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Prevalence of asymptomatic malaria infection and associated risk factors in Mizan-Aman town, Ethiopia: community-based cross-sectional study

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Abstract

Background Asymptomatic malaria parasitemia patients constitute an effective transmission pool for malaria infection in the community. However, less attention has been given to malaria control and elimination strategies. Therefore, to achieve a malaria elimination strategy, investigating the magnitude of asymptomatic malaria in different settings in Ethiopia is crucial. However, there is not enough information on the prevalence of asymptomatic malaria infection and associated risk factors in the Bench Sheko Zone, southwest Ethiopia. This study, therefore, aimed to provide information and help achieve sustainable malaria elimination.

Methods A community-based cross-sectional study was conducted from February to April 2019 in Mizan-Aman town, southwest Ethiopia. A semi-structured questionnaire was used to collect sociodemographic information. *Plasmodium* species were screened using microscopy and rapid diagnostic test (RDT). HemoCue was used to measure haemoglobin levels. Statistical Package for the Social Sciences (SPSS) version 20.0 was used for descriptive and logistic regression statistics to risk factors. A P-value of 0.05 was used as a cutoff-value for significance.

Results A total of 353 malaria-like symptom free participants were enrolled in this study. 17 seventeen (4.8%, 95% Confidence interval = 2.57, 7.03) asymptomatic malaria cases were revealed; among these, 12 (70.58%) (95% CI = 65.75, 75.25) were due to *Plasmodium vivax* and 5 (29.41%) (95% CI = 24.74, 34.25) were due to *Plasmodium falciparum*. Asymptomatic malaria was significantly associated with the presence of mosquito breeding sites [Adjusted odd ratio (AOR) = 6.06 (1.76–20.82)], insecticide-treated nets (ITN) use [AOR = 3.51 (0.97–12.68)], and indoor residual spraying (IRS) [AOR = 3.95 (1.26–12.37)]. Mild anaemia was found in 20% (3/15) of the asymptomatic malaria patients. Additionally, there was a significant association between malaria and anaemia [OR = 5.786 (1.46–22.85)] in this study.

Conclusions The population of the current study area will be at risk because asymptomatic malaria is present. Low coverage of the IRS, ITN, and proximity of stagnant water in residences had an impact on asymptomatic malaria. Further studies are needed on the burden of asymptomatic malaria via molecular methods, and the Bench Sheko regional health office is better able to scale-up malaria prevention and control tools.

Keywords Asymptomatic malaria, Hemoglobin level, Risk factors, Southwest Ethiopia

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Background

Globally, the World Health Organization (WHO) malaria report estimated 228 million malaria cases in 2019, with 9,000,000 more cases from the previous year. Among these, the WHO Africa region bears 213,000,000 cases. In contrast, deaths due to malaria decreased from the previous year, with an estimated 435,000 to 405,000 deaths [1]. In the Ethiopian context, malaria deaths declined in 2015 [2]. However, there was an increase in malaria transmission rates in 2016 compared with previous years [3]. A higher incidence of malaria contributes to school absenteeism in children and child labourers when a parent is sick [4], low productivity due to illness, permanent neurological damage, and other damage to infants [5]. Moreover, it has implications for anaemia, cognitive impairment, intrauterine growth retardation, prematurity in pregnant women as a result of both severe and uncomplicated episodes of malaria infection cause and acute kidney injury [6, 7].

The Ethiopian national malaria control strategy plans to achieve and sustain zero indigenous malaria transmission by 2020 in 239 low transmittance districts and eliminate it from Ethiopia by 2030. It has been suggested that this strategy could be attained through the scale up of vector control intervention, early diagnosis via microscopy and rapid diagnostic tests (RDT), and prompt treatment [8]. The Ministry of Health (MOH) has implemented different measures that can reduce the burden of malaria. The mass distribution of insecticide-treated bed nets (ITNs), together with increased utilization of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) [9] and adoption of artemisinin-based combination therapy (ACT) has resulted in a substantial decline in malaria-related morbidity and mortality in Ethiopia [10].

However, the lack of regular use of LLINs [11], a coverage gap in vector control tools, changes in mosquito behavioural patterns (exophagy), LLINs and IRS resistance [12], and the hypnozoite reservoir of infection in endemic communities [13] present major challenges for the elimination of malaria. Moreover, the presence of asymptomatic malaria cases in a community is a major challenge for the development malaria elimination strategies, such as the reservoir gametocyte stages of the parasites. These are the infective stages for the vectors. As a result, these asymptomatic cases are contribute to continuous malaria transmission in the community [14].

Asymptomatic infections are often undetected and untreated; this becomes a major source of gametocytes, which can subsequently be infectious to mosquitoes. This increases the morbidity and mortality of malaria in the seasonal malaria transmission areas of Africa [15, 16].

For example, the prevalence rate of asymptomatic malaria in western Cambodia, the China–Myanmar border, and India were 9.1% [17], 23.3% [18], and 20.7% [19], respectively. Additionally, a review of asymptomatic malaria in African countries indicated that asymptomatic cases were still a problem [20]. Hence, the prevalence in Gabon was 18.8% [21] and in Nigeria it was 69.9% [22]. Furthermore, the prevalence in Ethiopia ranges from 0.93% to 21.5% in various settings; 0.93% in Butajira [23], 8.2% in south-central Oromia [24], 21.5% in the Gambella region [25] and 4.1% in Arba Minch town [26].

Therefore, to address the 2030 zero malaria transmission goals, efforts should be made to investigate the burden of asymptomatic malaria cases in both high and moderate transmittance settings in the country. Mizan-Aman town is located in a moderate malaria transmission area and studies on the burden of asymptomatic malaria and associated risk factors are lacking. Therefore, the aim of this study was to determine the prevalence of asymptomatic malaria, the level of haemoglobin, and associated risk factors in the community Mizan-Aman town, southwest Ethiopia.

Knowing the prevalence of asymptomatic malaria infection and associated risk factors in communities in moderate and high transmission areas is critical for informing policymakers on how to design effective malaria elimination strategies. The results of the current study will be used by the local malaria control office, MOH, and other stakeholders to plan effective malaria prevention and control strategies. In addition, it will help to evaluate the effectiveness of malaria interventions being implemented in the study area. On the basis of these findings, the management of asymptomatic carriers could also help to reduce the risk of malaria transmission in communities. Furthermore, this study will be used as a baseline for further work on asymptomatic malaria in the area.

Methods

Study area and period

The study was conducted in Mizan-Aman town, which is located in the Bench-Sheko Zone, Southwest Ethiopia, between February 19 and April 19, 2019 Gregorian calendar. The Bench-Sheko Zone is one of the zones in the Ethiopian Southern Nations, Nationalities, and Peoples Region (SNNPR). The altitude of the town is 1451 m above sea level. The average annual temperature and rainfall range from 15° to 270 °C and 400–2000 mm, respectively. Coffee planters are the primary source of income. The capital of the zone is located at a distance of 574 km southwest of Addis Ababa, the capital city of Ethiopia. According to the national malaria report, the current study setting was a moderate malaria transmission area [3]. In the study setting, five health posts, one health centre, and one teaching hospital were found. Four

primary schools, one secondary school, and one secondary and preparatory government school, as well as one primary and secondary private school, are found in the town (Fig. 1).

Study design

A community-based cross-sectional study was conducted in selected kebeles of Mizan-Aman town, Southwest Ethiopia.

Population

Source population

The source population was the population residing in Mizan Aman town for at least the previous year.

Study population

All individuals who fulfill the inclusion criteria and provide consent are selected kebeles.

Sample size and sampling technique

Sample size determination

The required sample size was determined by using single population proportion formula.

$$n = \frac{(Z\alpha/2)^2 P(1-P)}{(d)^2} * DE$$

$$n = \frac{(1.96)^2 0.069(1-(0.067))}{(0.0335)^2} * 1.5 = 321$$

where: n = Sample size, P = Expected proportion of prevalence of malaria is 6.7% [27], Z $\alpha/_2$ = 1.96 (at the 95% confidence level), d = 0.0335% marginal error, if P is less than 0.1 (10%) and greater than 0.9 (90%), d is a half of P to obtain a large sample size [28]. DE = Design effect is 1.5

On the basis of above assumption, the minimum sample size was 321. To minimize errors arising from the probable occurrence of noncompliance (non-response rate), 10% of the sample size was added, and 353 study subjects were included in the study.

Sampling techniques

A multistage sampling technique was used to select the study unit. The town of Mizan-Aman has five *kebeles*; of those, two (Shesheka and Kometa) were selected

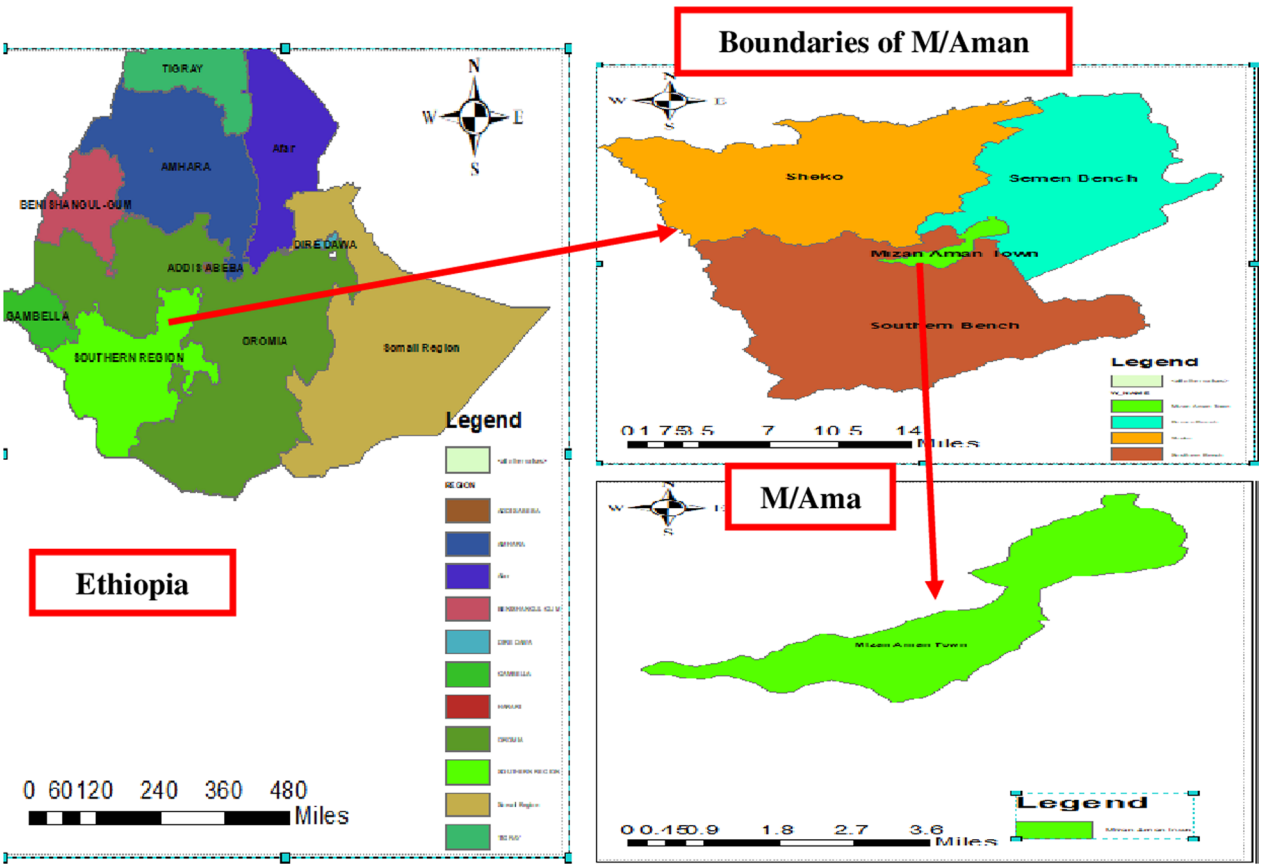

Fig. 1 Map of the study area

randomly for this study. The total populations of Shesheka and Kometa kebeles are 8455 and 7801, respectively, and the numbers of, households in each kebel are 1726 and 1592, respectively. The calculated sample size 353 was divided for SNNP average family size, that is, 4.9, so that 72 households (HHs) were estimated to include in the current study; however, 97 HHs were included. For each kebele 51 and 46 households were selected randomly from Shesheka and Kometa through a random table, respectively. All available individual family members in the selected households at the time of data collection were included until the sample size was reached. Venous blood was collected for the determination of asymptomatic malaria and haemoglobin levels; and semi-structured questionnaires were also used to assess socio-demographic, and socio-economic characteristics and associated risk factors for asymptomatic malaria infection from February 19 to April 19, 2019 G.C. Written consent was obtained from participants and their parents/guardians for under 5 children (Fig. 2).

Study variables

Dependent variable

Asymptomatic malaria infection.

Haemoglobin level.

Independent variable

Age by year, sex, educational status, place of residence (locality), occupational status, monthly estimated income, utilization of ITN, indoor residual spray coverage, replant utilization, presence of parasites in the blood, resting behaviour, previous history of fever, previous self-medication history, previous malaria history, presence of a breeding site around the residence, house wall type, house floor type, and latency type.

Eligibility criteria

Inclusion criteria

The current study included all individuals over the age of two, with an auxiliary body temperature of 37.5 °C, individuals who consented to the study, and individuals who had lived in the study setting for at least one year.

Exclusion criteria

Individuals who received anti-malarial treatment within two weeks at the time of data collection, had malaria-like symptoms, and resided less than one year in a community were excluded from this study.

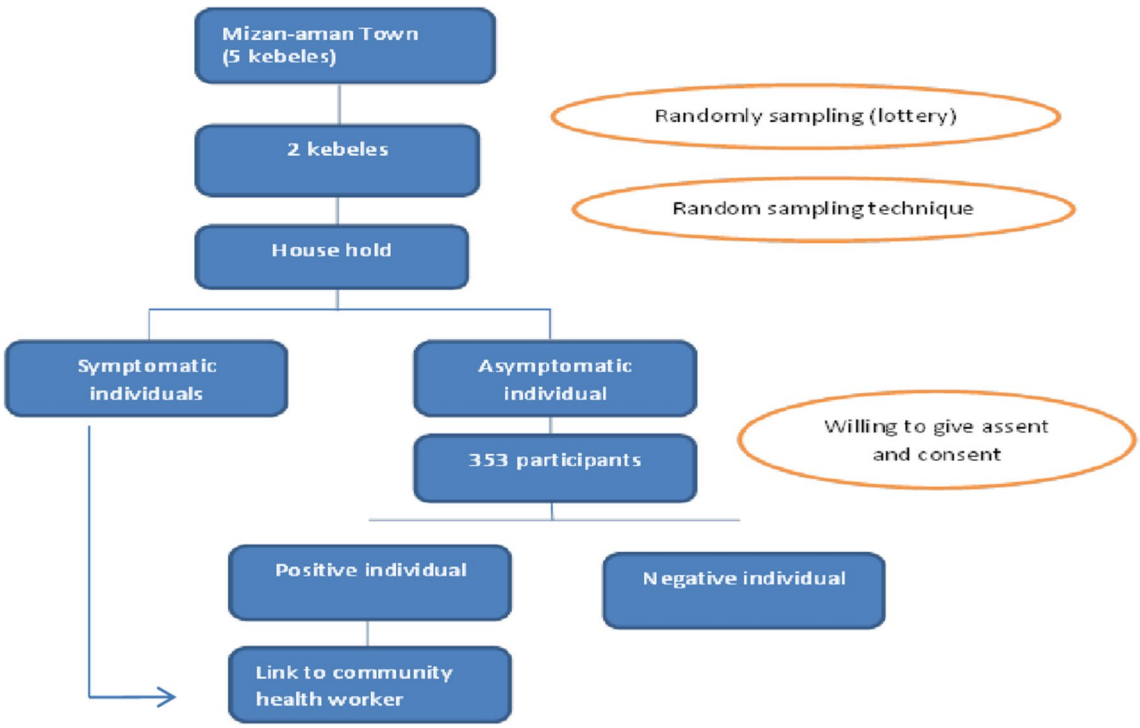

Fig. 2 Schematic presentation of sampling procedure in mizan aman town 2019

Data collection

Questionnaire

Sociodemographic, socioeconomic characteristics, and associated risk factor data were collected via semi-structured questionnaires (Annex-I).

Specimen collection and processing

Venous blood sample collection

Prior to blood sample were collected, participants were given enough information about the study; then, for those who were willing and deciding to participate in the study, auxiliary body temperatures were measured with a digital thermometer by community health workers; and from participants with body temperature37.5 °C and who had not received anti-malarial treatment within the previous two weeks, approximately 3–4 ml of venous blood was drawn with an EDTA tube (Annex-II).

RDT testing

Rapid diagnostic test (care start ™ Malaria HRP2/pLDH (Pf/PAN) Combo) was used on the spot to detect *Plasmodium* species. Rapid diagnostic test histidine-rich protein 2 (HRP2) was used to detect *P. falciparum* and lactose dehydrogenase (LDH) for was used to detect *P. vivax*.

Haemoglobin determination

The EDTA tube blood samples were taken to the Mizan-Tepi University teaching hospital laboratory, where the haemoglobin level was determined using the HemoCue® Hb 301 as directed by the manufacturer. The apparatus measures whole blood absorbance; dual wavelengths (506 nm and 880 nm) are used for Hb measurement and turbidity compensation. Quality control methods and optional liquid controls were used.

Microscopic examination

Thick and thin blood smears were also prepared and stained with 10% Giemsa for 10 min. Giemsa stain contains methylene blue, which stains the parasite cytoplasm, and eosin stain includes parasite nuclei. The stained parasite chromatid was red/pink colour in and was examined for the presence of malaria parasites by a laboratory technician. Parasite density was calculated by counting the number of asexual parasites per 200 leukocytes in the thick blood film, while a slide was considered negative after 500 leukocytes where counted. When thick films were positive, thin films were read for species differentiation. The normal reference range is assumed to be 8000/μL white blood cells. The parasite density per μ1 was calculated via the following formula:

$$\frac{Parasites/\mu L\ blood}{Number\ of\ parasite\ counted \times 8000\ WBC/\mu L\ blood} = \frac{}{Number\ of\ WBC\ counted}$$

Data quality assurance

Pre-analytical phase: A pretest was performed for a semistructured questionnaire, the data collectors were trained, and quality controls were performed for each test procedure before commencing the test.

Analytical phase: The manufacturer's instructions and standard operating procedures (SOPs) were strictly followed when running each test.

In the post-analytical phase: 10% of the negative slide and all positive blood film slides were reexamined by senior medical laboratory technologist blindly, and the completeness of the data was also checked regularly.

Data analysis

Data entry, data cleaning, and coding were performed by using Epi Data Manager Version 4.4.2.1 and then exported to the Statistical Package for Social Sciences (SPSS) version 20.0 software package for analysis. Bivariate and multivariate logistic regression models were used to assess the predictors that contributed to asymptomatic malaria infection. The results were then summarized and presented in tables, figures, and text.

Ethical considerations

Ethical clearance was obtained from institutional review board (IRB) of Jimma University Institute of Health (IHR-PGP/551/18). Written informed consent was collected from participants and parents/legal guardians of children under-5 year of age. All positive participant for malaria and had a low level of haemoglobin were linked to community health workers for treatment and management according to the national malaria treatment guidelines.

Definition of asymptomatic malaria

Individual harbouring malarial parasitaemia of any density, in the absence of fever or other acute symptoms, in individuals who have not received recent anti-malarial treatment.

Anaemia: Based on haemoglobin concentration anaemia classified in mild anaemia (≤11 g/dL), moderate anaemia (≤8 g/dL) and severe anaemia (≤5 g/dL).

Results

Characteristics of the study participants

A total of 353 individuals without malaria like symptoms participated in the current study, of whom 53% (n = 187) were from Shesheka and the remaining 47.0% (n = 166) were from Kometa kebeles. The proportion of sex was

approximately equal between females 53.5% (n = 189) and males 46.5% (164). The mean age of the participants was 26 years (± 11 SD).The majority of participants, 40.2% (n = 142) of the sample, were between the ages of 16 and 25. Primary school and students where the predominant educational and occupational statuses of the participants, with 46.5% (n = 164) and 32.6% (n = 115), respectively. The majority of the participants' estimated monthly income was less than 650.00 ETB, which was 32.30% (n = 114). More than 90% of the participants were not users of ITNs, replanted, had a history of fever, and had a history of malaria. More than half of the participants lived in IRS sprayed homes and had a history of self-medication; 66.0% (n = 233) and 64.3% (n = 227), respectively (Table 1).

Microscopy and RDT confirmation of asymptomatic malaria infection

Among 353 asymptomatic individuals, 8 (2.3%, 95% CI [0.74, 3.86]) with asymptomatic malaria infection were identified and differentiated through microscopic examination. Of these, 6/8 (75.0%, 95% CI [70.49, 79.51]) were *P. vivax,* and 2/8 (25%, 95% CI [24.44, 25.55]) were *P. falciparum*. One subject had a parasitaemia of 500 parasites per one microliter of blood, one subject had a parasitaemia of 920 parasites per one microliter of blood, and the other had a parasitaemia of 1080 parasites per one microliter of blood. The RDT detected n = 17 (4.8%, 95% CI [2.57, 7.03]) asymptomatic malaria infected participants, 12 (70.5%), 95% CI [65.75, 75.25]) due to *P. vivax* and 5 (29.41%, 95% CI [24.74, 34.25]) due to *P. falciparum*. All

Table 1 Characteristics of the study participants in Mizan-Aman town; Ethiopia, from February to April 2019

Characteristics	Frequency n (%)	Characteristics	Frequency n (%)
Address		Utilization of ITN	
Shesheka	187 (53.0%)	Yes	35 (9.91%)
Kometa	166 (47.0%)	No	318 (90.08%)
Gender		Use of replants	
Male	164 (46.5%)	Yes	11 (3.1%)
Female	189 (53.5%)	No	342 (96.9%)
Age in year		Indoor residual spray coverage	
≤ 5	3 (0.8%)	Yes	233 (66.0%)
6–15	52 (14.7%)	No	120 (34.0%)
16–25	142 (40.2%)	Previous history of fever	
26–35	88 (24.9%)	Yes	334 (94.6)
≥ 36	68 (19.3%)	No	19 (5.4)
Education Status		Previous malaria history	
Illiterate	74 (21.0%)	Yes	325 (92.0%)
Only able to write and read	32 (9.1%)	No	28 (8.0%)
Primary school (1–8)	164 (46.5%)	Presence of breeding site	
Secondary (9–12)	53 (15.0%)	Yes	133 (37.7%)
College/above	30 (8.5%)	No	220 (62.3%)
Occupation status		House wall type	
Government	40 (11.3%)	Wood	143 (40.5%)
Farmer	78 (22.1%)	Brick	13 (3.7%)
House wife	84 (23.8%)	Other	197 (55.8%)
Merchant	23 (6.5%)	House floor	
Daily labourer	9 (2.5%)	Wood	2 (0.6%)
House servant	4 (1.1%)	Cement	180 (51.0%)
Student	115 (32.6%)	Mud	171 (48.4%)
Estimated monthly income (ETB)		Resting behaviour	
< 650	114 (32.3%)	Indoor	353 (100%)
650–1300	37 (10.5%)	Outdoor	0
> 1300	37 (10.5%)		
Self-medication history			
Yes	227 (64.3%)		
No	126 (35.7%)		

microscopically detected malaria cases were confirmed through RDT. Therefore, the overall prevalence of asymptomatic malaria in this study was 4.8% (n = 17). No mixed infection was revealed with either of the diagnostic techniques (Table 2).

Distribution of asymptomatic malaria cases by age, sex and address/kebeles

The distribution of asymptomatic malaria among specific age groups was as follows: the > 35 years age group had the highest prevalence of asymptomatic malaria (7.4% (5/68), with the highest prevalence among that age group. In the male sex group, 6.1% (10/164) of the patients had asymptomatic malaria, whereas in the female sex group, 3.7% (7/189) of patients had asymptomatic malaria. The distributions of asymptomatic malaria differ among kebeles. 7% (13/187) of malaria cases were detected in the Shesheka kebele and 2% (4/166) were detected in the Kometa kebele (Fig. 3).

Risk factors associated with asymptomatic malaria infection

Only 13 variables were fitted for logistic regression (address, gender, age, educational status, occupational status, estimated monthly income, history of malaria, self-medication, use of antimalarial spray, house wall type, presence of mosquito breeding site, use of replants, and utilization of ITN). In Bivariate logistic regression analysis, all variables that are significant at a *P*-value of 0.25 and 95% CI was entered into a multivariate logistic regression analysis. Variables that are significant at *P*-value < 0.05 level and 95% CI are considered to be the determinant factors of asymptomatic malaria infection. Gender, age, educational status, occupational status, estimated monthly income, previous history of malaria, previous self-medication history, IRS coverage, and house wall type did not show a significant association with the outcome variable *(*P > 0.25*)* (Table 3).

Whereas five variables (address, use of replant, IRS coverage, presence of mosquito breeding sites, and utilization of ITN) were candidates for multivariate analysis as a result of *P* 0.25 in bivariate analysis As a result of a P value of 0.25 in bivariate analysis, five factors (address, usage of replant, IRS coverage, presence of mosquito breeding location, and use of ITN) were candidates for multivariate analysis. In the multivariate analysis, the utilization of the ITN, coverage of the IRS, and presence of mosquito breeding sites were significantly associated with asymptomatic malaria infection in the current study.

An individual who has no utilization of ITNs during bedtime has a 4.7 times greater likelihood of being infected with asymptomatic malaria than those who do [AOR = 3.51 (95% CI = 0.973–12.681)]. Individuals who live in homes that are not sprayed with indoor residual spray (IRSs) have a 4 times greater likelihood of being infected with asymptomatic malaria than do those who live in sprayed homes [AOR = 3.95, (95% CI = 1.26–12.37)], and those who are sprayed with a mosquito breeding site in their living environment have a 6 × greater probability of being infected than do those who live in the absence of a mosquito breeding site in the surrounding environment [AOR = 6.06 (95% CI = 1.76–20.82](Table 3).

Prevalence of anaemia and its association with asymptomatic malaria

In present study, fifteen (4.2%, 95.9% CI [2.11, 6.29]) participants were anaemic. Of these, fourteen (93.3%) were mildly anaemic and 1 (6.7%) was moderately anaemic. No severe anaemia was found. The minimum and maximum haemoglobin levels were 7.5 and 24.6, respectively, while the mean haemoglobin level was 15.76. Among asymptomatic malaria positive participants, n = 3 (18%) had mild anaemia, according to the WHO standard. In this study, a significant association was observed between asymptomatic malaria and anaemia; the individuals who were positive for *Plasmodium* parasites were 5.8 times more likely to be anaemic than were negative individuals [OR = 5.786 (1.465, 22.852), P = 0.012] (Table 4).

Discussion

Thus, the present study suggests the presence of asymptomatic malaria infection in the study settings, as confirmed through either of two conventional malaria diagnosis techniques (microscopy and/or RDT). The overall prevalence of asymptomatic malaria was 4.8% (17/353). None of the participants regularly used ITNs, living in an IRS sprayed home, and the presence of mosquito breeding sites increased the risk of asymptomatic malaria infection. In contrast, the presence of asymptomatic malaria was associated with a risk of anaemia. The greatest proportion of asymptomatic malaria infection was detected in the male sex group 6.1% (10/164), the

Table 2 Microscopy and RDT confirmed asymptomatic malaria infection in Mizan-Aman town; Ethiopia, from February to April 2019

		Microscopy results			
		P. falciparum	*P. vivax*	Negative	Total
RDT results	*P. falciparum*	2*	0	3	5
	P. vivax	0	6*	6	12
	Negative	0	0	336	336
	Total	2	6	345	353

*Number of malaria cases detected by both microscopy and RDT

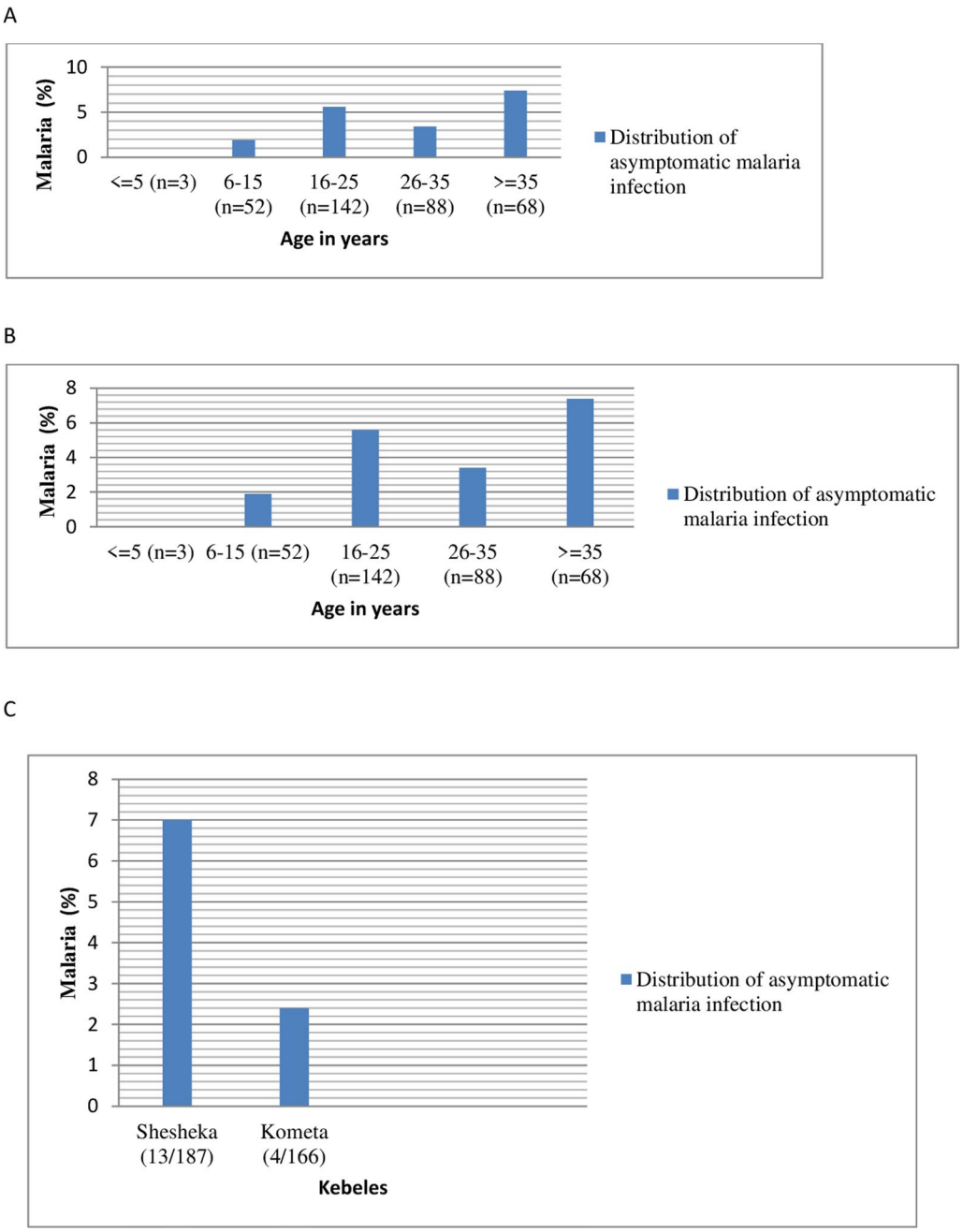

Fig. 3 Distribution of asymptomatic malaria Vs. gender (**a**), age (**b**) and kebeles (**c**) in Mizan- Aman town :Ethiopia ,February to April 2019

age group ≥ 35 years 7.4% (5/68), and the Shesheka kebele residence group 7% (13/187).

Thus, our finding was comparable with those of a study conducted in the Great Mekongi sub region, Haiti, and North Peruvian Amazon, which reported values of 5% [29], 3% [30], and 4.9% [31], respectively. Similarly, a study conducted in Ethiopia reported comparable findings: 6.7% in Dembia district [27], 4.8% in Debre Elias East Gojam [32] and6.1% in Benna Tsemay district [33]. However, this study findings were relatively greater than those of a study conducted in eastern Myanmar, which reported a 1.44% [34] rate of asymptomatic malaria infection; this difference might be due to the different in transmission intensity, which may be low, and different sampling techniques, which was used as a convenient sampling technique.

Table 3 Bivariate and multivariate analyses of risk factors associated with asymptomatic malaria infection in Mizan-Aman town; Ethiopia, from February to April 2019

Variables	No of infection/Total examined	COR (95%CI)	*P.value*	AOR (95% CI)	*P.value*
Address					
Shesheka	13/187 (7%)	1.00		–	
Kometa	4/166 (2.4%)	0.33 (0.106–1.034)	0.057*	0.324 (03097–1.084)	0.67
Gender					
Male	10/164 (6.1%)	1.00	0.3	1	
Female	7/189 (3.7%)	0.592 (0.22–1.593)			
Age					
< 5	0/3 (0%)	0.00	0.999		
6–15	1/52 (1.9%)	0.247 (0.028–2.182)	0.208		
16–25	8/142 (5.6%)	0.752 (0.237–2..392)	0.630		
26–35	3/88 (3.4%)	0.44 (0.102–1.93)	0.279		
> 36	5/68 (7.4%)	1		1	
Educational status					
Illiterate	0/74 (0%)	0			
Only able to write and read	4/32 (12.5%)	9 (0.339–11.817)	0.444		
Primary school (1–8)	7/164 (4.3%)	0.624 (0.123–3.161)	0.569		
Secondary (9–12)	4/53 (7.5%)	1.143 (0.197–6.641)	0.882		
College/above	2/30 (6.7%)	1		1	
Occupation					
Government	2/40 (5%)	1		1	
Farmer	4/78 (5.1%)	1.02 (0.18–5.862)	0.976		
House wife	2/84 (2.4%)	0.463 (0.063–3.415)	0.450		
Merchant	1/23 (4.3%)	0.864 (0.074–10.081	0.907		
Daily labourer	1/9 (11.1%)	2.375 (0.791–29.477)	0.501		
House servant	1/4 (25%)	6.33 (0.437–91.708)	0.176		
Student	6/115 (5.2%)	1.046 (0.202–5.404)	0.957		
Estimated monthly income (ETB)					
< 650	6/114 (5.3%)	2 (0.233–17.176)	0.528		
650–1300	3/37 (8.1%)	3.17 (0.315–32.09)	0.327		
> 1300	1/37 (2.7%)	1		1	
Previous malaria history					
Yes	15/325 (4.6%)	0.629 (0.136–2.901)	0.552		
No	2/28 (7.1%)	1		1	
Previous Self-medication history					
Yes	9/227 (4.0%)	0.609 (0.229–1.620)	0.32		
No	8/126 (6.3%)	1		1	
Spread anti-malarial spray (IRS)					
Yes	5/233 (2.1%)	1		1	
No	12/120 (10.0%)	5.06 (1.741–14.743)	0.003*	3.95 (1.261–12.374)	0.004
House wall type					
Wood	2/143 (%)	1		1	
Brick	0/13 (%)	0.00	0.999		
Other	15/197 (%)	5.8 (1.307–25.825)	0.021		
Presence of breeding site					
Yes	13/133 (9.8%)	5.85 (1.86–18.34)	0.002*	6.06 (1.765–20.826)	0.004
No	4/220 (1.8%)	1		1	
Use of replants					
Yes	3/11 (27.3%)	1		1	
No	14/342 (4.1%)	0.114 (0.27–0.476)	0.003*	0.153 (0.023–1.025)	0.053
Utilization of ITN					
Yes	6/35 (17.1%)	1		1	
No	11/318 (3.5%)	5.77 (1.99–16.752)	0.001*	3.51 (0.973–12.681)	0.05

**P.value* < 0.25, *CI* confidence interval, *AOR* adjusted odd ratio, *COR* crude odd ratio

Table 4 Univarate analysis of anemia Vs sociodemographic character and asymptomatics malaria infection in Mizan-Aman town; Ethiopia, from February to April 2019

Variables	No anemic/ Total examined	COR(95% CI)	*P-value*
Gender			
Male	7/164 (4.3%)	1	
Female	8/189 (4.2%)	0.99 (0.35, 2.8)	0.99
Occupation			
Government	3/40 (7.5%)	1	
Farmer	1/78 (1.3%)	0.16 (0.016, 1.59)	0.118
House wife	3/84 (3.6%)	0.45 (0.088, 2.37)	0.351
Merchant	3/23 (13.0%)	1.85 (0.34, 10.02)	0.476
Daily lobber	0/9 (0.0%)	0	0.99
House servant	1 /4 (25%)	4.1 (0.32, 52.7)	0.277
Student	4/115 (3.5%)	0.44 (0.095, 2.078)	0.303
Monthly estimated income			
< 650	4/114 (3.5%)	0.412 (0.088, 1.93)	0.26
650–1300	1/37 (2.7%)	0.315 (0.31, 3.175)	0.327
> 1300	3/37 (8.1%)	1	
Asymptomatic malaria			
Negative	12/336 (3.6%)	1	
Positive	3/17 (17.6%)	5.78 (1.46, 22.85)	0.012*

**P.value* < 0.05, *CI* confidence interval, *COR* rude odd ratio

Other African studies reported a low prevalence of asymptomatic malaria infection of 1.0% in Zanzibar and 0.8% in Namibia [35, 36]; this discrepancy may be due to the difference in transmission intensity and study area altitude. In the current study area, the transmission intensity was moderate, whereas in Zanzibar and Namibia, there was a low transmission area and an altitude difference. Additionally, in Namibia, vector control tools and treatment improved and it became equivalent to the pre-elimination phase. One survey conducted in the Oromia regional state of Ethiopia reported a low prevalence of malaria 0.56% [37]. This difference might be due to the study participants' age; the majority of our participants were older than 15 years, whereas in the Oromia regional state survey the participants were school children; this may be cause, that in an endemic area, children have the ability to develop protective immunity to against malaria infection. Therefore, this may decrease the density of parasites and make them unable to be detected through conventional diagnostic techniques whereas some studies conducted across African countries have shown a much higher prevalence of asymptomatic malaria as than to this study's findings did. In Gabon 18.8% [21] of asymptomatic malaria cases where revealed; this contrast might be due to the difference between the participants. On this study participants were recruited from the urban area and included all age groups, whereas in the Gabon study, the participants were recruited from different settlements, and came from the malaria-endemic area, and the transmission intensity of the area was perennial. Similarly, in Nigeria, two studies have shown a high prevalence of asymptomatic malaria, 69.9% and 26.1% [22, 38]. This could represent the difference in participant and weather conditions. In the study conducted in Abuja municipality, participants were from a malaria-endemic area, households were linked to malaria patients attending health facilities, and the temperature was slightly higher than normal, which may have increased the sporogony cycle. In another study conducted in Southwest Nigeria, participants were from a malaria-endemic area.

Similarly in Ethiopia, the prevalence of asymptomatic malaria was much higher found at 18.4% in Armachio district and 29.8% in Jimma town [39, 40]. This great difference may be due to the transmission intensity and the participants' differences. The Armachio district study area was experiencing hyperendemic malaria transmission and the participants were migrant labourers. However, in Jimma town, the enrolled participants were febrile and asymptomatic individuals, which may increase the chance of detecting more cases through conventional techniques. Similarly, a study conducted in the West Arsi Zone reported an incidence of 8.2% [24]: this contrast may be due to differences in topography; the presence of man-made rainwater storage in other study areas may contribute to the continuous transmission of malaria through the breeding of mosquito vectors. Another study conducted in Arba-Minch town also reported a high prevalence, which was 9.1% [41]. This difference may be due to the study participants, i.e., pregnant women. This may be because pregnant women have more high susceptibility rates to malaria infection than healthy individuals.

According to the national malaria report, 60% of the dominant *Plasmodium* species are *P. falciparum* and 40% are *P. vivax* [3]. Similarly, other studies conducted in Ethiopia revealed a similarly high proportion of *P. falciparum* species as *P. vivax* [24, 27, 33, 39]. This might be due to *P. falciparum* species being the most widely distributed in Ethiopia. However, this study revealed that *P. vivax* was responsible for 70.5% (12/17) of the cases and that *P. falciparum* was responsible for 29.5% (5/17) of the cases. Other studies conducted in Ethiopia support this findings by identifying a greater proportion of *Plasmodium vivax* species [40, 41]. This might be a result of the treatment, i.e., the current national malaria treatment guidelines focus more on the blood-stage of *P. falciparum* and in addition, climatic variability in the study area affects the sporogony cycle of the parasite.

The correlation analysis of parasite density with haemoglobin level did not reveal a significant correlation (>0.05). The regression analysis of the current study reveals that sexes, age in years, and locality or address were not significantly associated with asymptomatic malaria infection. Malaria was found in greater proportions in the male sex 6.1% (10/164), >35 years 7.4% (5/68), and Shesheka kebele residence 7% (13/187). This finding was similar to that of a study conducted in the North Gonder zone; there was a greater proportion of males than females and the age groups were different, adults ≥15 years of age; a greater proportion of males accounted for 20 [27]. Similarly, the Debre Elias Gojam study revealed a greater proportion of malaria in the male sex and 25–34 years of age groups [32].

Similarly, in multivariate analysis, previous history of malaria and self-medication had no significant associations with asymptomatic malaria infection. The proportion of individuals with no history of previous malaria infection was 7.1% whereas the percentage of those with a previous history of infection was 4.6% this could be because previous exposure to malaria infection aids in the development of acquired immunity, i.e., anti-parasite, anti-immunity, and anti-disease antibodies, which may reduce parasite density and symptoms. Additionally, individuals who do not have self-medication habits have a 6.3% higher infection rate than those who have self-medication habits. This approach might help prevent an increase in malaria parasite density as a result of self-medication. However, in the national malaria prevention and control strategy, an insecticide-based vector control mechanism is key strategy for the prevention and control of malaria transmission. Ensuring and maintaining universal access for at-risk populations through the distribution of LLINs and the IRS is the major vector controlling intervention. The current study confirmed that those who were regularly using ITNs at bedtime were 3.5 times more protected against malaria infection than those who were not. Other studies conducted across Ethiopia also support this results [27, 32, 37, 41, 42].

In present study, individuals who lived in non- IRS sprayed homes and were near stagnant water were more likely to be malaria positive than those who lived in sprayed home and were lacking stagnant water around their home. Other studies conducted in Nigeria [22] and Gabon [21] also support these study results, i.e. close proximity of stagnant water in residences is a significant risk factor for asymptomatic malaria. Similarly, a study conducted in the northwest Armachiho district [39] and North Gondar Zone Dembia district, northwest Ethiopia [27], showed that living in proximity to stagnant water and non- IRS sprayed houses was significantly associated with asymptomatic malaria; which was also similar to the finding of an Oromia regional state study [37]. This is because environmental management is a key preventive tool for mosquito breeding and transmmision.

The overall prevalence of anaemia in the current study was 4.2% (n=15), among malaria positive individuals, 18% (n=3) had mild anaemia. Another study conducted in Indonesia reported a much greater prevalence than the present study did; the prevalence was 32.8% [43]. This contrast may be due to the difference in parasite density and the number of asymptomatic malaria infected participants. In the present study, the number of infected individuals was lower than that in other studies. Additionally, a study conducted in Nigeria found reported a much greater prevalence of anaemia, of which 28.5%, 45.5%, and 14.6% had mild, moderate, and severe anaemia, respectively [44]. This greater discrepancy might be due to the study population difference; the current study was conducted among the community, whereas a study conducted in Nigeria was among health institutions and those who come for other medical issues.

In Benishangul Gumuz Regional State, Ethiopia, a much greater prevalence was found: 73.76% (194/263) [45]. This difference might be because the participants differece; the current study was asymptomatic healthy participants, but another study was conducted among symptomatic individuals, aged < 10 years, and may be due to nutritional differences (particularly iron deficiency). A study conducted in Arba Minch Town among pregnant women reported an overall prevalence of 34.6% anaemia (118/341), among those positive for asymptomatic malaria. 90% (27.9/31) had anaemia [41].

The bivariate analysis has revealed a significant association between asymptomatic malaria and anaemia. This result agreed with other studies conducted elsewhere [25, 41, 43, 44]. This result agreed with other studies conducted elsewhere. This agreement may be due to the fact that malaria causes anemia through different mechanism; impaired erythroposis, destruction of erythrocytes, and clearance of infected erythrocytes.

The limitation of this study is that the prevalence of asymptomatic malaria parasites was determined by conventional diagnostic techniques; this may not have truly detected a low density of asymptomatic malaria infections of sexual and asexual parasites in this study.

Conclusion

The findings of this study revealed that asymptomatic malaria is a public health problem in the community of Mizan Aman. A total of 4.8% (95% CI=2.6–6.99) of the patients were asymptomatic malaria cases. Low utilization of the ITN, low coverage of the IRS, and the presence of mosquito breeding sites in residences were significantly associated with asymptomatic malaria.

Asymptomatic malaria infection was one of the risk factors for anaemia in the present study, even though the cause of anaemia is multifactorial, particularly nutritional deficiency.

In this study, conventional malaria diagnostic techniques were used, but it may not be possible to determine the whole asymptomatic or low-density cases. Therefore, further studies, including molecular genotyping, are needed to determine the low density of sexual malaria parasites and immune system evaluations are needed to gain a better understanding of the epidemiology of asymptomatic malaria infection. Regional health offices and community health workers should scale up the coverage of ITNs and IRSs in study settings, and communities should be engaged in environmental management to prevent the establishment of mosquito breeding sites.

Abbreviations

ACT	Artemisinin-based combination therapy
AOR	Adjusted odd ratio
CI	Confidence interval
COR	Crude odd ratio
EDTA	Ethylene diamine tetra acetic acid
ETB	Ethiopian birre
HH	Household
HRP2	Histidine-rich protein 2
IRS	Indoor residual spray
LAMP	Loop-mediated isothermal amplification
LDH	Lactose dehydrogenase
LLIN	Long lasting insecticide treated net
MOH	Ministry of Health
PCR	Polymerase chain reaction
PI	Principal investigator
RDT	Rapid diagnostic test
SD	Standard deviation
SNNP	South nationality peoples
SOP	Standard operating procedures
SPSS	Statistical package social sciences
WBC	White blood cell
WHO	World health organization

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Author contributions
KD served as the study's lead investigator, collected, entered, analyzed, and interpreted the data: prepared the report; and acted as well as preparing the report and acting as a corresponding author. AZ, AB, DY and GG contributed to the article's drafting or critical revision for essential intellectual content. KD collected the data and helped with the interpretation of the results. The final document was read and approved by all the authors, and they agreed to be responsible for all parts of the work.

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Availability of data and materials
The paper and its supporting information files contain all necessary data.

Declarations

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Not applicable.

Competing interests
The authors declare no competing interests.

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