ADDIS ABABA UNIVERSITY

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE

Sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and KelewaniPrimary Hospital at Awra and Gulina district, Afar Region, Ethiopia

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This is to certify that the thesis prepared by Sintayehu Mehari entitled 'SERO-PREVALENCE OF HUMAN BRUCELLOSIS MALARIA-CO-INFECTION AND RISK FACTORS AMONG FEBRILE PATIENTS VISITED DERAYITU HEALTH CENTER AND KELEWANI PRIMARY HOSPITAL AT AWRA AND GULINA DISTRICT, AFAR REGION, ETHIOPIA' and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of Abbreviations

AAU	Addis Ababa University
CFT	Compliment Fixation Test
DMLS	Department of Medical Laboratory Sciences
DRERC	Department Research and Ethical Review Committee
ELISA	Enzyme Linked Immunosorbent assay
LPS	Lipopolysaccharide
PI	Principal Investigator
RBPT	Rose Bengal plate Test
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

Abstract

Background: *Brucellosis* is a zoonotic disease usually acquired through direct contact with the infected animals and consumption of contaminated milk and meat products. In humans Brucellosis presents similar signs with other febrile diseases like Malaria, typhoid and other febrile conditions.

Objective: In this study weaimed to determine Sero-prevalence of human brucellosis malaria co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia

Methods: A cross Sectional study was conducted among febrile patients visiting Derayitu Health center and Kelewani Primary Hospital at Awra & Gulina District of Afar region, Ethiopia from February to May 2019. After consent/assent obtained, demographic and clinical data were collected using structured questionnaire, 5ml venous blood was collected with plain vacutainer tubs. Serum was separated and stored in refrigerator after thick and thin blood films were prepared. Rose Bengal plate Test (RBPT) was performed and those positive sera were confirmed using ELISA. Giemsa stained thick and thin blood films, were made to look for the presence of hemo-parasites. Data were entered in Epi Data3.1 and exported to Stata 14 to analyze prevalence and potential risk factors at 95% confidence interval < 0.05 was considered as statistically significant

Results: A total 444 febrile individuals (females constituting of 61.1%), age ranged from 2 to 83 years (mean = 26.1, SD = ±11.8) were participated in this study. Among all (444) individuals tested, 31.5 % (140/444) were found reactive by Rose Bengal plate Test and only half of the reactive (70/140) were found reactive again by Enzyme Linked Immuno sorbent assay. The sero-prevalence of Brucellainfection based on the two tests was 15.8 % (70/444). Being males (23.3%, X^2= 13.05; p <0.001), illiterate (20.4%, X^2= 6.21; p = 0.045) and rural residents (17.6%; X^2 = 3.93; p = 0.047) were highly infected by Brucella infection than their respective comparison. Multivariate logistic regression model analysis revealed that drinking of raw milk (AOR=16.96, 95%CI: 2.27-126.69, p=0.006) and touching aborted fetus/discharges without protection

(AOR=2.13, 95%CI: 1.08-4.20, p = 0.029) were found significantly associated with having brucellosis where as being females (AOR = 0.42, 95 % CI: 0.44 - 0.74, P = 0.003) were found associated with not having brucellosis. Of all (444) individuals, 19 (4.3%) were found positive for malaria infection (only for P. falciparum).

Conclusion: Findings in this study clearly show that the sero-prevalence of human brucellosis among febrile patients is high (15.8%). The study also identified that consuming raw milk and touching of aborted material/fetus were the risk factors for brucellosis. Intervention focusing on awareness about the zoonotic nature of brucellosis and the role of raw milk in the transmission of the diseases is important to control the diseases.

Keyword: **Brucellosis, malaria, Sero-prevalence, Pastoralists, Afar Ethiopia,**

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1: INTRODUCTION

1.1 Background

Brucellosis is one of the important neglected bacterial zoonotic diseases that affecte animals and humans for decades [1]. The bacteria was first identified in 1887, by Sir David Bruce, a British military physician from the spleens of patients who died from undulant fever in Malta [2]. *Brucella* is a highly contagious zoonotic infection caused by ingestion of non-pasteurized or inadequately pasteurized milk or undercooked meat from infected animals, or close contact with their secretions [3, 4]. The causative bacterium is *Brucella with many speices- B. abortus B. canis B. melitensis* and *B. suis* These species are little, gram-negative, non-motile, non-spore-forming, rod-shaped bacteria known as Coccobacilli. They operate as facultative intracellular parasites that cause chronic infection.

The disease manifests with continued, intermittent or irregular fever (hence the name undulant fever), headache, weakness, profuse sweating, chills, arthralgia, depression, weight loss, hepatomegaly, and splenomegaly and generalized aching. Cases of arthritis, spondylitis, osteomyelitis, epididymitis, orchitis, and in severe cases neuro-brucellosis, liver abscesses, and endocarditis have also been reported in humans [1, 4, 5].

The disease affects are wide range of domestic and wild animals causing abortions, reduced milk yield, and infertility resulting in tremendous economic losses in livestock production [6]. It is one of the microbial occupational hazard to persons like veterinarians, laboratory workers, slaughterhouse workers and farmers, which is, acquired either through contact with infected animals, their tissues and products. Bacteria can also enter wounds in the skin/mucous membranes through contact with infected animals. Other risk factors for brucellosis are associated with consumption of raw or unpasteurized milk and dairy products processed from such milk [7].

Brucellosis remains endemic in areas like Europe, northern and eastern Africa, India, central Asia, Mexico, and Central and South America [8]. Though it is eradicated from several developed countries, brucellosis remains a serious neglected zoonotic

disease in low- and middle-income countries and a priority of reemergence in several countries with an increasing incidence of infectionin cattle[9]. Brucellosis is more prevalent in developing countries and considered to be a serious health problem due to lack of effective public health measures, domestic animal health programs, and appropriate diagnostic facilities. Furthermore, the situation is also worsen by the resemblance of the disease with other diseases leading to misdiagnosis and under-reporting [10].

1.2 Statement of the problem

Brucellosis is a recognized zoonotic public health hazard found in the world [11]. Globally, about half a million human brucellosis cases are annually reportable however, in sub-Saharan Africa, brucellosis prevalence is unclear and poorly understood with varying reports from country to country, geographical regions as well as animal factors [12] even though it is reported among animals as high or range from 10.2 to 25.7 % [13].

Human brucellosis has a wide clinical spectrum, presenting various diagnostic difficulties to poor or developing countries like Ethiopia because it mimics many communicable and non-communicable diseases like malaria, typhoid, rheumatic fever, joint diseases [14, 15]. Many cases remain undiagnosed either because they are not with apparently specific signs and symptoms or because physicians are unfamiliar with the disease and its epidemiology.

Many localities of Ethiopia are agro pastoralist or pastoral where people almost entirely depend on livestock for their livelihood, and many scholars have reported brucellosis from animals, but it does not considered yet as an important public health disease by health care stockholders. Hence, there is no clear statistical information available about the extent of infection in humans of the country. This posed a difficulty to estimate the prevalence of human brucellosis in Ethiopia.

The pastoralists of Afar Region depend on livestock and their products for their livelihood as milk and meat from cow, camel and goat are the main sources of their food and handling of aborted materials, manipulation of reproductive excretions with bare hands, and herding of a large number of animals collectively, are widely practiced [16]. The objectives of this study was be to determine the sero-prevalence and risk factors of human brucellosis febrile patients visiting Derayitu Health center and Kelwani Primary Hospital at Awra and Gulina District Afar region, Ethiopia.

1.3 Significance of the study

- The study generates information on the prevalence of human brucellosis and its risk factor among pastoralist community of Afar Ethiopia.
- This study was produce information about the extent of brucellosis that was help impact policy makers and stakeholders to control it.
- The result of the study was assist to determine the potential risk factors of the human brucellosis among pastoralist community of Afar Ethiopia.

2. LITERATURE REVIEW

Global Epidemiology

Human brucellosis is a major zoonotic disease of global health importance causing for more than 500,000 new infections every year [7]. The disease exists worldwide, with the highest prevalence in the Mediterranean countries, Asia, Africa and Central and South America. There are 10 identified *Brucella* species, which have preferential host specificities, amongst four are infective to humans. They are *B. melitensis*, which is found in goats, sheep and camels, is the most widespread and is the most virulent; *B. abortus*, which is found in cattle and camels, is less virulent; *B. suis*, which is found in pigs, is also less virulent; and *B. canis*, which is found in dogs, is the least common. In addition, rare cases of human infections with *B. pinnipediae* or *B. cetaceae* from marine mammals and *B. ovis* (from sheep), *B. neotomae* (from desert wood rats), B*. microti* (from voles), and *B. inopinata* (from unknown origin) have been reported [11, 17].

The study in Tanzania, a cross-sectional study was conducted Prevalence of Bacterial Febrile Illnesses in Children in Kilosa District, a total of 370 patients were enrolled presumptive acute brucellosis due to B. *abortus* was identified among 26 (7.0%) of patients while B. *melitensis* was detected in 57 (15.4%) of the enrolled patients. However, access to diagnostic tests for discrimination of febrile illnesses is needed. This would allow febrile patients to receive the correct diagnoses and facilitation of accurate and prompt treatment [18].

A cross sectional study conducted in Mexico to determine the Sero-prevalence of brucellosis among dairy farm workers. Their investigation showed that Sero-prevalence of brucellosis was 18.1% 13.3% of them corresponded to recent infection from high daily occupational exposure in contact with cows and living on-site. Besides, they found a high sero-prevalence of brucellosis among dairy farm workers, as well as a significant association among those with prolonged and close contact with cattle [2].

Similarly, another study on Prevalence and risk factors for brucellosis in prolonged fever patients in post-conflict Northern Uganda Sero-prevalence of brucellosis was calculated for i-ELISA IgG/IgM. A structured questionnaire was used to obtain data on possible risk factors for brucellosis. Brucellosis was confirmed in 18.7% of the 251 patients that tested positive for the disease, with the rapid Brucella Plate Agglutination Test, and ages 10-84 years (median age 47+0.86). Sex ($p = 0.001$; OR 3.79; 95% CI 1.75 - 8.24), rearing livestock ($p < 0.005$; OR 8.44; 95% CI 2.84-25.03) and consumption of unpasteurized milk ($p = 0.023$; OR 2.57; 95% CI 1.14-5.80) were factors associated with brucellosis. The Authors concluded that, control of brucellosis in animals, training and sensitization of the community on brucellosis is needed to stimulate action on human brucellosis control [19].

Study done in South-Eastern Nigeria co-existence and prevalence of another fever-causing condition—brucellosis, with malaria and typhoid. Involved 682 febrile patients referred to a private medical laboratory in Enugu metropolis in South-Eastern Nigeria for investigation for malaria and typhoid only. The number was made up of 295(43.3%) males and 387(56.7%) females, aged between 10 and 50 years. Identification of malaria parasites was done using thick films stained with Giemsa stain while typhoid and brucellosis were investigated serologically using Chromatest febrile antigen kits. Our results showed prevalence of 39.1%, 66.0% and 28.6% for malaria, typhoid and brucellosis respectively in the studied population. Prevalence among male and female patients was 46.1% and 33.9% for malaria, 80.3% and 55.0% for typhoid, and 34.2% and 24.3% for brucellosis respectively. The results also showed that prevalence of malaria decreased with age while typhoid and brucellosis increased with age [4].

Study done in Lahore district to determine sero-prevalence of Brucellosis in a high-risk occupational group and analysis of risk factors, 521.7% (95% CI 17.44% - 25.96%) were positive by ELISA test. The logistic regression model identified age

(OR 0.96, 95% CI 0.94-0.99), assistance in parturition of animal (OR 0.47, 95% CI 0.23-0.96), consuming raw milk (OR 2.25, 95% CI 1.04-4.87) and handling sheep (OR 0.30, 95% CI 0.09- 0.92) as risk factors for *Brucella* sero-positivity among slaughterhouse workers of Lahore district. The investigation recommended that to reduce the burden of brucellosis, a national brucellosis control programmer should be initiated with special emphasis on the high-risk population of slaughterhouse workers [20]. The study conducted in Northeastern Kenya, Human Brucellosis in Febrile Patients Seeking treatment at remote Hospitals. A total of Brucellosis was established in 146 patients (13.7%, 95% CI 11.7%–15.9%). Of these, 29 (2.7%) had negative serologic test results for *Brucella* infection. by the treating hospital clinicians in 119/146 (81.5%) cases in our study. Instead, these cases were mainly attributed to other causes of fevers or fevers of unknown origin [21]

A cross-sectional study done in Nigeria, the sero-prevalence and associated exposure factor among 224 abattoir workers, 54(24.1%) were identified positive. Of these, 32 (59.3%) were butchers, and 11 (20.4%) were meat-sellers. Slaughtering animals while having open-wounds occupational-exposure of >5years (AOR=2.45, CI=1.15 – 5.30) and eating raw meat (AOR=2.64, CI=1.14 - 6.14) were significantly associated with human brucellosis. The investigation concluded that, Sero-prevalence of human brucellosis among abattoir workers in Abuja was higher and recommended them to abstain from eating raw-meat and educate on adherence to safe animal product handling [22].

Study in Uganda to determine sero-prevalence and associated risk factors of human brucellosis in agro-pastoral communities was 17.0 % (n = 235). The prevalence was highest among males (20.5 %, n = 78) and the elderly - above 60 years (22.2 %, n = 18). Residence in rural areas (OR 3.16, 95 % CI: 1.16–8.56), consuming locally processed milk products (OR 2.54, 95 % CI: 1.12–5.78) and being single (OR 2.44, 95 % CI: 1.05–5.68), were associated with increased risk of

brucellosis. They conclude that, brucellosis highly prevalent in kiboga district, and therefore an important public health problem. The transmission risk was aggravated by consumption of unpasteurized milk product and there is a need to initiate screening and treating infected human early [1]. Similarly, another study on malaria negative febrile individuals showed the sero-prevalence brucellosis was high among participants aged 18–35 years (13.3%), Muslims 12 (14.0%), those with no formal education (33.3%) and divorced 2 (14.3%). Again consuming of raw milk (OR 2.162, 95% CI 0.021–1.379) and being a Muslim (OR 6.101, 95% CI 1.601–23.248) were associated with increased risk of *Brucella abortus* [7].

In Southern Ethiopia, a cross-sectional study was conducted on sero-epidemiology of human brucellosis among blood donors in Arba Minch Blood Bank Center, a total of 254 donors donated blood through regular programs and out-reach blood collection campaigns. Majority of the study participants were males (82.3%) and the mean age of the study participants was 26 years (standard deviation= ±8.26). Samples were screened sero-agglutinins reactivity to stained antigen of *Brucella abortus*. Standard tube titration test was performed for reactive serum to
determine the titer of the agglutinin. The sero-prevalence of human brucellosis among donors wasfound 10.6%. The Authors concluded that, the higher sero-prevalence of human brucellosis in the study area needs attention and additional confirmatory investigation [23].

Study done in Jimma, to determine the Sero-prevalence of human brucellosis, community awareness and practices on its zoonotic importance. Their investigation showed that a total of 48 blood samples, 24 from Chora botor and 24 from Jimma town were collected. The overall sero-prevalence of brucellosis in humans was 2.1% and 0.0% by RBPT and CFT, respectively. The majority (97.6%) of the respondents reported to have no awareness on brucellosis. They concluded that, to educating communities and creating awareness to prevent and control the disease [24].

Across sectional study conducted in Debre Ziet and Modjo, Central Ethiopia showed that 156 abattoir workers participated in the questionnaire survey and among them, 149 agreed for blood sample collection the overall sero-prevalence of brucellosis in abattoir workers was found to be 4.7 and 1.3% using Rose Bengal plate test and Compliment fixation test, respectively. Based on the questionnaire survey, 66 (44.2%) and 85 (53.21%) of abattoir workers were aware of brucellosis and other zoonotic diseases, and 29 (18.6%) and 21 (13.5%) were using gloves and cover their mouth while slaughtering, respectively. The investigators Concluded brucellosis in abattoir workers could be prevented by using protective closing and measures [25].

Across sectional study conducted in brucellosis among patients with fever of unknown origin in Jimma university hospital southwestern Ethiopia using Rose Bengal plate test and Complement fixation test from 56 subjects, 2 were positive giving a prevalence of 3.6%in cases with fever of
unknown origin in the study area. They concluded that, the study indicates that human Brucellosis could be the cause of fever of unknown origin in the study area
Thus, clinicians should consider it as one of the differential diagnoses for fever of unknown origin [26]. The study conducted in Borena and Hamer on prevalence of in traditional community the blood sample collected from 30 (34.1%) patient from Borena, 5(29.4%) Hamer
and 3(3%) from Metema tested positive in the *Brucella* ELISA. The disease common in particular among patients with recurrent unresolved febrile illness. They concluded that,
brucellosis should be included in disease education program and control measurement [27].

3. OBJECTIVE

3.1 General objective

To determine Sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia

3.2 Specific objective

- To determine the sero-prevalence of brucellosis
- To assess associated risk factors of brucellosis
- To determine the malaria and co-infection with brucella

4. Null Hypotheses

There is no relationship between the Sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia.

5. METHODS AND MATERIALS

5.1. Study Area and period

Afar National Regional state is one of the nine regions of Ethiopia and geographically located in northeast of the country between 39^0 34' and 42^0 30 28' east longitude and 8^0 30' north latitude. The total geographical area of the region is about 270,000 km. Afar National Regional State is characterized by an arid and semi-arid climate with low and erratic rainfall. The region has a total population of 1.5 million and administratively, divided into five zones, which are further subdivided into 32 weredas (administrative districts) and 358 pastoral associations [30].

The study was conducted from February to May 2019 in the Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar region, Ethiopia is one of five Zones of the Afar Region of Ethiopia. This zone is two largest ethnic groups reported in Zone 4 were the Afar (98.67%), and Amhara (1.09%); all other ethnic groups made up 0.24% of the population.

5.2 Study design

A cross-sectional study

5.3. Population

5.3.1. Source population

All patient who attending Derayitu Health center and Kelwani primary Hospital at Awra and Gulina District, Afar Region, Ethiopia at the study period.

5.3.2. Study population

The study populations were all febrile out patients that attending Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district Afar Region of Ethiopia and willing to participate in the study during the study period that fulfill the inclusion criteria.

5.4. Inclusion Criteria and Exclusion Criteria

5.4.1. Inclusion Criteria

All febrile patients who have fever and with axial body temperature ≥ 37.5°C during data collection period and able to provide consent/assent for participation, was included in the study

5.4.2 Exclusion Criteria

Patients taking any antibiotics and who was not provide consent/assent form.

5.5 Study variables

5.5.1 Dependent variables

- Prevalence of brucellosis
- Malaria Brucella co infection

5.5.2 Independent variables

- Socio demographic (Sex, age, marital status, educational status, occupational status, residence)
- Occupational type
- Potentials risk factors (consumption of raw or unpasteurized milk and dairy products processed from such milk, raw meat)

5.6. Sample size calculation and Sampling method

5.6.1. Sample Size calculation

Sample size was calculated by taking Community-based prevalence of brucellosis from previous study conducted among pastoralist community of Afar Ethiopia (4.4%) [5]. Using 95% Confidence interval and 2 % margin of error, the sample size is calculated using the following formula[31].

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

Provided that: n= sample size; Z/2 = to 95% confidence interval (1.96); P = Prevalence of brucellosis and *d* = desired level of precision (2%)

- $n = \frac{(1.96)^2 * 0.044 (1- 0.044}{(0.02)}$ n= 404

By taking additional 10% contingency for non-response rate, the sample size were = 444

5.6.2. Sampling Technique

Convenient sample technique was used to select study participants who visited health institute during study period.

5.7 Measurement and Data collection

5.7.1. Data collection procedure

After consent/assent was obtained and structured questionnaire was used to collect information about, the exposure status of potential risk factors and to assess the socio demographic characteristics of the study participants by the investigator. Five ml of venous blood was collected from each febrile patient using gel and clot activator tubes and thin and thick blood smears were prepared immediately to diagnosis malaria. Samples was left at room temperature for 30 minutes to facilitate clotting and was centrifuged at 3000rpm for 5 minutes to get clear serum to separate from whole blood. After separation, all sera collected in 1.5 ml null tubes were kept in a cool box and transported to the Addis Ababa Federals-police health center laboratory for storage and testing. All sera were screened for *Brucella* antibody using Rose Bengal Plate Test (RBPT). All rapid positive tests were confirmed using Brucella IgG Antibody ELISA Test at AHRI. All sera positive by RBPT were further tested by ELISA for confirmation.

5.7.2. Laboratory Methods Brucellosis diagnosis

Two types of serological tests were employed as a screening and confirmatory test for the detection of *Brucella* antibody in human serum, the Rose Bengal plate Test (RBPT) and Brucella IgG Antibody ELISA Test at Armore Hansson Research institute.

5.7.2.1. Rose bengal plate test

The rose bengal test (RBT) is a rapid, slide-type agglutination assay performed with a stained B. abortus suspension at pH of 3.6-3.7 and plain serum. Its simplicity made it an ideal screening test for small laboratories with limited resources.

5.7.2.2. Brucella IgG Antibody ELISA

The *Brucella* IgG Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Brucella in serum and plasma.

5.7.2.3. Thick and thin blood film preparation

Thick and thin blood smear study is the gold standard method for malaria diagnosis. The procedure follows these steps: collection of peripheral blood, staining of smear with Giemsa stain and examination of red blood cells for malaria parasites under the microscope.

Principle of the Rose Bengal antibody reaction

The smooth, attenuated stained *Brucela* antigen suspensions are mixed with the serum. Specific antibodies to Brucella antigens if present in the serum will react with the antigen suspension to produce agglutination reaction. No agglutination indicates the absence of specific antibodies to Brucella antigens.

INTERPRETATION OF RESULTS SLIDE TEST METHOD

- Agglutination is a positive test result and indicates the presence of specific antibodies to Brucella in the serum.
- No agglutination is negative test result and indicates the absence of specific antibodies to Brucella serum.

PRINCIPLE OF BRUCELLA IgG ELISA

The *Brucella* IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Brucella antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted in to the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Brucella antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugateis added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipette and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophoto-metrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

Qualitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of+/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

Thick and thin blood film preparation

Thick and thin blood smear study is the gold standard method for malaria diagnosis. The procedure follows these steps: collection of peripheral blood, staining of smear with Giemsa stain and examination of red blood cells for malaria parasites under the microscope.

Thick smear: It is not fixed in methanol; this allows the red blood cells to be hemolyzed, and leukocytes and any malaria parasites present was the only detectable elements. However, the hemolysis may lead to distorted plasmodia morphology making plasmodium species differentiation difficult. Therefore, thick smears are mainly used to detect infection and to estimateparasitemia.

Thin smear: It is fixed in methanol. Thin smears allow the examiner to identify malaria species, quantify parasitemia, and recognize parasite forms like schizonts and gametocytes.

5.8. Data Quality Assurance:

Quality assurance and quality control Standard operating procedures was strictly followed and internal quality controls materials were included from the test kits was performed based on manufacturer instructions. The questionnaires prepared was checked by advisors and pretest before the details work was done. Data collectors were carefully selected for their experience to collect biological as well as questioner data collection and also provide 2 days training prior to data collection. In addition, there was be daily follow up by the principal investigator and supervisor.

5.8.1. Pre-analytical Phases

Blood samples were collect from the study subjects and properly label with their unique identification number. The blood sample were collecting by the well be trained experienced laboratory personnel by using vacutainer SST. The laboratory personnel and principal investigator assured to collect enough amount and good quality sample for analysis to produce reliable and valid data.

5.8.2. Analytical phases The test was done by trained laboratory personnel according to standard operational procedures of each test methods. The reagents, kits and the methods was assessed with known positive and negative controls materials, well-trained and experienced laboratory professionals were participated in the laboratory analysis procedure. Finally the results was checked by the investigator and supervisors.

5.8.3. Post-analytical phases the results was recorded by unique identification numbers, repeatedly checked before recording in result log and also raptly checking the result from the result log in to instrument to avoid transcriptional error.

5.9. Data entry and analysis

Data was entered into Epi Data 3.1 and analyzed with Stata/SE 13.0. Descriptive analysis was

used to summarize the data in the form of frequencies and percentages of variables. Pearson

chi-square test was used to evaluate the statistically significant difference in the level of prevalence of brucellosis between male and female study participants and according to the reported clinical features. Bi variable and multivariable logistic regression analyses were

performed to explore associations of socio-demographic characteristics of the study participants with increased odds of having higher prevalence of brucellosis. P-value below 5% was considered as indicator of statistical significance.

5.10. Dissemination of results

This study on completion could serve as a reference material to researchers, experts or policy makers for intervention. To reach these bodies the finalized paper will be submitted to School of laboratory technology, Addis Ababa University. So it can serve as a reference in the library. In addition, a copy of this material will be given to Derayitu Health center and Kelwani Primary Hospital. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant workshops and seminars.

5.11. Ethical Consideration

An ethical review committee of the Department of Clinical Laboratory Sciences, School of Allied Health Sciences, College of Health Science, and Addis Ababa University was approved this study with an ethical letter. After written permission was obtain from the Derayitu Health center and Kelwani Primary Hospital. Names and any other sensitive personal information of individual study subject was not record during sample collection. Moreover the sample collectors were laboratory professional working in the laboratory department of the Derayitu Health center and Kelwani Primary Hospital Afar region, Ethiopia and were being monitored daily by the principal investigator. Sample was collected after getting consent/assent from the participant. All positive results were communicated to the attending physician. The confidentiality of the test result of the study was kept by investigator.

6. The overall work flow diagram

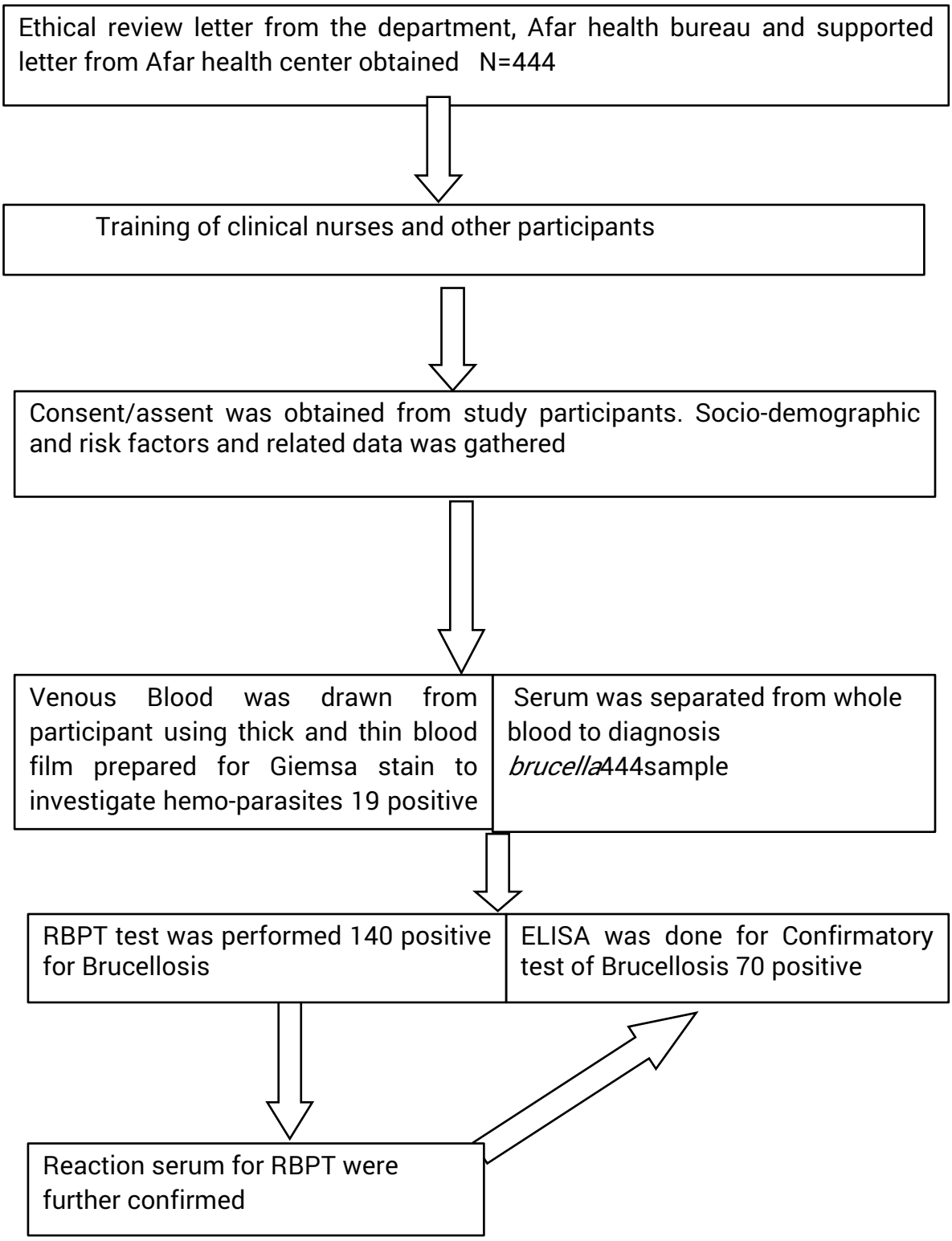

Figure 1: Work flow diagram of the study

7. RESULTS

7.1. Demographic characteristics of the study population

A total 444 febrile individuals were involved in this study. The age were ranged from 2 to 83 years with a mean age of 26.1(SD = ±11.8) years where majority(54.3%)were between 15–29 ages group. Most of the participants were female 59.5% (264), while Agro- and/or Pastoralist 56.1% (249), Illiterate 45.3% (201) and married 70.5% (313) were the major groups from their respective categories(Table 1).

Table 1: The socio- demographic characteristics of the study participants

Factors	Variables	Number (%)
Sex	Male	180(40.5)
	Female	264(59.5)
	Total	444(100)
Age	2-14	58(13.1)
	15-29	241(54.3)
	30-44	111(25.0)
	≥ 45	34(7.6)
Educational status	Illiterate	201(45.3)
	Primary school (1-8)	147(33.1)
	High school and above	96(21.6)
Marital status	Married	313(70.5)
	Non married	131(29.5)
Residents	Urban	97(21.9)
	Rural	347(78.1)
Occupation	Agro- and/ or Pastoralist	249(56.1))
	Others*	195(43.9)

7.2. Sero-prevalence of Brucella infection

The sero-prevalence for *Brucella* infection among the study participants was 31.5% (140/444) byRBPT and 50%(70/140) by ELISA. The combined sero-prevalence for *Brucella* infection by thetwo tests was 15.8% (70/444). The sero-prevalence for *Brucella*infection was relatively high inthe age group in 15-2915.4%(n=37/241) ,The sero-prevalence was also relatively highamong individuals who reported Illiterate in 20.4% (n=41/201) ,the prevalence was also higheramong marriedin16.6%(n=52/313) and the sero-prevalence was also higher among rural and among Agro-and/ or Pastoralistparticipants (Table 2)

Table 2: Demographic characteristics and distribution of brucellosis

Factors		**Number tested (%)**	**Number RBPT^{+ve} (%)**	**Number ELISA^{+ve} (%)**	**Over all sero prevalence**	**X^2, P value**
Sex	Male	180(40.5)	68(37.8)	42(61.8)	42(23.3)	**13.05; <0.001**
	Female	264(59.5)	72(27.3)	28(38.9)	28(10.6)	
	Total	444(100)	140(31.5)	70(50)	70(15.8)	
Age	2-14	58(13.1)	18(31.0)	7(38.9)	7(12.1)	3.58; 0.311
	15-29	241(54.3)	77(32.0)	37(48.1)	37(15.4)	
	30-44	111(25.0)	34(30.6)	17(50.0)	17(15.3)	
	≥ 45	34(7.6)	11(32.4)	9(81.8)	9(26.5)	
Educational status	Illiterate	201(45.3)	71(35.3)	41(57.8)	41(20.4)	**6.21; 0.045**
	Primary school (1-8)	147(33.1)	40(27.2)	19(47.5)	19(19.9)	
	High school and above	96(21.6)	29(30.2)	10(34.5)	10(10.4)	
Marital status	Married	313(70.5)	99(31.9)	52(52.3)	52(16.6)	0.57; 0.449
	Non married	131(29.5)	41(31.3)	18(43.9)	18(13.7)	
Residents	Urban	97(21.9)	23(23.7)	9(39.1)	9(9.3)	**3.93; 0.047**
	Rural	347(78.1)	117(33.7)	61(52.1)	61(17.6)	
Occupation	Agro- and/ or Pastoralist	249(56.1))	86(34.5)	46(53.5)	46(18.5)	3.13; 0.077
	Others*	195(43.9)	54(27.7)	24(44.4)	24(12.5)	

Others*(Daily laborer*, Govermental work*, students*)

7.3. Malaria

Among all febrile patients (444) suspected for malaria, 19 (4.3%) were found positive only for *Plasmodium falciparum* infection based on microscopic detection. Malaria cases were more common among males than females (7.2% vs. 2.3%, X^2= 6.14, p = 0.01) and non-married than married ones (7.6% vs. 2.9%, X^2= 5.10, p = 0.02). The relative positivity of *falciparum* malaria was high in the age group between 2-14 years (10.3%, X^2= 7.66, p = 0.05).). In addition, there was a dual occurrence of Brucella infection and malaria due to Plasmodium falciparum in five person.

Table 3: socio- demographic characteristics and malaria distribution

Factors		**Number (%)**	**Number positive (%)**	**X^2; p value**
Sex	Male	180	13(7.2)	6.40;**0.01**
	Female	264	6(2.3)	
Age	2-14	58	6(10.3)	7.66; 0.05
	15-29	241	12(4.0)	
	30-44	111	0(0.0)	
	≥ 45	34	1(2.9)	
Educational status	Illiterate	201	7 (3.5)	0.80; 0.67
	Primary school (1-8)	147	8 (5.4)	
	High school and above	96	4(4.2)	
Marital status	Married	313	9(2.9)	5.10; **0.02**
	Non married	131	10(7.6)	
Residents	Urban	97	5(5.2)	0.23; 0.63
	Rural	347	14(4.0)	
Occupation	Agro- and/ or Pastoralist	249	8(3.2)	1.57; 0.21
	Others*	195	11(5.6)	

7.4. Association of clinical feature with the sero-prevalence brucellosis

(Table 4) shows the clinical signs and duration of the illness with sero-prevalence brucellosis as reported by the study participants. The sero-prevalence brucellosis among patients with general weakness is 18.6% (n=22/118), Vomiting13.6% (n=19 /139),head ace 16.7%(n=57/340), Malaise

14.2% (n=18/127) and Joint pain 20.8% (n=26/125) were the frequently reported symptoms. The duration of the illness reported by the participants ranged between 1-3 and more than 3 days,

Table 4: Association of clinical feature with the sero-prevalence brucellosis

Clinical feature		Number tested	Number +ve (%)	COR(95% CI)	P value
General weakness	No	326	48(14.7)		
	Yes	118	22(18.6)	1.33(0.76;2.31)	0.318
Vomiting	No	305	51(16.7)		
	Yes	139	19(13.7)	0.93(0.51;1.69)	0.812
Headache	No	104	13(12.5)		
	Yes	340	57(16.8)	1.43(0.73;2.83)	0.300
Malaise	No	317	52(16.4)		
	Yes	127	18(14.2)	0.87(0.48;1.57)	0.653
Joint pain	No	319	44(14.0)		
	Yes	125	26(20.8)	1.68(0.97;2.91)	0.066
Onset days of febrile	1-3 days	289	43(14.9)		
	More than 3 days	155	27(17.4)	1.21(0.71;2.04)	0.484

7.5. Association of milk source and consumption experience to sero positivity of brucellosis

The sero-prevalence was also relatively high among individuals who reported drinking raw milk 19.8% (n=69/348) and touched aborted fetus/discharges from aborted animals without protection 35.8% (n=19/53) which was significantly associated with Brucellosis infection , drinking of raw milk (COR=27.71(3.59;213.68), p=0.001) and touching aborted fetus/discharges without protection (COR=2.82(1.16;6.86), p = 0.022) the rest of the potential risk factors did not have any significant influence(Table 5).

Table 5: Association of milk source and consumption experience to sero positivity of brucellosis

Factors		Number tested	Number +ve (%)	COR(95% CI)	P value
Milk from large ruminant	No	332	49(14.7)		
	Yes	112	21(18.7)	0.78(0.42;1.42)	0.419
Milk from small ruminant	No	168	18(10.7)		
	Yes	276	52(18.8)	1.12(0.57;2.23)	0.725
Milk from camel	No	214	29(13.5)		
	Yes	230	41(17.8)	0.64(0.35;1.17)	0.144
Drinking raw milk	No	96	1(1.0)		
	Yes	348	69(19.8)	**27.71(3.59;213.68)**	**0.001**
Drinking of boiled milk	No	248	38(15.3)		
	Yes	198	32(1602)	0.70(0.40;1.24)	0.222
Drinking of aborted animal milk	No	393	54(13.7)		
	Yes	51	16(31.3)	1.31(0.51;3.38)	0.579
Touching of aborted materials/fetus	No	391	51(13.0)		
	Yes	53	19(35.8)	**2.82(1.16;6.86)**	**0.022**

CI-Confident interval, COR-Crude odds ratio

7.6. Multivariate logistic regression model

(Table 6)shows the multivariable logistic regression analysis being female was associated with (AOR = 0.43, 95%CI: 044-0.75, P = 0.003), drinking raw milk (AOR=16.96, 95%CI: 2.27-126.69, p=0.006) and touching aborted fetus/discharges without protection (AOR=2.13, 95%CI: 1.08-4.20, p = 0.029) for *Brucella* infection and being Afar female is less affected than male because of male is more pastoralists and close contact in animals.

Table 6: Multivariate logistic regression modelamong the febrile study respondents

Factors		**Adjusted OR(95% CI)**	**P value**
Sex	Male	1	
	Female	**0.42(0.44;0.74)**	**0.003**
Educational status	Illiterate	1	
	Primary school(1-8)	0.76(0.40; 1.44)	0.399
	High school and above	0.55(0.24;1.22)	0.142
Resident	Urban	1	
	Rural	1.19(.53;2.64)	0.678
Drinking of raw milk	No	1	
	Yes	**16.95(2.27;126.60)**	**0.006**
Drinking milk from aborted animal	No	1	
	Yes	0.76(0.28;2.02)	0.577
Touching of aborted materials/fetus	No	**1**	
	Yes	**2.54(1.02;6.30)**	**0.045**

AOR= Adjusted odds ratioCI = Confidence interval

8. DISCUSSION

Brucellosis is an emerging zoonotic infectious disease, which has synonymous symptoms with many other febrile illnesses (e.g. Malaria, typhoid fever, typhus fever etc.) Predominately circulating among developing countries [4] On the other hand, Malaria is one of the killer diseases worldwide even though it is being over treating based on presumptive diagnosis

ignoring other febrile illness in these developing communities. In fact, as WHO report revealed that in 2018 malaria accounted around 219 million new cases and 435000 deaths globally, while most of the cases and deaths occurred in African [32].

Clinical symptoms of malaria and other tropical febrile diseases are overlapping that hinders the rate of diagnostic specificity and sensitivity of apparent presumptive diagnosis. This exposes to the wrong use of anti malarial drugs and reduces the effective management of patients with non-malaria febrile illness, especially in malaria endemic regions [33].

At this pastoralist study area, 444febrile patients visited health institutions were examined for the presence of brucellosis and malaria. The result revealed that among all febrile patients suspected for malaria, it was only4.3% found positive for malaria infection with the only species of *P. falciparum* malaria infection microscopically. The finding is in line with the resent study done in other area of Afar [5].

We investigated the prevalence of brucellosis and rule out of malaria among individuals reported signs of fever, headache, joint pain, malaise and General weakness in sero-prevalence and risk factors of human brucellosis among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar region, Ethiopia.

Since Brucellosis occurs naturally in animals, while humans get infected through contact with the infected animal and consumption of contaminated animal products [1]

hence this study area is important for *B.abortus* infection in these pastoralist population. Among examined febrile patients, the sero prevalence of brucellosis using RBPT and ELISA was found to be 31.5% and 15.8%, respectively. The overall sero prevalence was reported to be high (15.8 %) which is in agreement with findings in different countries such as: Bacterial Febrile Illnesses in Children Tanzania 15.4% [18], Northern Uganda prolonged fever patients 18.7% [19], North eastern Kenya in febrile patients 13.7% [21], Uganda agro-pastoral communities (17.0%) [1], Mexico dairy farm workers 18.1% [2], However, this study suggested that while human Brucellosis has expanded from the pastoral communities, consuming raw milk and touching aborted materials/fetus is a major risk factor.

However, in contrast to the present finding higher prevalence of human brucellosis was reported in Borena (Ethiopia) 34.9% [27], Nigeria butcher workers (24.1%) [22], Pakistan slaughter house
Worker (21.7%) [20], On the other hand, low sero-prevalence of brucellosis in humans was also reported by different studies; Uganda malaria negative febrile individuals (7.5%) [7], Jimma in febrile patients 3.6% [26], Southern Ethiopia blood donors in Arba Minch Blood Bank Center (10.6%) [23].The possible explanation for the differences in the sero-prevalence could be due to differences in the sampling design schemes used, the number of samples, exposure to Brucella species, the type of diagnostic tests used and the manner in which tests were interpreted.

In the current study, the sero-positivity recorded in humans among the different demographic risk factors (age groups, educational level and occupation) were not statistically significant (P> 0.05) with the Brucella sero-positivity which could be attributed different sample sizes and large within risk factors variations. Nevertheless, males (23.3%) were more affected than female (10.6%) which might be due to males have more contacts with animal than female at this study area. The finding is in line with Northern Uganda prolonged fever patients study [19]

On multivariate logistic regression analysis, being female was identified as less related with brucellosis with respect of being male. Similarly, consuming raw milk and touching of aborted material/fetus were identified as the risk factors for having brucellosis. Similar findings have been reported by the study of prolonged fever patients in post-conflict Northern Uganda [19].Consumption of unpasteurized milk products was associated with the occurrence of brucellosis in line with various studies that reported similar findings [1,19].

There was no statistically significant correlation between occurrence of Brucellosis and having Milk from large ruminant, Milk from small ruminant, milk from camel, drinking of aborted animal milk. However, we are unable to substantiate these factors with literatures.

Consumption of unpasteurized milk products was associated with the occurrence of brucellosis in line with various studies that reported similar findings [1, 7, 19]. This strengthens the argument that humans are infected through consumption of unpasteurized milk products as reported in other studies [1, 7, 18, 19].

We found that having human brucellosis was associated with handling aborted fetuses and ($p = 0.029$). This is consistent with the reports of studies done in Pakistan slaughterhouse worker indicating that brucellosis in humans was strongly associated with handling aborted fetuses and

placenta of infected animals [20]. In these studies get was a dual occurrence of Brucella infection and malaria due to Plasmodium falciparum in five Person. The finding is in line with the resent study done in other area of Uganda [36].

In fact, this group of diseases always underreported and misdiagnosed in many countries in Africa especially in Sub- Saharan Africa due to lack of basic infrastructures facilities, diagnostic materials and well-trained personnel to perform the diagnosis [12,34]. Misdiagnosis of brucellosis may be due to lack of awareness on brucellosis by medical staff, limited diagnostic facilities, lack of experience with laboratory testing [35].

9. STRENGTH AND LIMITATION OF THE STUDY

9.1. Strength of the study

- This serological study finding would produce a proper clue for microbiologists before employing the expensive, tiresome and hazardous culture method or molecular techniques

- The study was employed among pastoralist, which is important area to acquire brucellosis and fine to produce the potential data to influence policy makers to include brucellosis in their public health as a one health.

9.2. Limitation of the study

- This study was based on serological test method hence; it did not differentiate the current and previous infections.

- The purposive cross-sectional study design was employed which is difficult to inference for the general population.

- Since study respondents were acutely ill, the information they provided about their experience that may have predisposing factors for brucellosis were not genuine enough and there might be recall bias.

10. CONCLUSION AND RECOMMENDATION

10.1. Conclusion

The sero-prevalence of human brucellosis among febrile patients is high (15.8%) unlike malaria which was low. The study revealed that being male, drinking raw milk and touching of aborted materials/fetus were the risk factors for acquiring *Brucella*infection.

10.2. Recommendation

- Public health interventions through principle of one health among pastoralists on the control and prevention of brucellosis need to be implemented by community health workers and other stakeholders including public awareness of the diseases and the role of raw milk in the transmission of Brucellosis.
- Physician and clinicians should also consider and taken into account brucellosis during patient case managements especially in pastoralist area.
- The need of further community based investigations and studies designed to identify the circulating *Brucella*species, similarity and difference of the species among humans and animals and the characteristics of drug profiles need to be employed at this study area and other similar localities

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12. ANNEXES

Annex 7.1. English version of participant is information sheet

Department of medical laboratory, college of health science, Addis Ababa University, Addis Ababa, Ethiopia

Title: Sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia

Introduction

First, I would like to thank you in advance for your cooperation and consent in participation in this study. Please listen when I read for you about the general information of the study. If you have any question regarding the study, please ask freely.

Background information: Febrile illness has significant overlap of symptoms, signs and basic clinical diagnosis parameters. Such character leads to inadequate clinical evaluation, delay in the diagnosis, and use of inappropriate antibiotics, extended morbidity and possibly mortality

Aim of the study: The objective of this study is to determine the Sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia

Procedure of the sample collection

Sample will be collected from the vein of arm after the site is cleaned with 70% alcohol and the sample collected will be used for research purposes after patients or guardian's willingness to participate is confirmed in their signature.

Benefits for participants:

Study participants will not have any financial incentives or other inducements from participating on this study. However, their results will be given and will be treated by the prescribing physician based on the diagnosis results and depending on the nature of the disease and the physicians decision; patients may be appointed to await complement fixation tests results for better treatment of Brucella infection.

Risks and complication

There is no considerable risk to the study subjects in participating in the study. Taking 5ml of blood doesn't have any harm to your health except minor needle brick injury pain which lasts only for micro second. However if you have any discomfort you will be seen by physician.

Confidentiality

In order to maintain the confidentiality of participants' information, the name will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time

from the study. Only interested participants can retrieve their own lab result using their code number. No personal identifier will be disclosed to third party or will not appear in any report from this study.

Right to refuse

Since participation in this study is entirely voluntary. You can refuse to participate in this research at any time. Your refusal to participate in this study will not affect any of the benefits you are supposed to get from the center

Principal Investigator: Sintayehu Mehari

Contact Address: Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences. Phone (Cell Phone): +251-913 26 33 64. **E-mail**:sinte000@gmail.com

Annex 7.2. Information sheet Amaric verion

ለጥናቱ መረጃ እና ተሳታፊነት መግለጫ ቅጽ

የጥናቱ ዓላማ

የቡርሴላ ባክቴሪያ ስርጭት በአፋር ክልል ለማጥናት የታቀደ ነዉ ።

በጥናቱ ስለ መሳተፍ

በዚህ ጥናት መሳተፍ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡ ስለሆነም በጥናቱ እንዲሳተፉ ፈቃደኛነትዎን እንጠይቃለን፡ ለመሳተፍ ከፈቀዱ፤5ሚሊ ሊትር የደም ናሙና ከክንድዎ ተወስዶ የላቦራቶሪ ምርመራ ይደረግሎታል። የላቦራቶሪ ምርመራውም የቡርሴላ ባክቴሪያ በደም ዉስጥ መኖርና አለመኖር ማረጋገጥ ይሆናል።ደምከመወሰዱ በፊት እና ከውጤት በሁዋላ በሰለጠነ ባለሙያ የምክር አገልግሎት ያገኛሉ ። የደም ናሙናዉም የሚወሰደዉ ንጽህናዉ በተጠበቀ አዲስ እና በታሸገ መርፌ ነዉ ።

በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች

5ሚሊ ሊትር የደም ናሙናዉን ለመዉሰድ መርፌ ሲገባ ከሚፈጥረዉ የቅጽበት የህመም ስሜት በስተቀር የጎላ ችግር አያመጣም ነገር ግን ምቾት ካልተሰማዎት ሀኪም እንዲያይዎት ይደረጋል ።

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

የደም ናሙና የላብራቶሪ ዉጤት ምንም አይነት ችግር ካሳየ የመድሃኒት ትእዛዝና የባለሙያ ምክርይ ሰጥዎታል ።

የጥናቱ መረጃዎች ሚስጥራዊነት

በጥናቱ ውስጥ የተሰበሰቡ ማናቸዉም ግላዊ መረጃዎች ሚስጥራዊነታቸዉ የተጠበቀ
ይሆናል ።

ከማንነትዎ ጋር በቀጥታተያያዥነት ያላቸዉ መረጃዎች በሙሉ በዋና ተመራማሪዉ ሚስጥራዊ በሆነ የመረጃ ጥንቅር ዘዴ ከተቀየሩ በኃላ ብቻ ለምርምር ሂደቱ የሚዉሉ ይሆናሉ።

የጥናቱን ውጤት ስለ ማሳወቅ

የዚህ ጥናት ዉጤት በተለያዩ የህትመት ውጤቶች የሚቀርብ ሲሆን ይህ ከማንነትዎ ጋር የተያያዘ ምንም አይነት መረጃን አያካትትም ። ስለዚህም የጥናቱን ውጤት በሪፖርት እናቀርበዉ ዘንድ ፈቃዶን እንጠይቃለን ።

ከጥናቱ ስለ መውጣትና ስለ ማቋረጥ

ይህ ጥናት በፈቀደኝነት ላይ የተመሰረተ እንደ መሆኑ መጠን በማንኛውም ወቅት በፈቃድዎ ከጥናቱ መውጣት ይችላሉ ። ከጥናቱ ቢወጡም እንኳን የተለመደዉን የህክምና እርዳታ በጤና ተቋሙ ውስጥ በማንኛውም ጊዜ የማግኘት መብት አልዎት

Annex 7.3. Consent for adult participants (≥18 years): English version

I have been requested to participate in a research project that aims to determine the Sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia I have been informed that all information I will be giving will be kept confidential will be provided the opportunity to ask questions and given adequate time to think the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time and without supplying reasons. I am fully aware that the results of this Study will be used for scientific purposes and may be published. Agree to this, provided my privacy is guaranteed. And I confirm my agreement by putting my signature below. I here by give my consent for giving of blood specimens.

Name of Participant/ code: _____

Address: _____ Tel: _____

Signature of Participant _____ Date: ___/____/_____

Study staff name _____Signature _____Date: ___/____/_____

ስምምነት ማረጋገጫ ቅፅ

የጥናቱ ዓላማ የቡርሴላ ባክቴሪያ ስርጭት በአፋር ክልል ለማጥናት የታቀደነው በአፋር ክልል በአውራ እና ጉሊና ወረዳ ውስጥ ዶራይቱ ጤና ጣቢያ እና ከሌዋን የመጀመሪያ ደረጃ

ሆስፒታል ከላይ የተጠቀሱት በሽታዎች ዙሪያ ጥናት ለማድረግ ከላይ በመግቢያ ውላይ የተጠቀሰውን መረጃ አንብቢያለሁ ወይም በቃል የተሰጠኝን ማብራሪያ ተረድቻለሁ።በዚህ መሰረት ከእኔ የሚጠበቅብኝን ድርሻ በሚገባ አውቄያለሁ እናም በዚህ ጥናት ላይ በመሳተፌ ሊከሰቱ የሚችሉትን ሁኔታዎች ተገንዝቢያለሁ ። ከዚህ ጥናት በማንኛውም ሠዓት ያለምንም ቅድመ ሁኔታና ምክንያት እራሴን ከተሳታፊነት የማግለል ሙሉ መብት እንዳለኝ ተረድቻለሁ።ይህን ውሳኔዬን ተከትሎ በእኔም ላይ በምንፈልገው የጤና አገልግሎት ላይ ምንም አይነት አሉታዊ ተጽዕኖ እንደማይደርስብኝ ተረድቻለሁ።በመሆኑ ምስለጥናቱ ማብራሪያ የተሰጠ መሆኑን በተለመደው ፊርማዬ አረጋግጣለሁ ።

የአመልካች / ኮድ ስም: _____

አድራሻ: _____ ስልክ: _____

የአሳታፊው ፊርማ _____ ቀን: ___ / ____ / _____

የጥናት ቡድኑ ስም _____ _____
ፊርማ _____

Address of the investigator

Name: Sintayehu Mehari

Address: Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences.

E-mail: sinte000@gmail.com

Annex 7.4. Assent for children participants (12-17 years): English version

Study title Sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia

Purpose of the study

This study will help to identify the magnitude of Sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia available services.

I have read or someone has read to me, and fully understands the Participant Information Form and have had the opportunity to ask questions related to this study. I agree to give the required sample for diagnosis. I understand that the sample will not be used for any other purpose than to perform the same test that I would receive under normal circumstances. All information regarding my sample will remain completely confidential and served for stated study only. I understand that I am not forced to participate in this study, and I can decide not to participate at any time or withdraw from the study. I understand that this study does not place me at any greater medical risk than is customary with the test that I am receiving, nor does it interfere with the medical care that I am entitled to. I have read the above document and I understand that I have agreed to participate in this study.

Name of Participant/ code: _____

Address: _____ Tel: _____

Signature of Participant _____ Date: ___/____/_____

Study staff name _____Signature _____Date: ___/____/____

ስምምነት ማረጋገጫ ቅፅ

የጥናቱ ዓላማ የቡርሴላ ባክቴሪያ ስርጭት በአፋር ክልል ለማጥናት የታቀደነዉ
በአፋር ክልል በአውራ እና ጉሊና ወረዳ ውስጥ ዶራይቱ ጤና ጣቢያ እና ከሌዋን የመጀመሪያ ደረጃ ሆስፒታል ከላይ የተጠቀሱት በሽታዎች ዙሪያ ጥናት ለማድረግ.

አንብቤያለሁ ወይም አንድ ሰው ያነብብኛል እና የተሳታፊ የመረጃ ቅጽን ሙሉ በሙሉ ተረድቻለሁ እናም ከዚህ ጥናት ጋር የተያያዙ ጥያቄዎችን የመጠየቅ ዕድል አግኝቻለሁ. ለምርመራ አስፈላጊ የሆነውን ናሙና ለመስጠት እስማማለሁ. ናሙናው ለምንም ለሌላ ዓላማ እንደማይውል ይገባኛል. የእኔን ናሙና የሚመለከቱ መረጃዎች ሙሉ በሙሉ በምስጢር ይጠበቁ እና ለተገለጸው ጥናት ብቻ ያገለግላሉ በዚህ ጥናት ለመሳተፍ የግድ እንዳልሆነ ተረድቼያለሁ እናም በማንኛውም ጊዜ ላይ ላለመሳተፍ ወይም ከጥናቱ ለማውጣት መምረጥ እችላለሁ. ከላይ ያለውን ሰነድ አንብቤያለሁ እናም በዚህ ጥናት ለመሳተፍ እንደተስማማሁ ተረድቻለሁ.

የአመልካች / ኮድስም: _____

አድራሻ:_____ስልክ:_____

የአሳታፊው ፊርማ _____ ቀን: ___ / ____ / _____

የጥናት ቡድኑ ስም _____ _____ፊርማ _____

Address of the investigator

Name: Sintayehu Mehari

Address: Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences.

E-mail: sinte000@gmail.com

Annex 7.5. Parental consent for (below 12 years): English version

I have been requested to participate about this study, which plans to determine sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia. I have read or someone has read to me and my child, and fully understands the Participant Information Form and have had the opportunity to ask questions related to this study. My child will provide the required sample for diagnosis. I understand that the sample will not be used for any other purpose than to perform the same test that we would receive under normal circumstances. All information regarding my child sample will remain completely confidential and served for stated study only. I understand that my child is not forced to participate in this study, and she can decide not to participate at any time or withdraw from the study. I understand that this study does not place my child at any greater medical risk than is customary with the test that my child is receiving. I have read the above document and understand that I witnessed my child to participate in this study.

Parent/guardian Name _____

Parent/guardian signature or mark_____ Date: ___/___/___

ስምምነት ማረጋገጫ ቅፅ

የጥናቱ ዓላማ የቡርሴላ ባክቴሪያ ስርጭት በአፋር ክልል ለማጥናት የታቀደነዉ
በአፋር ክልል በአውራ እናጉሊና ወረዳ ውስጥ ዶራይቱ ጤና ጣቢያ እና ከሌዋን የመጀመሪያ ደረጃ ሆስፒታል ከላይ የተጠቀሱት በሽታዎች ዙሪያ ጥናት ለማድረግ.

አንብቤያለሁ ወይም አንድ ሰው ለእኔ እና ለልጄ ካነበበኝ እና የተሳታፊ የመረጃ ቅጽን በሚገባ ተረድቻለሁ እናም ከዚህ ጥናት ጋር የተያያዙ ጥያቄዎችን የመጠየቅ እድል አግኝቻለሁ. ልጄ ምርመራውን ለመፈለግ የሚያስፈልገውን ናሙና ያቀርባል. በተለመደው ሁኔታ ውስጥ የምንቀበለውን ተመሳሳይ ናሙናው ለምንም ለሌላ ዓላማ እንደማይውል ይገባኛል. የእኔን ናሙና የሚመለከቱ መረጃዎች ሙሉ በሙሉ በምስጢር ይቀራሉ እና ለአንዴ ጥናት ብቻ ያገለግላሉ. ልጄ በዚህ ጥናት ለመሳተፍ እንደማይገደድ ይገባኛል, እና በማንኛውም ጊዜ ላይ ላለመሳተፍ ወይም ከጥናቱ ለመልቀቅ ትችላለች. ይህ ጥናት ልጄን በሚቀበለው ላይ ከተለመደው ይልቅ በልዩ ሁኔታ ከሚያስከትለው የጤና ችግር ይልቅ ልጄን መመርመር እንደማይችል ተገንዝቤአለሁ. ከላይ የተሰጠውን ሰነድ አንብቤ ልጄ በዚህ ጥናት እንዲሳተፍ በተለመደው ፊርማዬ አረጋግጣለሁ ::

የወላጅ / አሳዳጊ ስም _____

የወላጅ / ሞግዚት ፊርማ ወይም ምልክት _____ቀን: ___ / ___ / ___

Address of the investigator

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Annex 7.6. Questionnaire English version

Addis Ababa University department of Medical laboratory technology questionnaire for sero-prevalence of human brucellosis malaria-co-infection and risk factors among febrile patients visited Derayitu Health center and Kelewani Primary Hospital at Awra and Gulina district, Afar Region, Ethiopia**l. Socio demographic data**

Name of Keble _____Date_____

Name of the patient……sex----------age------- code----------

1. Address: Village/town---------- phone----------

2. Body temperature_____

3. Educational status: 1. Illiterate 2. School (1-8)

3. High school (9 – 12) 4. Higher institute (college or university)

3. Resident? 1. Urban 2. Semi urban 3. Rural

4. Occupation? 1. Pastoralist 2. Agro pastoralist 3. Daily laborer

D. Other specify_____

5. Ethnicity: _____ 1. Afar 2.Amara 3. Tigray 4. Others_____

6. Marital status 1. Married 2.Divorced 3.widowed 4. non-married

7. Duration of residence in the area _____ (years)

8. Which of the following animals do you have

1= cow, 2= goat 3= sheep 4= camel 5= all 6. None

9. What is the source of milk for you and your family

1= cow 2=goat 3=sheep 4=camel 5= all 6. None

10. How do you drink milk?

1=Raw milk 2= boiled milk 3= fermented milk (yogurt) 4= all form 5= none

11. Do you /your family drink raw milk from aborted animals? 1= yes 2= no

12. Do you have ever touch aborted fetus/ retained placenta or uterine discharge with your

Hand without proper protection?

1= yes 2= no 3= do not remember

II. Clinical data

1. Onset of febrile illness? _____..... (Day/weeks/month/year)
2. Other Clinical symptoms.

1. Weakness 2. Vomiting 3.Headache 4.Malaise 5.Joint pain

6. Others specify _____

3. Physical sing _____

III. Laboratory Results

1. RBPT _____
2. BF Result Malaria _____ RF, PF, PV,

ELISA_____

Annex.7.7. Questionnaire Amaric version

አዲስ አበባ ዩኒቨርስቲ የህክምና ፋኩልቲ የላቦራቶትሪ ትምህርት ክፍል የቡርሴላ ባክቴሪያ ስርጭት ለማጥናት ለመረጃ ሰብሳቢዎች ጥያቄዉን ከጠየቃችሁ በኃላ መልሱን በተሰጠው ሳጥን ውስጥ ከተሰጡት አማራጮች አንዱን የኤክስ ምልክት ይጻፉ ።

I. የማህበራዊ ሁኔታ የሚገልፅ

ቀበሌ ስም ---------- ቀን ----------

የበሽተኛ ስም ---------- ፆታ ---------- እድሜ ---------- መለያ ---------

1. አድርሻ ገጠር/ከተማ ---------- ሞባይል ----------
2. የሰውነት ሙቀት ----------
3. የትምህርት ደረጃ 1 . መደበኛ ት/ት ያልተማረ 2 . መፃፍ እና ማንበብ የሚችል
   3. ከ 1ኛ እስከ 8ኛ 4. ከ 9ኛ እስከ 12 ኛ 5. ከ 12 በላይ
4. ነዋሪ 1. ከተማ 2. የገጠር ከተማ 3 .ገጠር

5. ስራ 1. ከብት አርቢ 2. ከብት እርባታ እና እርሻ 3. የቀን ስራ 4.ሌለ

6. ብሔር 1. አፋር 2.አማራ 3.ትግሬ 6. ሌላ (ይገለጽ)
7. የጋብቻ ሁኔታ 1. ያገባ/ች 2 . ያላገባ/ች 3 .የፈታ /ች 4. የሞተበት/ባት

8. የቆይታ ጊዜ ----------? (ዓመት)

9. ከሚከተሉት እንስሳት በቤት ውስጥ የሚኖር የተኛው ነው

   1. ከብት 2. ፍየል 3. በግ 4. ግመል 5. ሁሉም 6. ምንም የለም

10. ከሚከተሉት በለወተት ምንጭ የሆነው የተኛው ነው

    1.ከብት 2.ፍየል 3.በግ 4.ግመል 5.ሁሉም 6.የለም

11. እንደት አድርገው ነው የምትጣቀመው

1. ጥሬ ወተት 2. የፈላ ወተት 3. የተጠራ ወተት 4. ሁሉም 5. የለም

12. አንተ ሆና ቤተሰብ ውርጃ ከደረገት ወተት ትጠጣለ ወይ?

1. ጥሬ ወተት ትጠጣለ 2. የፋላ ወተት 3. የተጣራ ወተት 4. ሁሉም 5.የለም

13. ያለምንም መከላከያ ያለጊዜ የተወለደ ወይንም እንግዴ ልጅ መልሰህ ተውቃለህ ወይ?

1. አዎ 2. አይደለም 3. ማስታወስ አልችልም

II. ስለጤና መረጃ ያለው አሰባሰብ

1. ትኩሳት የለው ህመም የተከሰተበት ጊዜ ---------- ቀን/ወር/ዓ/ም

2. ሌላ የጤና ምልክቶች ----------

1. መድካም 2. መስተወክ 3. ራስምታት 4. የህመምስሜቶች 5. የመገጠጣምያ የህመም ስሜት 6. ደምመናስ 7. ሌላ ወይ የተለየ

3. አካላዊ ምርመራ……..

III. <u>የላብራቶሪ ውጤት</u>

1.የቡርሴላ ----------

2.የወባ ደም ምርመራ ----------

Annex 7.8. Laboratory method

Principle of *burcella* Ab test

The smooth, attenuated stained *Brucella* antigen suspensions are mixed with the serum. Specific

antibodies to *Brucella* antigens if present in the serum will react with the antigen suspension to produce an agglutination reaction. No agglutination indicates the absence of specific antibodies to *Brucella* antigens

Procedures for *brurcela* Slide test method

- Bring all reagents to room temperature.
- Shake and mix the BRUCEL antigen suspension well before dispensing.
- Place 80ul of saline onto the next reaction circle of the glass slide.
- Place 80ul of the serum to be tested onto the next reaction circle.
- Add one drop of the appropriate BRUCEL antigen suspension in each of the above circle (containing positive control, saline, and the patient serum to be tested
- Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
- Gently rock the slide back and forth, observe for agglutination macroscopically at one minutes against a white background.

INTERPRETATION OF RESULTS

- Agglutination is a positive test result and indicates the presence of specific antibodies to Brucella in the serum.
- No agglutination is negative test result and indicates the absence of specific antibodies to Brucella in the serum

PRINCIPLE OF *BRUCELLA* IgG ELISA TESTS

The *Brucella* IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Brucella antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted in to the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Brucella antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate(TMB) solution is pipette and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye ismeasured spectrophoto-metrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

Materials Supplied

microtiter strips

- 12 strips with 8 breakable wells each, coated with a Brucella antigen (Brucella abortus, strain W99). Ready-to-use.
- **Calibrator A (Negative Control):**2mL, Protein solution diluted with PBS, contains no IgG antibodies against Brucella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.
- **Calibrator B (Cut-Off Standard):**2mL, Human serum diluted with PBS, contains a low concentration of IgG antibodies against Brucella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.
- **Calibrator C (Weak Positive Control):**2mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Brucella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use

- **Calibrator D (Positive Control):**2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Brucella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.
- **Enzyme Conjugate:**15mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxaned 5 mg/L Proclin. Ready-to-use.
- **Substrate:**15mL, TMB (tetramethylbenzidine). Ready-to-use
- **Stop Solution:** 15 mL, 1N acidic solution. Ready-to-use
- **Sample Diluent**: 60 mL PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use
- **Washing Buffer**: 60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
- **Plastic bag**: Resealable, for the dry storage of non-used strips.

Assay Procedure

- Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
- Pipet 100 µL each of the diluted (1:101) samples and the ready-to-use standards and controls respectively into the wells. Leave one well empty for the substrate blank.
- Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.

- Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted wash in solution. This procedure is repeated totally three times. Rests of the washing buffer are after wards removed by gentle tapping of the microtiter plate on a tissue cloth.
- Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
- Cover plate with the enclosed foil and incubate at room temperature for 30 minutes

- Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
- Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
- Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
- To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
- After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

Qualitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of

+/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

- **Thick and thin blood film preparation**

Thick and thin blood smear study is the gold standard method for malaria diagnosis. The procedure follows these steps: collection of peripheral blood, staining of smear with Giemsa stain and examination of red blood cells for malaria parasites under the microscope.

Thick smear: It is not fixed in methanol; this allows the red blood cells to be hemolyzed, and leukocytes and any malaria parasites present will be the only detectable elements. However, the hemolysis may lead to distorted plasmodial morphology making plasmodium species differentiation difficult. Therefore, thick smears are mainly used to detect infection and to estimateparasitemia.

Thin smear: It is fixed in methanol. Thin smears allow the examiner to identify malaria species, quantify parasitemia, and recognize parasite forms like schizonts and gametocytes.

Procedure

- Place the labelled slide on the template below.
- Using a micropipette, place 6µl of blood for the thick film and 2–3µl for the thin film as shown on.
- Using the ground edge of the spreader slide spread the blood for the thin film.
- Using the beveled corner of the spreader slide spread the blood for the thick film until the entire circle of 12 mm diameter is covered evenly.
- Dry the films on a flat surface, protected from dust and insects. Slides must be completely dry before staining by drying on a slide warmer at 37–40 ºC for 1 hour or overnight in a dehumidified chamber at ambient temperature.
- Fix the thin film by dipping it in absolute methanol for a few seconds and then letting the slide air dry. Dry the thin film at an acute angle, with the film-side of the slide facing up and the thin film downwards. This protects the thick
- film from being fixed by methanol fumes and run-off. The thick film must not be fixed

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

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Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors

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Signature: _____

Date: _____

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Advisor: **Biruk Zerfu (M.SC, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Dr Megistu Legesse (Associate professor,PhD)**

Signature: _____

Date: _____