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**ORIGINAL ARTICLE**

# MALARIA MICROSCOPY PERFORMANCE IN SELF-PRESENTING FEBRILE PATIENTS AT FOUR HEALTH FACILITIES IN FENTALE DISTRICT OF EAST SHEWA, ETHIOPIA

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## ABSTRACT

***Background:*** *Correct and reliable microscopic examination results are vital in appropriate treatment of malaria in endemic areas, mainly where Plasmodium falciparum and P.vivax co-exist in Ethiopia. Thus, evaluation of regular malaria microscopy performance is needed.*

***Objectives****: To evaluate the performance of regular malaria microscopy and antimalarial drug prescription practices for self-presenting febrile patients at health facilities located in malaria endemic areas of upper Awash Valley, eastern central Ethiopia.*

***Methods:*** *A cross sectional study design was used to recruit 260 febrile patients at four health facilities in Fentale district. All slides collected at health facilities were rechecked in reference laboratories and Kappa score was calculated to see the slide reading agreement.*

***Results:*** *Malaria parasites from clinical cases were found in 19.6% (51/260) of the total febrile patients of which 82.4% (42/51) were infected with P. vivax and 17.6% (9/51) with P. falciparum. Overall sensitivity, specificity, positive and negative predictive values of regular malaria microscopy readings were 92.2%, 83.7%, 58% and 97.8%, respectively. Artemether-lumefantrine over prescription rates was 50.8% by the regular microscopy and 53.6% by reference microscopy. There was only a moderate agreement between regular malaria microscopy and reference microscopy with the Kappa value of 0.52.*

***Conclusion:*** *The overall reading agreement and agreement on species identification of the regular and reference microscopy were low. There was variability in performance in the different health facilities. Sensitivity, specificity, and positive predictive value of regular malaria microscopy need to be improved for accurate diagnosis and prompt treatment of malaria cases in Fentale district health facilities. There should be rational use of antimalarials especially on slide negative subjects.*

## INTRODUCTION

Malaria is a major public health problem in Ethiopia causing 828,415 annual malaria cases in 2009/2010 (1). In 2009/2010, parasitological examination microscopy and rapid diagnostic tests) was done only for 462,623 cases (55.8% of the total malaria patients) among which 256,487 (55.4%) were found positive for malaria. The peak of malaria incidence generally occurs following the main rainfall season (July-September) in October and November every year. However, many areas in the south and west of the country have a rainfall season which begin in April and May or have no clearly defined rainfall season (2).

*Plasmodium falciparum* and *P. vivax* are the two common species in Ethiopia contributing to the Countrywide prevalence estimate which was 1% in 2007 of which parasitemia prevalence for *P. falciparum* and *P. vivax* was 0.7% and 0.3%, respectively (3). According to the malaria diagnosis and treatment guidelines (4), the first- line drug for the treatment of uncomplicated *P. falciparum* malaria is Artemether-lumefantrine (AL). Chloroquine is used for the treat-

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ment of *P.vivax*. Rectal artesunate (or intramuscular [IM] artemether when rectal artesunate is unavailable) is used as prereferral treatment for severe malaria in the health posts. At the health center and hospital levels, intravenous (IV) artesunate infusion or IM injection (or, alternately, quinine IV infusion when artesunate is not available) is the first line antimalaria drug for management of severe malaria.

The health service delivery in Ethiopia is organized in four tier system as primary, secondary and tertiary levels. Health centers together with health posts are the primary health care units in the tier system followed by the most advanced medical care at hospitals (primary, general and referral hospitals). Microscopic examination of blood slides is in place as the regular method of diagnosis in health centers and hospitals. Moreover, rapid diagnostic tests are the main diagnostic tools at the health posts (4).

Microscopy has the advantage of enabling the examiner to identify the species, stage and density of an infection. However, the technique requires skilled personnel, precision instruments and a source of electricity (5) Accurate parasitological diagnosis of malaria by microscopy could provide precise estimates of prevalence and incidence to evaluate the impact of malaria control interventions (5). Estimates made on the attributable benefits of a diagnostic test showed that with 95% sensitivity and 95% specificity a diagnostic test requiring minimum infrastructure would avert more than 100,000 deaths and nearly 400 million unnecessary treatments, thus reducing drug pressure (6).

Studies which assessed the performance of malaria microscopy in Ethiopia are quite few (7-10). A study by Mitiku *et al.* in North Gonder zone reported low sensitivity, specificity, positive predictive value, and reading agreement between the operational and reference reader on blood slides stained and read by the laboratory personnel at the primary level (7). A recent study by Endeshaw et al (10) elsewhere at rural health centers also reported low sensitivity in identifying parasite positivity in one out of the ten health centers studied.

The present study assessed malaria microscopy while health facility laboratories perform using their own resources and procedures without being given any kind of instruction, procedure, reagent, microscope or refresher training. The study has been designed to measure the performance of malaria microscopy in the usual investigation practice. It should be remembered that the regular microscopy results guide the treatment of malaria especially the costly AL in Ethiopia since 2004 (4) and hence it requires close monitoring (5, 10). Therefore, the objective of this study was to evaluate the performance of malaria microscopy in the regular practice of malaria diagnosis at four health facilities in Fentale District of East Shewa, Ethiopia using reference microscopy as gold standard for malaria diagnosis. This study also assessed the antimalarial drug prescription practices.

## MATERIALS AND METHODS

This study was done in four health facilities in Fentale Dstrict, East Showa Zone, Oromia Regional State, Ethiopia between March and April 2011. This area is located adjacent to Awash River in the Great Rift Valley of Ethiopia. This district has a surface area of about 1170 $Km^2$ with a total population of 82,225 (11), residing in 18 rural and two town kebeles (the smallest administrative unit of the government structure). Metehara Town is the administrative capital of the district. In 2008-2011, the district on average had about 2137 confirmed cases per annum of which 1074 cases were due to *Plasmodium falciparum*, 699 due to *Plasmodium vivax* while 364 were mixed infections. Confirmed malaria cases in 2012 were 95 in March and 112 in April (retrospective data from district health office). The district has moderate and seasonal malaria with frequent occurrence of epidemic (12-13). Public health services are obtained at one hospital, three health centers and eighteen health posts, private facilities and at Catholic Church Clinic. The four health facilities (namely Metehara Hospital, Metehara Health Center, Metehara Catholic Clinic and Sabure Sugar Estate Clinic) were selected in consultation with the district health bureau as they were easily accessible health facilities to supervise the study and they had functional malaria microscopy in place during the study period.

The design of the study was cross-sectional survey. 260 self presenting acute febrile patients presenting with symptoms of uncomplicated malaria (14) mainly fever (the inclusion criteria) at the time of presentation (body temperature>37.5) or history of fever in the past 48 hours were included in the study. The patients were recruited by clinical staffs Physicians, Health Officers and Nurses) in the outpatient departments. Patients less than one year old, pregnant women, and patients with other febrile conditions like otitis media, tonsilities, measles and absesses were not included.

Individuals doing the regular malaria microscopy were one laboratory technologist and five laboratory technicians in Metehara Hospital, one laboratory technologist and one laboratory technician in Metehara Health Center, two laboratory technicians in Metehara Catholic Clinic and one laboratory technician in Sabure Sugar Estate Clinic. All the laboratory personnel had a minimum laboratory experience of one year. All the laboratories in the health facilities were using their own reagents and microscope (Olympus). They prepared blood films following their own procedure which was being used in their own laboratory in the regular practice. Each of the laboratory personnel in each facility participated in malaria microscopy diagnosis alternately according to their schedule.

All slides were transported to Nazareth Malaria Control Laboratory and Ethiopian Health and Nutrition Research Institute (EHNRI) Malaria and Other Parasitic and Vector- Borne Research Team. Two experienced malaria microscopists at Nazareth Malaria Control Laboratory served as two independent first and second reference readers and each of them re-examined all the slides independently, identified the species, quantified and determined parasite density against 200 WBCs or 500 WBCs on thick films according to the WHO procedure (15).

The third reference reader at EHNRI re-diagnosed and resolved discrepant results which occurred only in four slide readings between the two reference readers. In addition, the reference reader at EHNRI performed parasite density estimation for all positive slides. The "gold standard" readings were established by taking the concordant readings between the first and the second reference readings plus the third reference reading (which was in agreement with one of the reference readings).

Epi Data version 3.1 (http://www.epidata.dk) and STATA version 8.2 (StataCorp.2003.Stata Statistical Software: Release 8. College Station, TX: StataCorp LP) were used for data entry and analysis, respectively. The performance of health facility regular microscopy was determined by calculating the sensitivity, specificity, positive and negative predictive values against the reference microscopy as the gold standard. Kappa score was calculated to quantify the existing agreement between readings by regular malaria microscopists in the health facilities and the "gold standard" reference readings established by the three reference readers.

The cut-off values for the agreement were less than 0.20, 0.20 - 0.40, 0.40 - 0.60, 0.60 - 0.80 and 0.80 - 1.00 for poor agreement, fair agreement, moderate agreement, good agreement and very good agreement, respectively (16). Geometric mean parasitemia levels were defined as lower level (< 500 asexual parasite/μl), middle level (500–5000 asexual parasites/μl) and upper level (>5,000 asexual parasites/μl) parasitemia (17) to investigate how good readers are at low, middle and higher parasitemia level. Overprescription was defined as the rate of malaria microscopy negative subjects treated on the total of malaria microscopy negative subjects. Categorized data were compared by Pearson's Chi-square test and a *P*-value below 0.05 was considered statistically significant.

This study obtained ethical approval from Aklilu Lemma Institute of Pathobiology and Ethiopian Health and Nutrition Research Institute. Signed informed consent was obtained from each study participant. Guardians' consent as well as assent was obtained for non-adult participants. All malaria positive cases were treated at the health facilities by the clinical personnel according to the malaria treatment guidlines in Ethiopia (4).

## RESULTS

A total of 260 self-presenting febrile patients were diagnosed for malaria by microscopy in the four health facilities (Table 1). Of those, 164 (63.1%) were males and the rest were females. The mean age of patients was 19.5 years (ranging from 1 to 62 years). More than half of patients (57%) were older than 15 years (Table 1). Most of the patients (more than 98%) reported fever. Poor appetite, headache, sweating, chills and nausea were the most frequently reported signs and symptoms [data not shown]. Paracitamol was the most prescribed medicine (69.6%) followed by Artemether-lumefantrine (51.5%), Chloroquine (21.5%) and antibiotic (17.7%) [data not shown].

Table 1: Characteristics of study participants, Fentale District, Metehara, March to April 2011, Ethiopia.

| Characteristics | Health facitlities | | | | Total, n (%) |
|---|---|---|---|---|---|
| | Metehara catholic clinic, n (%) | Metehara Health Centre, n (%) | Sabure Sugar Estate Clinic, n (%) | Metehara Hospital, n(%) | |
| **Age groups (years)** | | | | | |
| 1-4 | 28 (30.4) | 1 (3.3) | 7 (20.0) | 9 (8.7) | 45 (17.3) |
| 5-9 | 12 (13.0) | 1 (3.3) | 4 (11.4) | 22 (21.4) | 39 (15.0) |
| 10-14 | 7 (7.6) | 1 (3.3) | 4 (11.4) | 16 (15.5) | 28 (10.7) |
| ≥15 | 45 (49.0) | 27 (90.0) | 20 (57.1) | 56 (54.4) | 148 (57.0) |
| **Sex** | | | | | |
| Male | 48 (52.0) | 25 (83.3) | 24 (68.6) | 67 (65.0) | 164 (63.0) |
| Female | 44 (48.0) | 5 (16.7) | 11 (31.4) | 36 (35.0) | 96 (37.0) |

Microscopic examination of 260 slides at four health facilities in the study district is shown in Table 2. The health facilities found malaria parasites in 31% of slides they examined in which 51.8% of slides were presumed to be positive for *P.falciparum*, 43.2% of slides positive for *P.vivax* and 5% of slides positive for mixed (*P.falciparum* and *P.vivax*) infections. Reference microscopy detected malaria parasites in 19.6% of the total febrile patients (which is lower than the regular microscopy by 11.7%) of which 82.4 % were infected with *P. vivax* and 17.6% with *P. falciparum*. However, no mixed infection was detected by the reference malaria microscopy unlike the regular microscopy in the health facilities. The sensitivity, specificity, positive predictive value and negative predictive value of regular microscopy in detecting malaria parasites is shown in Table 3.

The regular microscopy detected malaria parasites with a sensitivity of 92.2% (95% CI: 81.1-97.8) and a specificity of 83.7% (95% CI: 78.2-88.5). The positive and negative predictive values were 58% (95% CI: 46.5-68.9) and 97.8 % (95% CI: 94.4-99.4), respectively. The regular microscopy and reference microscopy agreed only in 80% of the readings with the overall Kappa coefficient of 0.52. The reading agreement between the reference and the regular microscopy in detecting *Plasmodium* parasites was good (Kappa=0.62). However, health facilities individually had different level of agreement with the reference microscopy.

Table 2: Malaria parasite investigation results at different health facilities, Fentale District, March to April 2011, Ethiopia

| Health facilities | Slides examined, n (%) | Parasite positive, n (%) | Parasite species | | |
|---|---|---|---|---|---|
| | | | P. falciparum, n (%) | P. vivax, n (%) | Mixed (P.f + P.v), (n %) |
| Metehara Catholic Clinic | 92 (35.4) | 17 (18.4) | 16 (94.1) | 1(5.9) | 0 (0) |
| Metehara Health Center | 30 (11.5) | 7 (23.3) | 5 (71.4) | 2 (28.6) | 0 (0) |
| Sabure sugar estate clinic | 35 (13.5) | 11 (31.4) | 10 (90.9) | 1 (9.1) | 0 (0) |
| Metehara Hospital | 103 (39.6) | 46 (44.7) | 11 (23.9) | 31 (67.4) | 4 (8.7) |
| Total | 260 (100) | 81 (31) | 42 (51.8) | 35 (43.2) | 4 (5.0) |

Table 3: Malaria microscopy performance in detecting malaria parasites in self-presenting febrile patients at four health facilities, March to April, Fentale District, Ethiopia

| Regular microscopy | | Reference Microscopy | | Sensitivity (95%CI) | Specificity (95%CI) | Positive predictive value (95%CI) | Negative predictive value 95%CI) | % Agreement | Kappa Value |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | | |
| Metehara Catholic Clinic | Positive | 0 | 17 | 0 (0-70.76)* | 80.90 (71.2-88.5) | 0 (0-19.5)* | 96 (88.8-99.2) | 78 | -0.06 |
| | Negative | 3 | 72 | | | | | | |
| Metehara Health Center | Positive | 7 | 0 | 100 (59.4-100)* | 100 (85.2-100)* | 100 (59.4-100)* | 100 (85.2-100)* | 100 | 1.00 |
| | Negative | 0 | 23 | | | | | | |
| Sabure sugar estate clinic | Positive | 4 | 7 | 80 (28.4-99.5) | 76.67 (57.7-90.1) | 36.36 (10.9-69.2) | 95.83 (78.9-99.9) | 77 | 0.38 |
| | Negative | 1 | 23 | | | | | | |
| Metehara Hospital | Positive | 36 | 10 | 100 (90.3-100)* | 85.07 (74.3-92.6) | 78.26 (63.6-89.1) | 100 (93.7-100)* | 90 | 0.79 |
| | Negative | 0 | 57 | | | | | | |
| Overall | Positive | 47 | 34 | 92.16 (81.1-97.8) | 83.73 (78.2-88.5) | 58.02 (46.5-68.9) | 97.77 (94.4-99.4) | 85 | 0.62 |
| | Negative | 4 | 175 | | | | | | |

* one-sided, 97.5 % confidence interval

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Table 5: Overall antimalarial prescription in different health facilities by age categories, Fentale District, March-April, 2011, Ethiopia

| Health Fa-cilites | MCC | | | MHC | | | SSEC | | | MH | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age groups | AL | CQ | QU | AL | CQ | QU | AL | CQ | QU | AL | CQ | QU | |
| 1-4 | 17 /28 (61) | 5 /28 (18) | 0 /28 (0) | 1 /1 (100) | 0 /1 (0) | 0 /1 (0) | 6 /7 (86) | 0/7 (0) | 1 /7 (14) | 1/9 (11) | 1/9 (11) | 0/9 (0) | 32/45 (71) |
| 5-9 | 10 /12 (83) | 2 /12 (17) | 0 /12 (0) | 0 /1 (0) | 0 /1 (0) | 0 /1 (0) | 4 /4 (100) | 0/4 (0) | 0 /4 (0) | 4/22 (18) | 8/22 (36) | 0/22 (0) | 28/39 (72) |
| 10-14 | 6 /7 (86) | 1 /7 (14) | 0 /7 (0) | 1/1 (100) | 0 /1 (0) | 0 /1 (0) | 3/4 (75) | 0/4 (0) | 0/4 (0) | 3/ 16 (19) | 6/16 (37) | 1/16 (6) | 21/28 (75) |
| ≥ 15 | 39 /45 (87) | 4 /45 (9) | 0 /45 (0) | 6 /27 (22) | 8 /27 (30) | 0 /27 (0) | 19/20 (95) | 1/20 (5) | 0/20 (0) | 12/56 (21) | 20/56 (36) | 3 /56 (5) | 112/148 (76) |
| Total | 72/92 (78) | 12/92 (13) | 0/92 (0) | 8/30 (27) | 8/30 (27) | 0/30 (0) | 32/35 (91) | 1/35 (3) | 1/35 (3) | 29/103 (19) | 35/103 (34) | 4/103 (4) | 193/260 (74) |

MCC= Metehara Catholic Clinic, MHC= Metehara Health Center, SSEC=Sabure Sugar Estate Clinic, MH=Metehara Hospital, AL= Artemether-lumefantrine, CQ= Chloroquine, QU= Quinine

Over prescription of Artemether-lumefantrine was 50.8% by regular microscopy and 53.6% by reference microscopy. Overprescription of Chloroquine was 12.8% by the regular microscopy and 15.7% by reference microscopy [data not shown].

Comparison of slide readings by species detected is shown in Figure 1. Health facilities reported 33 more *P.falciparum* slides compared to reference microscopy. In Figure 2, parasite prevalence per ranges of parasite density is shown as determined by reference microscopy on 49 positive slides.

Figure. 1. Comparison of slide readings by species detected.

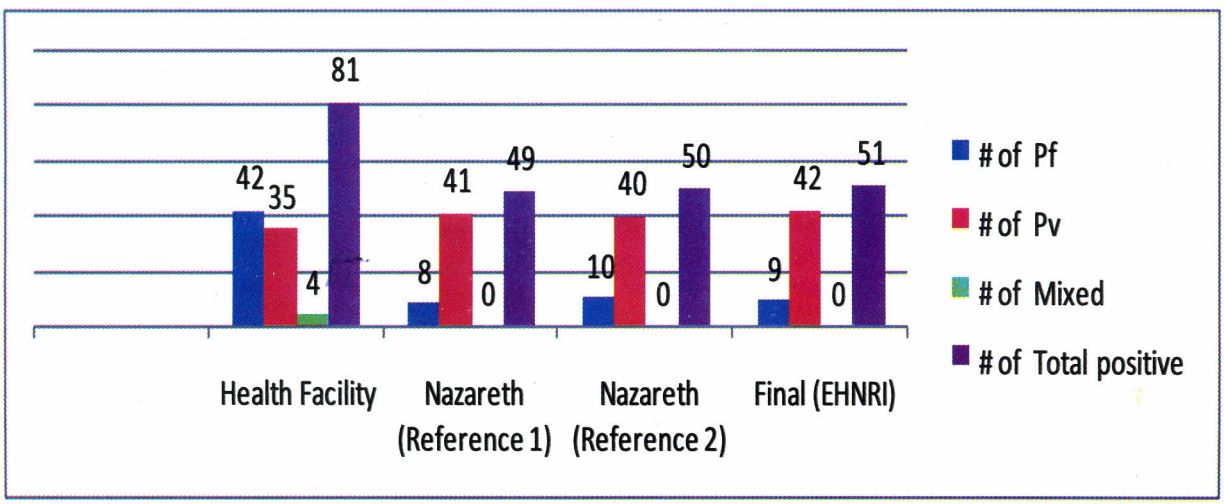
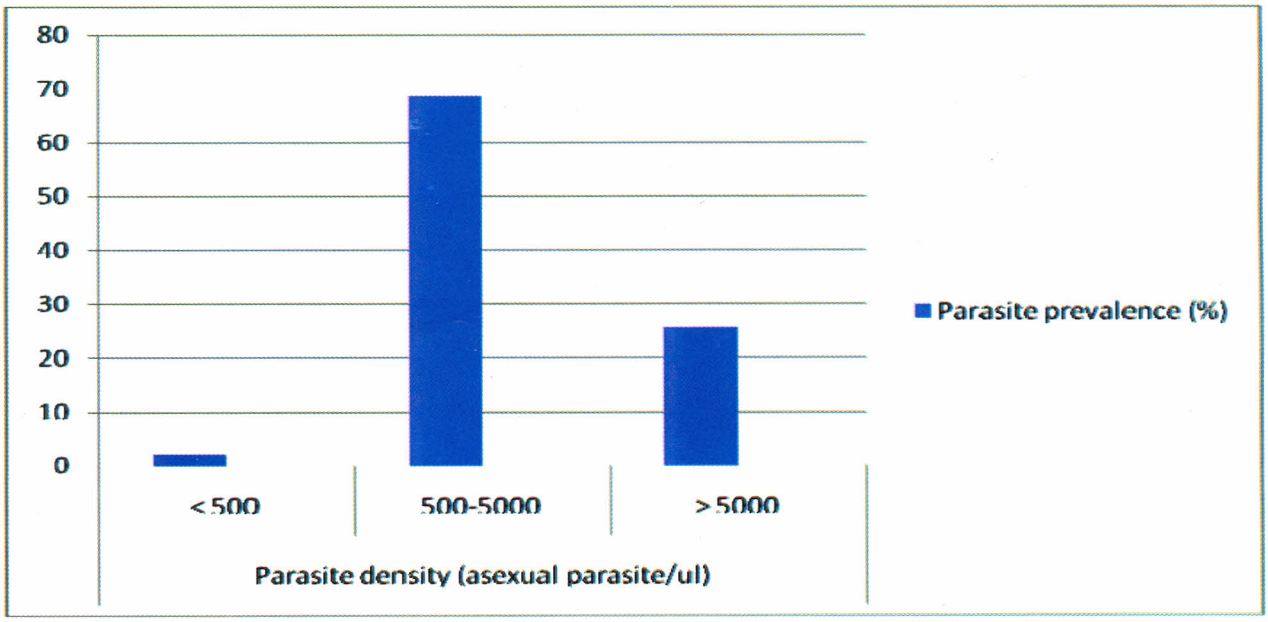


Figure. 2. Parasite prevalence per ranges of parasite density




## DISCUSSION

This study examined the sensitivity, specificity, positive and negative predictive values of regular malaria microscopy in four health facilities compared to the gold standard reference microscopy. Overall, there was only moderate agreement between the regular microscopy and gold standard reference microscopy. This reduced overall reading agreement was mainly due to the low performance in species identification of the regular microscopy as it had only fair agreement in species identification while it had good agreement in detection of *Plasmodium* parasites with gold standard reference microscopy. This finding confirmed the frequent occurrence of misidentification of malaria species by the regular microscopy in the health facilities studied. Misidentification of malaria species is a major problem which leads to initiation of incorrect treatment to patients consequently increasing malaria related morbidity and mortality (18-20).

The regular microscopy in the health facilities studied in Fentale district performed similarly with the previous report from north Gonder where the overall Kappa score was about 0.53 (7). However, the difference observed in the performance of regular malaria microscopy between the different health facilities in the present study (a very good performance by Metehara Health Center and good performance by Metehara Hospital versus no agreement in performance by Metehara Catholic Clinic and poor performance by Sabure Sugar Estate Clinic) could be explained by the presence of slide rechecking scheme in some of them while absent in the others (21). MS observed Metehara Health Center and Metehara Hospital receiving feedback from rechecking schemes during the study period. The two slide rechecking schemes by Metehara Health Center done once a month and every two weeks by two different institutions might explain why it performed better than Metehara Hospital which participated only in one slide rechecking scheme once a month.

Generally, the regular malaria microscopy in Fentale district health facilities showed good sensitivity in detection of *Plasmodium* parasites with the exception of Metehara Catholic Clinic which did not detect all malaria positive slides (there were 3 malaria positive slides identified by the reference gold standard). The positive predictive value in identifying *Plasmodium* parasites in the two health facilities (Metehara Catholic Clinic and Sabure Sugar Estate Clinic) was very poor, fair in Metehara Hospital and very good in Metehara Health Center. As a result, the overall positive predictive value of the regular malaria microscopy for identifying *Plasmodium* parasites was very poor.

The sensitivity and negative predictive value of regular microscopy obtained in this study is similar with a recent study from Northwest Ethiopia (10). Unlike this study, the study from Northwest Ethiopia reported very good specificity and positive predictive value of regular microscopy. In the present study, the overall sensitivity of the regular microscopy in detecting *P. vivax* was very poor. Again, this finding was similar with one of the health centers in the above mentioned recent study from Northwest Ethiopia.

In the present study, the positive predictive value of regular microscopy for identifying *P. falciparum* was very poor and it was only fair for identifying *P.vivax*. Thus, the poor positive predictive value of regular malaria microscopy caused considerable false positives resulting unnecessary antimalarial drug prescription which leads to drug resistance and drug pressure (22). This could partly be due to the lack of uniform supportive supervision, quality assurance mechanisms and refresher trainings (19, 23).

More than one-third (over 30 slides) of the overall microscopic results were found negative by the reference centers and EHNRI (Figure 1). It also appeared identifying *P. falciparum* and *P.vivax* is also a problem. The number of *P. falciparum* reported by the health facilities dropped extremely at reference centers and EHNRI. These variations could be due to the much involvement of the reference centers at slide rechecking works and EHNRI's participation in continuous slide quality assurance (EQA) scheme. Therefore, putting in place quality assurance mechanisms like participation in slide EQA (5) is needed to improve the performance of regular malaria microscopy. Thus, the malaria control programme (MCP) at different level should address such gap in order to be successful in the programmatic aspects.

Accurate malaria diagnosis is a prerequisite when considering malaria elimination from a certain area as the case which will be in Ethiopia in the near future, 2015 from some areas and from all over the country in 2020 (24). As in the present study, the tendency of overestimating *P. falciparum* infections on blood slides was also seen in a study done elsewhere (18). Moreover, apart from overestimating *P. falciparum*, there was also a tendency of overlooking of malaria parasites on slides with higher parasite density at the health facilities. This could again be the area of focus by the MCP at different level to bring improvements in this aspect when considering malaria elimination.

The prescription of antimalarials observed in the study facilities for self-presenting febrile patients shows that the clinical staffs in the health facilities often make decision treatment for malaria based solely on clinical diagnosis for slide negative subjects. Thus, this practice indicates that malaria slides are requested only to confirm the clinical suspicion rather than for guiding malaria treatment (25). However, another cause of fever must be thought in slide negative cases before prescribing anitmalarials as indicated in the malaria treatment guidelines (4).

***Conclusion:***

In conclusion, the findings in this study inform the need for renewed effort in improving the quality of malaria microscopy diagnosis especially when considering malaria elimination from an area as precise estimates of parameters like prevalence and incidence, which are essential to evaluate the impact of malaria control interventions, are obtained only through accurate diagnosis. This study also informs the need to do other studies in many areas of similar settings.

The overall reading agreement and agreement on species identification of the regular and reference microscopy were low. There was variability in performance in the different health facilities. The sensitivity, specificity and positive predictive value of regular malaria microscopy needs to be improved for accurate diagnosis and prompt treatment of malaria cases in Fentale district health facilities. There should also be rational use of antimalarials especially on slide negative subjects.

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