000×*g*. Next, 150 µl of a 20% Chelex-100 resin (Bio-Rad Laboratories, Hercules, CA) and 100 µl of DNase/RNase-free water (ddH2O) were added to the discs and incubated for 10 min at 95 °C. After high-speed centrifugation (14,000 rpm), the supernatant containing the gDNA was either used immediately for PCR amplification reactions or stored at − 20 °C.

Quantitative PCR (qPCR) detection

The genomic DNA of each sample was amplified using 18S rRNA genes-based primers. The qPCR was run on the Applied Biosystems Quant Studio 3 Real-Time PCR System (Thermo Fisher Scientific Inc., USA). Briefly, *P. falciparum* and *P. vivax* qPCR were done with the final volume containing 2 µl of sample DNA and a reaction master mix containing 6 µl of Perfecta® qPCR Tough-Mix™, Low ROX™ Master Mix (2X),0.4 µl of each primer and 0.5 µl of each probe (10 µM). 1.4 µl of double-distilled water. To quantify *P. falciparum* and *P. vivax*, qPCR employed a standard curve generated with a serial diluted MRA-177 (*P. falciparum)* and MRA-178 (*P. vivax)* plasmid controls (BEI Resources, https://www.beiresources.org/). Each qPCR run included these positive controls and three negative controls [35, 36]. The thermoprofile was set as follows; 50 °C for 2 min, 95 °C for 2 min, 95 °C for 3 s, and 58 °C for 30 s) for 45 cycles.

Variables

Outcome variables

The primary outcomes were microscopy and RDT positive, as well as qPCR-confirmed *P. vivax, P. falciparum,* and mixed infections) on days 0, 30 and 60 of the study period. The *Plasmodium vivax* infection incidence rate per 1000 person-months was calculated by dividing the number of new cases of *P. vivax* individuals from the RCD follow-up visits by the total number of months per individual (person-time) in the study.

Individual and household-level variables

Individual level variables included study participants' basic characteristics and others. The socio-demographic characteristics of study participants included sex, age group, educational status, duration in the study area, migration status, and use of vector control such as LLINs. Household variables included vector control measures such as IRS, household heads educational levels, and housing conditions. Household conditions included house distance to index cases, wall and roof materials, eaves, and distance from vector breeding sites.

Data management and analysis

All data collected during the survey were recorded in electronic forms on a tablet installed with Open Data Kit (ODK) and statistical analyses were carried out using STATA version 17.0 (Stata Corp., College Station, Texas 77845 USA). Descriptive analysis and 95% confidence intervals were used to summarize the percentage, prevalence and infection incidence as measured by qPCR, RDT, and microscopy disaggregated by household type. Proportions were compared by applying standard chi-square and Fisher's exact tests to determine the significant difference. In this study, symptomatic malaria infection was defined as malaria infection (RDT and/or microscopy positive) with measured fever (temperature > 37.5 °C).

To determine the infection incidence rate, PCR-positive individuals were removed from the follow-up visits (censored) if they had an infection by PCR at baseline and during the follow-up visits. Microscopy and RDT positive were also removed if they had an infection and positive PCR at baseline and the follow-up visits. In the study context, to calculate the incidence rate, all microscopy, RDT, and PCR-negative individuals re-entered the study during the first visit after baseline and exited when they tested positive for microscopy and /or RDT and confirmed positive PCR results. Index cases were also excluded from the analysis.

The generalized estimating equations (GEE) with a logit model with an exchangeable working correlation structure matrix were used to identify individual and household risk factors associated with *P. vivax* infections. The GEE model was chosen for its ability to account for the clustering of secondary households around the index household, individuals are nested within households and to more accurately estimate robust standard errors as well as repeated malaria status through follow-up visits. Variables with *P* values, of 0.25 in univariate models were eligible for inclusion in the multivariate model. The level of statistical significance was set at the stricter *P* values below 0.05 in the multivariate model. For every analysis statistical significance level of $p < 0.05$.

Results

Baseline characteristics of the study participants

From 63 *P. vivax* index cases and their neighbouring and control households visited for the RCD study, 52 malaria index cases from different households were selected as eligible for the initiation of a reactive case detection study and completed follow-up visits. Two hundred fifty-seven neighbouring households were recruited between 1 and 200 m away from the index case households. Also, 160 control households were recruited between 201 and 500 m away from the index case households. Thus, the proportion of index case households, neighbouring households, and control households was 11.0%, 54.7%, and 34.0%, respectively.

In this RCD study, a total of 2003 residents were enrolled, and 5185 visits were made. At baseline, we screened and enrolled 143 residents of the 52 index case households, 672 individuals from 257 neighbouring households, and 484 individuals from 160 control households using RDT. After 3 months follow-up period, a total of 1,299 in 469 households received screening in

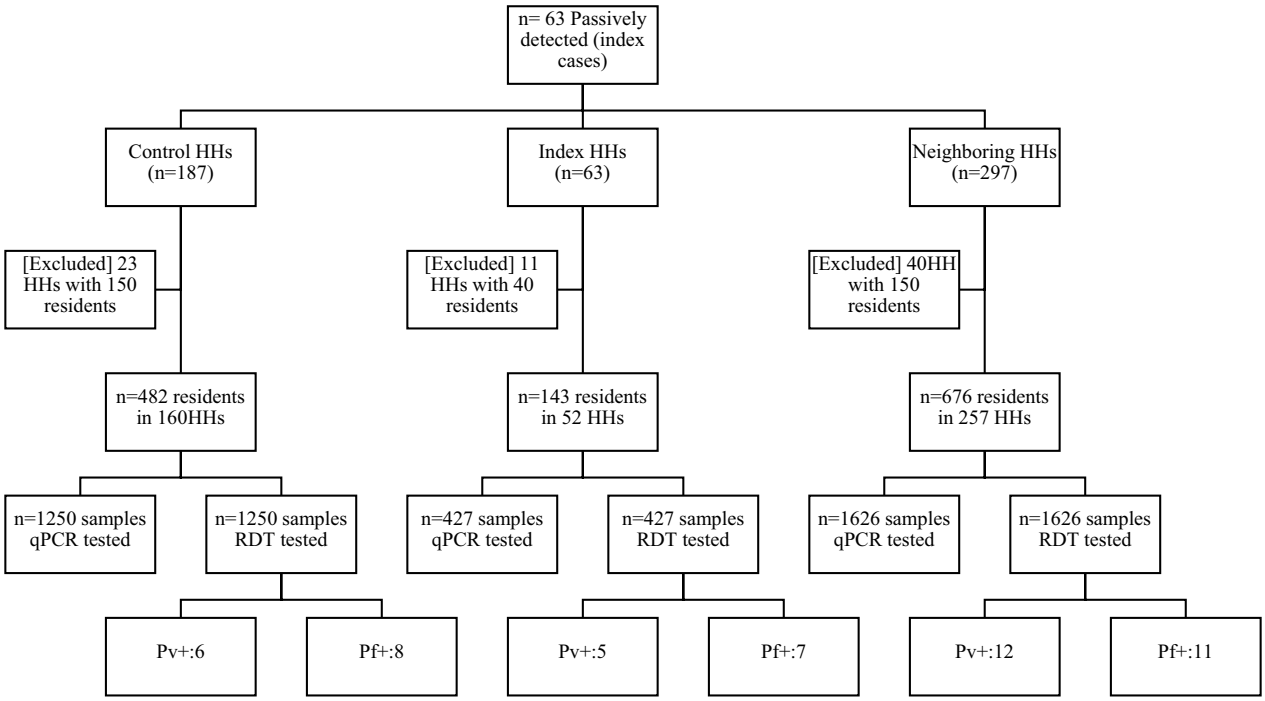

Fig. 3 Flow chart of households recruited around the index cases and 3 rounds of samples collected. Those excluded households and their residents from this program are due to Covid-19 pandemic. All the collected blood samples were tested with both RDT and qPCR for confirmation. All RDT *Pv* + cases were treated by CQ and all RDT *Pf* + cases were treated by ACT. *HH* household, *qPCR* quantitative polymerase chain reaction, *RDT* rapid diagnostic test, *Pf* + *P. falciparum* positive; *Pv* + *P. vivax* positive

one or two visits and 3,303 blood samples were collected from the index, neighbouring, and control household members and checked for malaria infection using RDT, qPCR, and microscopy (Fig. 3). The age of the RCD study population was between 6 months and 70 years (mean, 19 years) (IQR: 8, 30) (639 or 49.2%) were females and 660 (50.8%) were male. At baseline, the rapid diagnostic test positivity among cohort participants is as follows: index households: 3.9%, neighbouring households: 1.9%, and control households: 0.9% (Table 1).

Overall positivity of *Plasmodium* spp. infections

The overall positivity rate of *Plasmodium* infection by qPCR analysis was 10.2% (n = 334/3303), comprising 5.8% (n = 194/3303) *P. falciparum*, 3.9% (n = 130/3303) *P. vivax,* and 0.3% (10 of 3303) mixed *P. falciparum /P. vivax* in the follow-up visits. At the first visit (Day 0), the prevalence of *P. falciparum* infections was 9.9%, 6.8%, and 7.4% in the index, neighbour, and control households, respectively. Moreover, the prevalence of *P. falciparum* infections were similar across the households' types in each visits. There was no statistical significance difference across the household types ($p = 0.6$). However, a significantly higher *P. vivax* positivity rate was observed in the index households (5.6%) and neighbour (4.7%) compared to control households (2.3%) ($p = 0.0006$) (Table 2). These findings suggest that residents in index and neighbour households have a greater risk of *P. vivax* infection, compared to those in control households, because they live closer to an active focus of *P. vivax* transmission, whereas the prevalence of *P. falciparum* infection was similar across household types.

Monthly prevalence of *P. vivax* by qPCR

Residents of index case households consistently had a higher positivity rate of *P. vivax* infection by qPCR (5.6%, 24 positive samples among 427 examined), followed by neighbouring households (4.7%, 77 positive samples among 1626 examined), and it was lower in control households (2.3%, 29 positive samples among 1250 examined, Table 2). A significantly higher prevalence of *P. vivax* was observed when comparing index and neighbouring households to control households (Fisher exact test, $p < 0.01$ in both comparisons). A persistent and comparable prevalence of *P. vivax* infection was found across months by household types, ranging from 6.4 to 4.2% ($p = 0.8$) in index case households, 5.4% to 3.4% ($p = 0.4$) in neighbouring households, and 2.1 to 2.4% ($p = 0.4$) in control households using qPCR. Furthermore, both *P.vivax* infected members of index cases household

Table 1 Individual and household level characteristics for the study subjects in index, neighboring, and control households in Arjo Didessa, Southwestern, Ethiopia, November 2019–October 2021

Characteristics	Index households	Neighboring households	Control households
Individual characteristics, % (n/N)			
Number of study participants (n)	143	672	484
Baseline prevalence of *Pv* by RDT (%)	3.9%	1.9%	0.9%
Gender Female	45.5 (65/143)	52.0 (349/672)	50.8 (246/484)
Male	54.5 (78/143)	48.0 (323/672)	49.2 (238/484)
Age (years), median (IQR)	19 (1.62)	20 (1.70)	16 (1.70)
Migrant	18.4 (26/143)	19.0 (128/672)	6.4 (31/484)
LLINs usage	42.0 (60/143)	48.0 (323/672)	41.0 (198/484)
How long have you lived in the study area			
> 3 years	60.1 (86/143)	58.8 (395/672)	75.8 (367/484)
1–3 years	20.3 (29/143)	22.1 (149/672)	17.7 (86/484)
7–12 months	9.1 (13/143)	5.9 (40/672)	3.0 (14/484)
< 7 months	9.1 (13/143)	13.1 (88/672)	3.5 (17/484)
Household level characteristics, % (n/N)			
Number of households	52	257	160
IRS in this year	49.1 (25/52)	44.0 (113/257)	41.8 (67/160)
Wall structure Iron sheet	28.8 (15/52)	34.6 (89/257)	10 (16/160)
Mud wall	57.7 (30/52)	52.1 (134/257)	75.6 (121/160)
Wood only	13.5 (7/52)	13.2 (34/257)	13.1 (21/160)
Roof structure Iron sheet	49.1 (25/52)	53.7 (138/257)	34.3 (55/160)
Grass thatched	50.9 (27/52)	46.3 (119/257)	65.7 (105/160)
Distance to larval breeding sites			
> 500 m	28.3 (15/52)	19.0 (49/257)	21.8 (35/160)
200–500 m	35.8 (19/52)	41.6 (107/257)	33.7 (54/160))
< 200 m	35.5 (18/52)	39.3 (101/257	43.1 (69/160)
Animal pens near/inside the household	44.2 (23/52)	33.5 (86/257)	37.5 (60/160)
Opened eaves	84.6 (44/52)	80.5 (257)	65.0 (104/160)

(median parasite density by qPCR, 68 copies/μL; IQR, 38–90) and neighbouring had higher parasite density (median, 23 copies/μL; IQR, 11–48) than infections detected in households control (median, 16 copies/μL; IQR, 16–23) without statistical difference (p = 0.240).

At the first visit (Day 0), the highest prevalence of *P. vivax* was in index case households (6.4%) (excluding the index case) and neighbouring (5.4%) households, and the lowest was in the control households (2.1%, Table 2). In this respect, a significant difference was observed when comparing index cases to control households (Fisher exact test, $p = 0.023$) and neighbouring to control households (Fisher exact test, $p = 0.006$). However, there was a similar prevalence of vivax malaria when comparing the index case to neighbouring households (Fisher exact test, $p = 0.6$). Furthermore, there was no statistically significant variation in the prevalence of infection among index, neighbour, and control households on day 60 (Table 2; Fig. 4).

Plasmodium vivax positivity rate by RDT

The prevalence of *P. vivax* malaria in the index case households was 4.9% (day 30) and 3.5% (days 0 and 60) by RDT (4.0%, 17 RDT positive among 427 examined). Index case households had a higher infection prevalence (range, 3.5–4.9% than control households (range, 0.9–1.4%), with a significant difference observed at days 30 and 60 (Fisher exact test, p = 0.02). There were more asymptomatic carriers in the index case household members than control household members on days 30 and 60. Moreover, persistent prevalence of *P. vivax* was observed in the first visit and the subsequent RCD visits in the index cases household (Table 3).

This RCD study found that *P. vivax* infection prevalence among the residents of neighbouring households ranged from (1.8–2.5%) across all RCD visits. Additionally, there was no statistically significant difference in the prevalence of *P. vivax* in the index case and neighbouring households in all RCD visits (Fisher exact test > 0.05) (Table 3).

Table 2 The positivity rate of *Plasmodium* infections using qPCR in RCD household types by each visit in Arjo, Southwestern Ethiopia, November, 2019–October , 2021

RCD rounds	*Plasmodium* species	Index HHs		Neighbor HHs		Control HHs		
		No. of infections	%	No. of infections	%	No. of infections	%	*P value**
Day 0	*P. falciparum*	14	9.9%	46	6.8%	36	7.4%	0.4
	P. vivax	9	6.4%	36	5.4%	10	2.1%	0.009
	Mixed	0	0.0%	6	0.9%	1	0.2%	< 0.0001
	Total tested	141		672		484		
Day 30	*P. falciparum*	4	2.8%	19	3.9%	15	3.8%	0.8
	P. vivax	7	4.9%	24	5.0%	10	2.5%	0.1
	Mixed	0	0.0%	2	0.4%	1	0.2%	0.6
	Total tested	144		482		394		
Day 60	*P. falciparum*	4	2.8%	28	5.9%	28	7.5%	0.1
	P. vivax	8	5.6	17	3.6%	9	2.4%	0.1
	Mixed	0	0.0%	0	0.0%	0	0.0%	–
	Total tested	142		472		372		
Total	*P. falciparum*	22	5.2%	93	5.7%	79	6.3%	0.6
	P. vivax	24	5.6%	77	4.7%	29	2.3%	P < 0.001
	Mixed	0	0.0%	8	0.5%	2	0.2%	0.1
	Total tested	427		1626		1250		

HHs household, *P. falciparum Plasmodium falciparum*, *P. vivax Plasmodium vivax*, *RCD* reactive case detection

* p values for χ^2 tests on 3 × 2 contingency tables testing a positive result across rows (HHs types)

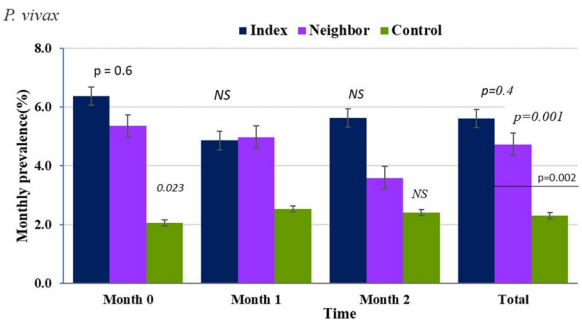

Fig. 4 Monthly *Plasmodium vivax* infection rates by household types in Arjo-Didessa, Southwestern Ethiopia. *NS* not significant

P. vivax asymptomatic infections

In this study, index case households' members had higher rates of asymptomatic infections (3.7%, 16 qPCR positive, among 427 examined; index cases excluded in all comparison) than control households (1.8%, 23 qPCR positive, among 1250 examined) (Fisher exact test, $p = 0.04$) and neighbouring households (4.0%, 66 qPCR positive, among 1626 examined) compared to control households (1.8%, 23 qPCR positive, among 1250 examined) (Fisher exact test, $p = 0.001$). There was no significant difference prevalence of asymptomatic *P. vivax* infections between the index case and neighbouring households (Fisher exact test, $p = 0.8$). Of the overall *P. vivax* infections detected, 81.0% (105/130) (95% CI 72.9–87.1%) were asymptomatic *P. vivax* infections by qPCR (Table 4).

A 4.2-fold more asymptomatic than symptomatic infections were diagnosed in this RCD study. In index case households, both asymptomatic and symptomatic *P. vivax* infections were significantly higher when compared to control households (as determined by the Fisher exact test, with p values of 0.04 and 0.012, respectively). Furthermore, index case households had higher symptomatic vivax malaria compared to neighbouring households (Fisher exact test, p = 0.041 (Table 4). In general, 77.7% of asymptomatic infections were found in both index case and neighbouring households while 22.3% of asymptomatic infections were found in control households.

Also, in a comparison of diagnostic tests, higher proportion of additional *P. vivax* infections were detected by qPCR (3.9%, 130 positives among 3304 examined) than by RDT (1.9%, 64 positives among 3304 examined) and microscopy (1.5%, 52 positives among 3304 examined). The detection rate of *P. vivax* by qPCR was higher compared to RDT (p < 0.002), indicating that most infected individuals would not be detected by using RDT. More than two-thirds of PCR-positive *P. vivax* infections were missed by RDT (sensitivity 37.0,

Table 3 The prevalence of symptomatic and asymptomatic *P. vivax* infection using RDT in RCD household type by each RCD visit, in Arjo-Didessa, Ethiopia

RCD rounds	Types of infections	Index HHs		Neighbor HHs		Control HHs		P Value[a]
		No. of infections	%	No. of infections	%	No. of infections	%	
Day 0	Symptomatic	2	1.4%	6	0.9%	2	0.4%	0.2
	Asymptomatic	3	2.1%	6	0.9%	5	1.0%	0.4
	Total	5	3.5%	12	1.8%	7	1.4%	0.1
	No. tested	141		672		484		
Day 30	Symptomatic	2	1.4%	5	1.0%	3	0.7%	0.8
	Asymptomatic[b]	5	3.5%	7	1.4%	3	0.7%	0.06
	Total[b]	7	4.9%	12	2.5%	6	1.4%	0.08
	No. tested	144		482		394		
Day 60	Symptomatic	1	0.7%	1	0.2%	1	0.2%	0.6
	Asymptomatic[b]	4	2.8%	6	1.3%	2	0.5%	0.1
	Total[b]	5	3.5%	7	1.5%	3	0.8%	0.07
	No. tested	142		472		372		

[a] p values for χ^2 tests on 3 × 2 contingency tables testing a positive result across rows (HHs types)

[b] Pairwise comparisons across rows with 0.05 significance: index HHs = neighbor HHs; index HHs > control HHs

Table 4 The prevalence of asymptomatic and symptomatic *P. vivax* infection using qPCR in RCD household type by each RCD visit, in Arjo-Didessa, Ethiopia

RCD rounds	Types of infections	Index HHs		Neighbor HHs		Control HHs		*P*-Value[a]
		No. of infections	%	No. of infections	%	No. of infections	%	
Day 0	Symptomatic	3	2.1%	6	0.9%	2	0.4%	0.1
	Asymptomatic[b]	6	4.2%	30	4.4%	8	1.6%	0.02
	Total[b]	9	6.4%	36	5.4%	10	2.1%	0.009
	No. tested	141		672		484		
Day 30	Symptomatic	4	2.8%	4	0.8%	3	0.7%	0.1
	Asymptomatic[c]	5	3.4%	20	4.1%	7	1.8%	0.1
	Total[b]	9	6.2%	24	5.0%	10	2.5%	0.08
	No. tested	144		482		394		
Day 60	Symptomatic	1	0.7%	1	0.2%	1	0.2%	0.6
	Asymptomatic	5	3.5%	16	2.1%	8	2.1%	0.5
	Total	6	4.2%	17	3.6%	9	2.4%	0.4
	No. tested	142		472		372		

[a] p values for χ^2 tests on 3 × 2 contingency tables testing a positive result across rows (HHs types)

[b] Pairwise comparisons of *Pv* positivity rates across rows with Fisher exact test (at 5% significance): index HHs = neighbor HHs; index HHs > control HHs; neighbor HHs > control HHs

[c] Pairwise comparisons across rows with Fisher exact test (at 5% significance): index HHs = neighbor HHs; neighbor HHs > control HHs

95% CI 28.6–45.8, specificity 99.5, 95% CI 99.2–99.7) (Additional file 1. Table S1).

Additionally, RDT positivity rate was higher in other members of households where the index case resided (3.9%) and lower in neighbouring (1.9%) and control households (1.3%) of study participants ($p < 0.002$). Across the type of households, sensitivity and specificity were highest in the index case and followed by neighbour and control households using qPCR and microscopy as a reference. All tests recorded > 97% specificity across the different household types (Additional file 1. Table S1).

Table 5 *Plasmodium vivax* infections positivity rate based on microscopy and qPCR in Arjo Didessa Southwestern, Ethiopia, (November, 2019–October, 2021)

Household types	Index	Neighbor	Control	Total
Total blood samples (n)	427	1626	1250	3303
Microscopy n (%)	13 (3.0%)	25[a] (1.5%)	16[a] (1.3%)	54 (1.6%)
qPCR, n (%)	24 (5.6%)	77 (4.7%)	29 (2.3%)	130 (3.9%)
Sub-microscopy	11 (2.6%)	55 (3.5%)	18 (1.5%)	84 (2.5%)

[a] 4 (each) samples were positive by microscopy but negative by qPCR

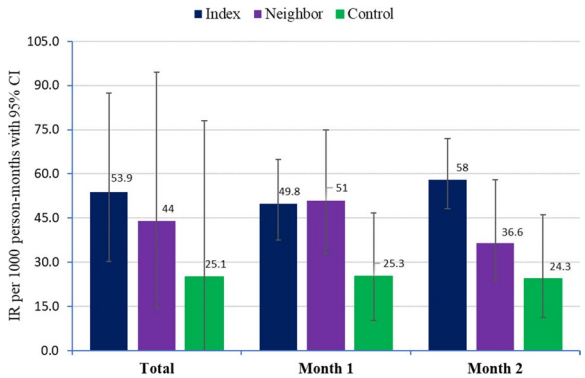

Fig. 5 *Plasmodium vivax* infection incidence rates (IR) per 1000 person-months in index, neighboring and control households across months in Arjo-Didessa, Southwestern Ethiopia, November 2019–October 2021

Sub-microscopic *P. vivax* infections

Plasmodium vivax malaria positivity rate was 1.6%) as determined by microscopy. In three rounds of RCD visits, microscopy-based RCD would identify only 33.8% (46/130) of the infections detected by qPCR. Of the overall asymptomatic infections detected 80.0% (84/105) were submicroscopic (qPCR positive and blood smear negative) *P. vivax* infections. This indicates that 64.2% of the infections were missed by microscopy (sensitivity 35.4, 95% CI 27.2–44.5, specificity 99.7, 95% CI 99.5–99.9) (Additional file 1: Table S1). Significantly, neighbouring households had a higher prevalence of submicroscopic *P. vivax* compared to control households (Fisher exact test, p = 0.001) (Table 5).

Plasmodium vivax infection incidence

Overall, the infection incidence of *P. vivax* was 39.1 cases per 1000 person-month, as measured by qPCR. In this study, infection incidence was higher in index case households (53.8 cases per 1000 person-months) and (44.0 cases per 1000 person-months) in neighbouring households. The lowest infection incidence (25.1 cases per 1000 person-months) was recorded in control households. There was a significant difference between the infection incidence rate of the index case and control households (p = 0.02). In addition, there was a significant difference in infection incidence rate between neighbouring and control households (p = 0.04). However, there was no significant difference in infection incidence between index cases and neighbouring households (p = 0.5) (Fig. 5).

In index case households (72.4 versus 35.6 cases per 1000 person-months) and neighbouring households (50.9 versus 35.9 cases per 1000 person-months), the incidence rate was higher in males than in females. There was a significant difference between index cases and control households (p = 0.01). Additionally, when comparing index case households to neighbouring households, the infection incidence rate for children < 5 was higher (133.4 versus 43.4 cases per 1000 person-months), respectively (p = 0.04). Furthermore, when compared to control households, children under 5 and of school age in index case households had a significantly higher incidence (133.4 versus 40.8 cases per 1000 person-months and 89.9 versus 31.3 cases per 1,000 person-months, respectively).

Index households, which resided in 200–500 mosquito breeding habitats, had the highest infection incidence rate (85.4 cases per 1000 person-months) compared to control households in a similar location (29.5 cases per 1000 person-months) (p = 0.008). In addition, higher infection incidence was observed in index case households not utilizing ITN compared to control households not utilizing ITN (69.4 versus 26.9 cases per 1000 person-months) (p = 0.02). Similarly, index case households that did not receive IRS in the previous year had a higher infection incidence rate (73.6 versus 34.5 cases per 1,000 person-months) (p = 0.03) than control households that did not receive IRS) (Table 6).

Risk factors associated with *Plasmodium vivax* infection

According to the results of univariate and multivariate generalized estimated equation (GEE) logit model (Additional file 2: Table S2) , socio-demographic characteristics, malaria preventive measures, and local environmental factors were assessed to determine predictors of *P. vivax* risk of infection.

In the index case households, children under 5 years old and of school age (AOR: 6.3, 95% CI: 2.24–18.02, p = 0.001 and AOR: 2.7, 95% CI: 1.10–6.44, p = 0.029) were risk factors associated with *P. vivax* infection, respectively. *P. vivax* infections were more likely associated with neighbouring households with opening eves, doors, or windows that were located near index case households (AOR: 1.9. 95% CI: 1.01–3.87, p = 0.046). Also, the results further indicated control households that did not receive IRS in the previous year were

Table 6 *Plasmodium vivax* infection incidence rates per 1000 person-months by individual and household level factors in Arjo-Didessa Southwestern, Ethiopia, November 2019–October 2021

Factors	Variables	Index HHs		Neighboring HHs		Control HHs	
		PCR + /Person-months	IR Per 1000 person-months	PCR + / Person-months	IR Per 1000 person-months	PCR + Person-months	IR Per 1000 person-months
Gender	Male	(10/138)	72.4	(23/432)	50.9	(8/341)	23.4
	Female	(5/140.5)	35.6	(18/501)	35.9	(11/415.5)	26.4
Age (years), n (%)	> 15	(2/152)	13.1	(24/547)	43.8	(7/405.5)	17.2
	5–15	(8/89)	89.8	(10/244.5)	44.9	(8/255)	31.3
	< 5	(5/37.5)	133.4	(6/138.5)	43.3	(4/98)	40.8
Vector breeding sites	> 500 m	(2/92)	21.7	(13/398.5)	32.7	(6/309)	19.4
	200–500 m	(11/128.5)	85.4	(19/394.5)	48.1	(11/372.5)	29.5
	< 200 m	(2/58)	34.5	(8/141.5)	56.5	(2/75)	26.6
ITN usage	Yes	(5/123.5)	40.4	(19/446.5)	42.5	(7/312.5)	22.4
	No	(10/144)	69.4	(21/507.5)	41.3	(12/446)	27.0
Openings /eaves	Yes	(13/228.5)	56.9	(34/708.5)	47.9	(15/273.5)	54.8
	No	(2/50)	40.0	(6/231)	35.9	(4/283)	14.1
IRS in the study year	Yes	(2/102)	19.6	(11/366.5)	30.0	(3/193.5)	15.5
	No	(13/176.5)	73.6	(29/567.5)	51.1	(16/463)	34.5
Season	Dry	(9/209.5)	42.9	(26/733)	35.4	(10/500)	20.0
	Wet	(6/69)	86.9	(14/200.5)	69.8	(9/148.5)	60. 6

HH household, *IRS* indoor residual spraying, *ITN* insecticide treated net, *PCR+* polymerase chain reaction test positive

Table 7 Results of multivariate Generalized Estimation Equations logit model showing predictors of *P. vivax* risk of infection by household type in Arjo-Didessa, Southwestern, Ethiopia, November 2019–October 2021

Factors	Category	Index households			Neighboring households			Control households		
		AOR	95% CI	*P* value	AOR	95% CI	*P* value	AOR	95% CI	*P* value
Sex	Female (ref)									
	Male	0.8	0.36–2.12	0.766	1.3	0.85–2.10	0.204	1.3	0.56–3.08	0.528
Age in years	> 15 (ref)									
	5–15	2.7	1.10–6.44	0.029*	1.2	0.70–2.04	0.500	1.5	0.68–3.29	0.308
	< 5	6.3	2.24–18.02	0.001*	0.9	0.43–2.15	0.936	1.6	0.53–4.95	0.387
LLINs usage	Yes (ref)									
	No	1.2	0.50–2.90	0.667	0.9	0.54–1.61	0.814	0.4	0.16–1.22	0.116
Season	Dry (ref)									
	Wet	1.4	0.52–3.87	0.491	1.4	0.88–2.43	0.136	1.9	0.74–5.20	0.170
Eaves (opening)	No (ref)									
	Yes	1.4	0.30–6.37	0.633	1.9	1.01–3.87	0.046*	1.3	0.45–3.71	0.663
Larval breeding habitat	> 500 m (ref)									
	200–500 m	2.9	0.33- 25.47	0.332	1.4	0.51–4.10	0.476	1.8	0.50–6.47	0.362
	< 200 m	1.1	0.13–9.50	0.899	1.3	0.46–4.08	0.567	1.4	0.49–4.25	0.501
IRS received	Yes (ref)									
	No	1.9	0.48–7.60	0.293	1.2	0.71–2.28	0.417	11.5	2.20–60.10	0.004*

CI confidence interval, *OR* odds ratio, *LLINs* long lasing insecticidal net, *IRS* indoor residual spraying, *HH* households, *AOR* adjusted odds ratio

*Significant at P < 0.05

Table 8 Individual and household level characteristics associated with vivax malaria infection using GEE model in RCD households in Arjo, Southwestern Ethiopia, November,2019–October, 2021

	Bivariate			Multivariable		
	COR	95% CI	P value	AOR	95% CI	P value
Individual-level factors						
Study participants residing in HHs						
Control HHs (ref)	–	–	–			
Index HHs	2.29	1.08–4.87	0.030	2.16	1.06–4.41	0.03*
Neighbor HHs	2.05	1.20–3.48	0.008	1.88	1.13–3.11	0.01*
Gender						
Female (ref)	–	–	–			
Male	1.29	0.89–1.88	0.17	1.26	0.86–1.82	0.22
Age						
Adult (age > 15 years) (ref)	–	–	–			
School-age children (age 5–15)	1.44	0.96–2.17	0.07	1.36	0.92–2.01	0.11
Children younger than 5 years	1.25	0.68–2.26	0.46	1.56	0.89–2.71	0.11
LLINs usage						
Yes (ref)	–	–	–			
No	1.12	0.73–1.71	0.59	–	–	–
Duration of stay in the area						
> 3 years (ref)	–	–	–			
1–3 years	1.45	0.86–2.44	0.15	–	–	–
7–12 months	1.39	0.59–3.26	0.44	–	–	–
≤6 months	1.14	0.51–2.55	0.74	–	–	–
Household-level factors						
Season						
Dry (ref)	–	–	–			
Wet	1.46	1.09–2.46	0.07	1.57	1.05–2.35	0.025*
Highest level of education of HH head						
Secondary school or higher (ref)	–	–	–	–	–	–
Primary school	0.89	1.09–2.46	0.73	–	–	–
No formal education	0.66	0.34–1.29	0.22	–	–	–
Larval breeding habitat						
> 500 m (ref)	–	–	–	–	–	–
200–500 m	1.81	0.80–4.13	0.15	1.66	0.72–3.83	0.22
< 200 m	1.50	0.64–3.51	0.34	1.49	0.65–3.41	0.34
Household-level factors						
IRS received						
Yes (ref)	–	–	–	–	–	–
No	1.62	0.95–2.77	0.07	1.69	1.02–2.82	0.04*
Animal pens						
Yes (ref)	–	–	–	–	–	–
No	1.55	0.72–3.30	0.25	–	–	–
Eaves (Openings)						
No (ref)	–	–	–	–	–	–
Yes	2.04	1.17–3.55	0.01	1.79	1.06–3.01	0.02*

* p value-significant level

AOR adjusted odds ratio, *COR* crude odds ratio, *CI* confidence interval, *HHs* households, *IRS* indoor residual spraying

associated with a higher risk of *P. vivax* infection (AOR 11.5, 95% CI 2.20–6.10, p = 0.004) (Table 7).

In unstratified GEE analysis, compared to control households, study participants in the neighbour HHs had 1.88 higher odds (AOR = 1.88; CI 1.13, 3.11) of being vivax malaria positive, and index household members had almost 2.16 times higher odds (AOR = 2.16; CI 1.06, 4.41) of being vivax malaria positive. Other household-level variables, such as seasonality, households receiving IRS in the past 6 months and households with an opening or eaves, which were significant in the univariate analyses, were significantly associated with vivax malaria positivity in the full GEE model. However, age was not associated in multivariable analysis in this model (Table 8).

Discussion

The present study shows incidence of *P. vivax* was highest among residents living within the same household and neighbouring households of the index case and decreased with distance away from the index case houses. Furthermore, asymptomatic *P. vivax* infections were more common in index and neighbouring households than in the control households. Compared to the conventional RDT and microscopy, more than two-fold of *P. vivax* infections were detected by qPCR. The finding of this study implies that malaria elimination efforts could be challenged due to the presence of asymptomatic and submicroscopic *P. vivax* infections in the study area.

This current study also documented additional *P. vivax* infections detected by RDT, microscopy, and qPCR among individuals from the index case households, neighbours, and control households. Of the qPCR-positive *P. vivax* infections, 50.9% were negative by RDT. In line with the findings from other studies conducted in low transmission settings [37–40], this study demonstrated that both microscopy and RDT exhibit lower sensitivity in detecting vivax infections compared to qPCR. This suggests that RCD study using molecular methods has an advantage over RDT and microscopy to detect additional infections in low transmission settings, presumably asymptomatic infections around the proximity of index cases households. Furthermore, this study demonstrated the diagnostic limitations of RDT and microscopy for low levels of parasitaemia, and their impact on achieving malaria elimination [41, 42].

The majority of the parasitaemic individuals were found to reside in index case households [43]. Interestingly, during both the 30-day and 60-day follow up visits, the incidence of *P. vivax* infection remained persistent both the index case and neighbouring households. However, despite treating all positive cases at baseline and during the follow-up visits, there was no reduction in the incidence of *P. vivax* infections. In contrast, RCDs targeting *P. falciparum* showed a significant decline in follow-up visits [44]. Interestingly, a similar incidence of *P. falciparum* was observed across the household types of this study. These differences may not necessarily be exposed to more vector bites [45]. Instead, this could result from the biological features of *P. vivax* infections, including the activation of hypnozoites leading to relapse [46]. On the other hand, a study indicated that *P. vivax* RCD might not efficiently identify infection clusters following active infection in a single visit [28]. Therefore, multiple rounds or an extended *P. vivax* RCD strategy may be necessary to detect additional *P. vivax* infections that could have been acquired simultaneously with the index case and to uncover hidden reservoirs of infections as well as relapses [19, 47].

In the current study, unlike another study conducted in Ethiopia [28], a significant clustering of asymptomatic *P. vivax* infections was detected within a 200-m radius of index case households. This clustering may be attributed to the repeated *P. vivax* RCD approach of the study. However, genetic clustering was not performed to determine the transmission networks. In agreement with this study, higher asymptomatic *P. vivax* infections were detected within a 200-m radius of index cases [19]. In this regard, the higher presence of asymptomatic *P. vivax* infections could enable more sustained residual vivax malaria transmission than *P. falciparum* [16]. Furthermore, asymptomatic *P. vivax* infections have gametocyte carriage that significantly contributes to persistent parasite transmission [48, 49]. In general, the findings suggest how long and where to target the interventions, considering the asymptomatic *P. vivax* reservoir, to enhance vivax malaria elimination efforts.

In the present study, 80% of *P. vivax* infections were submicroscopic. This finding is consistent with other studies in low transmission settings [6, 40, 50], *P. vivax* is more likely to be submicroscopic and asymptomatic in low transmission settings [51]. It is challenging to achieve malaria elimination if microscopy and RDT are used for surveillance during the pre-elimination and elimination phases. The other challenge is that individuals with asymptomatic *P. vivax* infections will not receive treatment and this would create submicroscopic malaria carriers and ultimately facilitate malaria transmission [17]. Studies have shown that submicroscopic infections can contribute up to 28–69.8% of mosquito infections [16], and serve as a source of residual malaria transmission and maintain the transmission [49, 52]. To achieve complete malaria elimination of *P. vivax*, more sensitive, cost-effective, and robust molecular diagnostic methods are needed [37, 53].

In the current study, a higher infection incidence rate was observed through the RCD study compared to another study that reported an infection incidence rate in Ethiopia of 46.3 cases per 1000 population at risk [54]. Young children and those of school age exhibited a higher infection incidence of *P. vivax* infection in both index cases and neighbouring households. This phenomenon due to, low parasite virulence or the rapid acquisition of clinical immunity in children, rendering them asymptomatic carriers of *P. vivax* [55, 56]. Supporting this notion, the detectability of *Plasmodium* infections was low in older age groups and low transmission settings [57]. The present study also found, an incidence of *P. vivax* in children under-5 years of age, and being in this age group and of school age were identified as a risk factors associated with *P. vivax* infection [58].

Recent evidence supports targeted test and treat (TTaT) and targeted mass drug administration (tMDA) depending on the local context of transmission and G6PD status. However, tMDA has a safety concern related to PQ and the unknown hypnozoite carrier status of individuals [59]. In conclusion, multiple and extended rounds of RCD, tailored approaches involving targeted IRS and (TTaT) strategies using a point-of-care quantitative G6PD test for radical cure treatment are required to increase efforts towards residual vivax malaria elimination. In agreement with other studies, this study's finding suggests that in low transmission settings, implementation of targeted intervention may be more feasible and cost-effective in a delimited radius. Additionally, molecular tools for the detection of asymptomatic and submicroscopic infections, considering index cases and neighbouring households, could also enhance elimination in low transmission settings. Furthermore, vector and malaria surveillance targeting the index and neighbouring households along with RCD with a defined radius, as well as improving the household and environmental conditions, are recommended.

The limitation of the study is molecular genotyping such as using deep amplicon sequencing or microsatellites was not analysed to determine the genetic clustering or local transmission networks among *P. vivax* index cases with vivax malaria-infected neighbours and control households members.

Conclusions

This study found clustering of asymptomatic and submicroscopic *P. vivax* infections in the index case household and their neighbours using RCD and molecular methods. Children under 5 years and of school age were significantly associated with *P. vivax* infection in index case households. Thus, tailored RCD approaches and targeted interventions for interrupting residual *P. vivax* transmission networks are needed to eliminate *P. vivax* malaria in low transmission settings.

Abbreviations

DNA	Deoxyribose nucleic acid
ELISA	Enzyme-linked immunosorbent assay
ICEMR	International Center Excellence for Malaria Research
IRS	Indoor residual spraying
LLINs	Long lasting insecticidal net
MOH	Ministry of Health
PCD	Passive case detection
RCD	Reactive case detection
qPCR	Quantitative polymerase chain reaction
RDT	Rapid diagnostic tests
TIDRC	Tropical Infectious Diseases Research Centre
WHO	World Health Organization

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Author contributions

AA, DY, GY, CK, and JK conceived and designed the study; AA, HG, AD, AT, and KH were collected and processed field data. AA, HG AD, GZ, DZ, and XW analyzed and interpreted the data. AA drafted the main manuscript text. MCL developed a map of the study area. The manuscript was critically reviewed by DY, GY, CK, and TD for significant intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The protocol was reviewed and approved by the National Research Ethics Review Committee (NRERC) of Ethiopia (Ref. no.: 10/31/2018) and Jimma University Institute of Health, Institutional Review Board (Reference No. IHR-PGD/362/2). Permission was obtained from the relevant Buno Bedele and East Wollega health offices, and Arjo-Didessa Sugar Factory, Oromia regional state, Ethiopia. A written consent was obtained from the study participants and the parents and guardians of children. All malaria-positive index cases and positive study participants identified by microscopic examination of blood film and Rapid Diagnostic tests (RDT) at PCD clinics were treated as per the national malaria treatment guideline for free by health professionals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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