
# Assessement of Typhoid Fever in Masha Town and its Surrounding, South Wollo, Northeast Ethiopia

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## Acronyms

| | |
|---|---|
| **AAU** | Addis Ababa University |
| **AOR** | Adjusted Odds Ratio |
| **COR** | Crude Odds Ratio |
| **CSA** | Central Statistical Agency |
| **NGO** | Non-Governmental Organization |
| ***S. typhi*** | Salmonella typhi |
| **CI** | Confidence Interval |
| **TSAP** | Typhoid fever surveillance in Africa program |
| **GPAQ** | Global Physical Activity Questionnaire |
| **TF** | Typhoid Fever |

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## Abstract

Typhoid fever (TF) caused by *Salmonella enterica* subsp. *enterica*, serovar Typhi is a major public health concern in low-income countries. In Ethiopia, like any other sub-Saharan African country, the condition is worsened due to shortage of safe potable water and toilet access, low health education and overall high illiteracy level. The objective of this study was to estimate the prevalence of TF and its established risk factors in Masha town and its surroundings, northeast Ethiopia. In this health facility-based cross-sectional study design, all patients attending Masha Health Center (MHC) between October 2016 and February 2017 formed the source population and those clinically suspected of TF were successively recruited. A structured questionnaire was administered to capture socio-demographic, dietary and knowledge-related variables in association to the risk of TF. Blood samples collected, sera separated and tested by the slide-agglutination (Widal) method using commercially available *S. typhi* somatic (O) and flagella (H) antigens. Univariate and multivariate logistic regression models were used to test the association between socio-demographic variables and seropositivity for salmonella antigens used with p-value ≤0.05 considered statistically significant. Out of 490 patients diagnosed 346(70.6%) were TF seropositive the highest proportion (74.5%) of cases occurring among children 1-15 years followed by 16-45 years old adults (72.2%). Lower age (adjusted odds ratio (AOR) 2.259, 95% confidence interval (CI) 1.227-4.161, p=0.009), males 246(74.8%) TF positive with (AOR 2.064, 95% CI 1.301-3.275, p=0.002), lack of toilet 270(55.1%) from this 202(74.8%) TF positive with (AOR 1.713, 95% CI 1.331-2.906, p=0.037), and illiteracy 173(77.2%) TF positive with (AOR *3.940, 95% CI 1.926-8.063,* p<0.0001), family member 5 and more 215(75.5%) with *(AOR) 2.103 95% CI 1.213-3.171 p=0.029* were independent significant predictors of TF seropositivity. TF is a serious public health burden in the locality calling for scale-up of intervention strategies including provision of safe water supply, toilet coverage and health education.

**Keywords**: typhoid fever, *S. typhi*, slide agglutination test, widal test

## 1. Introduction

Typhoid fever (TF) or enteric fever is a systemic bacterial disease. The global impact of TF is tremendous that an estimated 12-33 million clinical cases and 216,000-600,000 deaths occur annually (Pang et al. 1995; DeRoeck 2007). Even in industrialized countries a case fatality rate up to 2% has been reported for TF showing the great health threat of the disease in this region as well. The disease is endemic to areas of Africa, India, South and Central America that are characterized by rapid population growth, increased urbanization, and limited safe water, infrastructure and health system (Uneke 2008, Allen and Honest 2010). In South-central and Southeast Asia there were an estimated 22 million cases and 0.22 million deaths in 2000 (Crump 2014). In general, conservative estimates indicate that case fatality rate of 12-32% is reported in some nations in Asia, Africa, and the Far East (Worku 2000).

Sub-Saharan Africa is carrying the highest TF burden with an incidence of 10-100 per 100,000 cases per year (Tadesse 2014). A study conducted in Singida Region of Tanzania showed that the annual burden of TF increased from 771 cases per 100,000 persons in 2003 to 1,402 per 100,000 in 2004 (Allen and Honest 2010). A study in a rural village of Agogo, Ghana also showed an incidence of over 200 cases per 100,000 persons per year (Marks et al. 2010, Breiman et al. 2012).

It is difficult to estimate the real impact of the disease as the clinical symptoms may be confused with other febrile illnesses and specific laboratory confirmation may not be available in these areas (Sudharshan 2014). Children, the elderly and immune-compromised individuals are the high-risk groups and case fatality rates of 38% in children and 47% in adults were recorded in some regions (Tadesse 2014). The disease is observed at a great frequency in acquired immunodeficiency syndrome (AIDS) patients than the general population.

The biggest challenge is the emergence and spread of multidrug-resistant strains of bacteria causing TF and the complication with malaria co-infection, leading to significant morbidity and mortality (Gupta 1994; Bhutta 1996; Bhan et al. 2005, Siddiquia et al. 2006). Resistance has

been developed to primary TF drugs chloramphenicol, trimethoprim, ampicillin and quinolones (Pirisi et al. 2006, Getamesay et al. 2014).

The prevalence rate of TF varies in different region of Ethiopia; it also varies in different age group, sex due to different types of research design conducted by different researchers. For example, a case fatality rate of 15.7% reported in hospital admitted Ethiopian children (Worku, 2000). In another study on patients with febrile illnesses, typhoid fever was recorded in 5.85% of patient with a higher occurrence in children aged 3 to 14 years (6.6%) compared to children aged 15 to 17 years (1.1%) (Animut et al. 2009).

Definitive diagnosis of typhoid is by isolation of bacteria from blood, bone marrow or other study fluids, most developing nations like Ethiopia due to limited access to laboratory facilities, use the old widal test ( Beyene et al., 2008). While respect to antigens 33% for O and 34.4% for H represents the proportion of patients with positive test results that are correctively diagnosed, it is considered as the most important clinical diagnosis method (Andualem et al., 2014 and Keddy et al., 2011). On the other hand, study undertaken in Hawassa revealed 22 (8.1%) Salmonella typhi positive food handlers out 272 from blood sample by widal test, while there was no Salmonella identified by microbiological methods from stool specimen (Desta, 2010). As the study indicated in Bahir Dar, 1.6% of food handlers out of 384 were found positive for Salmonella typhi (Andargie et al, 2008). However, in Gonder town, no Salmonella species were isolated in food handlers (Abera et al, 2010).

In Ethiopia, many researchers were interested to study about drugs to limit Salmonella typhi and how the bacteria resist the drug rather than prevalence of the disease, different risk factors, opportunistic disease that aggravate the disease, life styles of people that is poor or rich and types of test for the disease. Published data regarding about prevalence and determinant factors of TF among people in all age were limitted in Ethiopia especially in Amhara region, particularly in Mekdela district was totally absent. It shows that, there is a research gap on prevalence of TF, different risk factors, related disease that aggravate TF and types of test that indicates test positive and test negative which are applied on people in all age in Ethiopia. The bacteria use human as a reservoir host which makes it worse. So that, the emphasis of this research is onto

screen out which age and sex more susceptible to typhoid fever, risk factors of each location, life styles of people (feeding habit) in all ages of people that attend between October 2016 and February 2017 in Mekdela woreda masha health center, northeast Ethiopia. It is obvious that TF is among the top health challenges in Ethiopia, like any other sub-Saharan African country. But studies on the magnitude of TF burden and associated socio-demographic or environmental factors are limited. Thus, this study was designed to generate a preliminary data on the status of TF in Mekedela District Mash town and its surrounding, northeast Ethiopia.

## 2. Objectives

### 2.1 General objective

The general objective of this study was to assess the prevalence of TF and associated factors among people attending Mekedela District Masha Health Center MHC, northeast Ethiopia

### 2.2 Specific objectives

The specific objectives of the current study were:

to estimate the prevalence of TF among different age- and sex- groups in MHC,

to determine the association between TF and socio-demographic, dietary and related variables

## 3. Literature review

TF is an acute illness associated with fever caused by *Salmonella enterica serotype typhi* bacteria. It can be also caused by Salmonella paratyphi a related bacterium that usually causes a less severe illness. Typhoid fever is almost always acquired by ingestion food or water contaminated with excreta from a patient with typhoid or from chronic carriers. Human beings are the only reservoirs of salmonella typhi, and control of typhoid fever has been achieved in many countries by limitation of the faecal-oral transmission of the organism. Nevertheless, the disease continued to be a major health problem in developing countries ( Worku 2000).

### 3.1 Epidemiology

All *Salmonella* serotypes are considered potentially pathogenic and host-specific, but the majority can affect different hosts. *S.* Typhi and *S.* Paratyphi A, B and C are the most common causes of enteric fever in humans. *Salmonella* species are found in faeces, blood, bile, urine, food and feed and environmental materials. The type of species is *S. enterica* (WHO 2003a). *S. enterica* subsp. *enterica*, serovar Typhi is a human-restricted pathogen and which is transmitted primarily through the fecal-oral route. Asymptomatic carriers may play an essential role in the evolution and global transmission of *S. enterica* (Roumagnac 2006). TF is almost always acquired by ingestion of food or water contaminated with excreta from a patient with typhoid or from chronic carriers.

As man is the only host for *S. enterica*,, transmission is by ingestion of contaminated food or water by human feces. The exact determinants and factors associated with endemic disease and outbreaks of drug-resistant causes of typhoid are uncertain, there does seem to be an association with crowding, lack of sanitation and access to street foods and the infection is transmitted directly by feco-oral route or urine-oral route with feces or urine or indirectly by ingestion of contaminated water, milk, food, or through flies (Fig 1). Contaminated ice, ice cream, and milk products are a good source of infection (Levantesi et al. 2012).

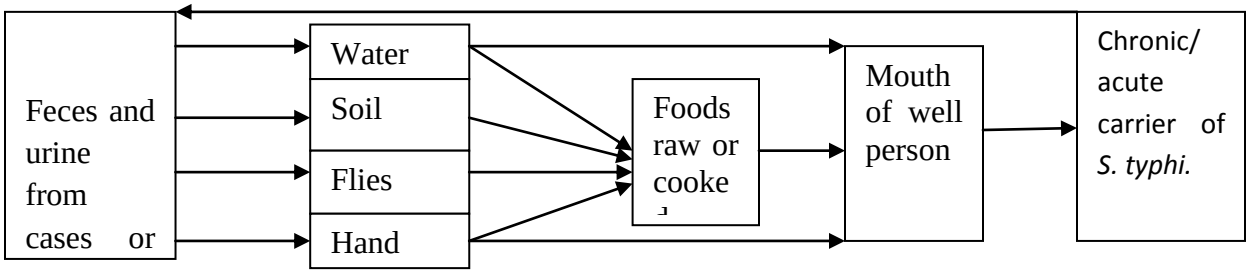



Figure 1 Salmonella transmission routes (developed by researchers).

### 3.2 Pathogenesis of TF

Following ingestion, the organism passes the gastric barrier and reaches the duodenum where it invades the intestinal epithelium and is engulfed by phagosomes in the Peyer's patches and the bacilli multiply and enter the bloodstream and cause transient bacteremia which then dissemination throughout the body, and other phagosomes, via the lymphatics and colonize tissues/organs of the reticuloendothelial system such as the liver, spleen, lymph nodes and bone marrow (House et al. 2001). The infectious dose of *S. enterica* varies between 1000 and 1 million organisms (Hornick et al. 1970, House et al. 2001). Signs and symptoms result when a critical number of bacteria have replicated. Major clinical expressions include prolonged fever and abdominal pain, sustained bloodstream infection (bacteremia and septicemia), activation of the endothelial system, metastatic infection and immunologic problems due to immune complex deposition leading to multi-organ dysfunction (Teh et al. 2014, Denise et al. 2004). In short salmonella has many virulence factors that enable to it to cause disease like acid resistant cell wall, fimbriae for cell mediated attachment, toxin production, antigen variation, and biofilm development.

Infection may become persistent and invade the gallbladder that shows clinical phase of disease depending on host defense and bacterial multiplication. Chronic carriers are the source of transmission by harboring the organisms in their gallbladder (especially in the presence of gallstones) and rarely at other sites (House et al. 2001).

### 3.3 Taxonomy

Salmonella are rod-shaped, flagellated (motile) bacteria with diameters of around 0.7-1.5μm and lengths of 2-5μm, with a few exceptions. They are gram-negative and non-spore-forming. The

TF agent is assembled under kingdom *bacteria*, phylum: *proteobacteria*, class *gammaproteobacteria*, order *enterobacteriales*, family *enterobacteriaceae*, and genus: *Salmonella*, species *S. enterica*, subspecies *S. enterica* subsp. *enterica*, serovar *typhi* (Langridge et al 2014).

*Salmonella* species are classified and identified into serovars (or serotypes) and so far there are more than 2,500 serovars (Park et al. 2009, Wattiau et al. 2011). New serovars are being discovered each year, adding to the complexity of this large bacterial population. Primary subdivision is into O serovars (those which share a common somatic antigen), and these are then subdivided on the basis of H (flagella) antigens (Wattiau et al. 2011). Of these several *Salmonella* species and subspecies and serovars the one that can cause TF commonly is *S. enterica* subsp. *enterica*, serovar Typhi (WHO 2003b). Serovar Typhi may produce Vi antigen, which is an acidic polysaccharide layer outside the cell wall. When fully developed, it renders the bacteria agglutinable with Vi antiserum and inagglutinable with O antiserum. Antigens similar to Vi may also be found in some serovars of *S.* Paratyphi C and *S.* Dublin (Park et al. 2009).

## 3.4 Characteristics and Identification

Salmonella can be detected and identified directly from water, food, feces and other related body fluids including urine. Various methods and techniques can be used to recover the bacilli from target samples. The gram-negative bacilli can be detected and identified by the gram stain using light microscopy. Further, the bacteria in clinical samples can be cultured and isolated using primary isolation media like blood agar, cystine-lactose-electrolyte-deficient agar, xylose-lysine-desoxycholate agar, desoxycholate citrate agar, brilliant green agar and other commercial validated media (WHO 2003b). In all cases the bacterial culture is incubated in air at 35-37ºC except blood agar which is incubated in 5-10% carbon dioxide for 18-24 hours. The cultures are examined thereafter.

### 3.4.1 Culture and colonial appearance

While in blood agar Salmonella colonies are moist with 2-3mm in diameter; in cystine-lactose-electrolyte deficient agar and xylose-lysine-desoxycholate agar, desoxycholate citrate agar and in

brilliant green agar the colonies are red, colorless and red-pink with 1-3mm respectively (https://www.gov.uk/uk-standards-for-microbiology).

### 3.4.2 Biochemical tests

Cystine-lactose-electrolyte deficient agar-*Salmonella* species are generally non-lactose fermenters except some serovars like *Salmonella arizonae* and *Salmonella indiana* which may ferment lactose. The lactose non-fermenting bacteria obtain their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes. They produce acid from glucose usually with the production of gas, and are oxidase negative (Le Minor 1984). From same source, most produce hydrogen sulphide except *S. Paratyphi A* and *S. enteric subsp. enterica serovar typhi*, which is a weak producer. They are identified with a combination of serological and biochemical tests like urease TP36-urease test, oxidase test and TP19-indole test. *Salmonella* species urease, oxidase and indole negative.

### 3.4.3 Rapid diagnostics

Identification of *Salmonella* species by commercial systems is also widely used. Many rapid confirmation and identification methods have been developed for *Salmonella* and a large number have been developed into commercial products. Biochemical confirmation can be accomplished using commercial identification systems. Rapid immunological identification and confirmation tests based on latex agglutination, enzyme immunoassay and enzyme-linked immunosorbent assay have been developed for *Salmonella*, and simple-to-use lateral flow test strips using immune-chromatographic technology have also been developed into commercial products by a number of manufacturers (https://www.gov.uk/uk-standards-for-microbiology).

A serologcal test involving agglutination is popular for the diagnosis of suspected TF in an acute phase as it is simple, rapid, easily available and affordable for low-income countries (Wilke et al. 2002). Two types of agglutination techniques are available: the slide-agglutination and tube-agglutination tests. The slide-agglutination test first discovered by Max von Gruber and Herbert Edward Durham (1866-1945) and in 1896 by Georges-Fernand Widal (1911-1929) developed a procedure for diagnosing TF based on the fact that antibodies in the blood of infected individuals cause the bacteria to bind together into clumps, widal reaction, (Welch et al. 1936) is a rapid test and used as screening procedure. The Widal test is the bi-important serological test used for

diagnosis of antibodies to two *S. typhi* antigens. While the somatic (O) antigen shows fine agglutination the flagella (H) antigen shows coarse and very fine agglutination reactions (WHO 2003a, Sudharshan 2014).

Although definitive diagnosis of TF is by isolation of the causative bacteria from blood, bone marrow or other body fluids most low-income countries like Ethiopia due to limited access to laboratory facilities use the old widal test. Wassihun et al. (2015) reported that (66.7%) febrile patients with widal false-positive and treated wrongly as TF while only 1.6% patients were culture-proved TF cases.

### 3.4.4 Antibiotic / drug resistance

The antibiotics that have been traditionally incorporated in to the therapy of TF have been ampicillin, chloramphenicol, sulfamethoxazole-trimethoprim and tetracycline. However, the evolution of plasmid-encoded multi-drug resistant to these drugs in the 1970s and 1980s, ciprofloxacin was introduced as first line therapy for *Salmonella enterica serotype typhi* and *paratyphi A* (Threlfall et al.1999). antimicrobial therapy for the treatment of TF and the complication associated with it. Penicillin (amoxicillin, ampicillin), Cephalosporins (ceftriaxone, cefuroxime,aminoglycosides such as streptomycin, and gentamicin), Macrolide (erythromycin, fluoroquinolones such as ciprofloxacin, ofloxacin and perofloxacin and tetracyclines are used for treatment of *S.typhi* infection (Richared et al. 2007). Mechanism of antibiotic resistance in *S. typhi* is mediated by two factores which were acquisition of foregn genes via plasmids and mutation of chromosome (Holt et al. 2008).

## 1.5 TF prevention and vaccination

At the frontline of measures advocated to be taken against TF is health educational at community and household levels. Community members are sensitized on the transmission of TF and importance of personal and community sanitary practices, particularly hand washing before eating and preparing food and after latrine visit to combat it. The educations include eating fresh and cooked and vegetables. Further, the need for the availability of basic facilities like safe drinking water and proper toilet is emphasized.

However, because of behavioral and socio-economic problems TF remained a great health challenge to stop its transmission by preventive measures. Therefore, the disease widespread in poor settings and the common way of countering it remains passive case detection and treatment. Nevertheless, the limited resources available for case detection and management including prescribing appropriate antimicrobial therapy of multi-drug and quinolone-resistant strains forms additional bottleneck in TF control in low-income countries.

Two anti-salmonella vaccines have been developed and functioned, live oral vaccine based on Ty21a (an attenuated strain of *S. Typhi*) that is well-tolerated and the other is Vi-based parenteral subunit vaccine (based on the purified capsular polysaccharide *S. Typhi* Vi antigen). Both these vaccines are well-tolerated but are only moderately protective (Garmony et al. 2002).

## 4. Materials and Methods

### 4.1 Study site

The study area is Mekedela, northeast Ethiopia. Mekedela having an area of 152,100 hectares is among 21 Districts (*Woredas*) in South Wollo Zone (Amhara Region) and is bordered by *Walo Shabatala* River in the southwest, by Semein Gonder Zone in the west, by *Beshilo* River, which separates it from Semein Wollo Zone, in the north and by *Tenta* District in the east (Fig 2). Mekedela is located at 552km north of Addis Ababa having geographic coordinates of $11^0$ 30′ N latitude and $38^0$ 45′ E longitude. Based on the July 2014 national census projection by the central Statistical Agency of Ethiopia, the District had a total population estimate of 161,648 (80,854 males and 80794 women) with 8,875 urban inhabitants.

Mekedela District is subdivided into 29 smaller units named *kebeles*. At the time of this study there were 8 health centers serving the population in the entire district by clustering the *kebeles* and attaching specified appropriate health centers. MHC is located in Masha town which is the administrative center of Mekedela District was selected for the current study purposely based on its comparative better transport facility, laboratory service, and higher populations clustered to it but other 7 health centers were not will organized, recently build, inadequate professional personnel, laboratory service and others . The selected health center serves 8 *kebeles* (7 rural and 1 urban (Masha town) clustered to it. These are *kebeles* Dederie (029), Gonderoch (027), Anjewa (013), Tebi (012), Ganatite-Sankeha (06), Kibtia (03),Wofeche (02) and Masha town *kebele* 01 (Fig 2).

Masha town and its surrounding clustered 7 *kebeles* are characterized by an average annual rainfall of 1331.42 mm, temperature 17.83°C and mean maximum and minimum temperatures 20.57°C and 15.13°C, respectively, in the past five years (new-locclim 1.10 local climate estimator: http://www.fao.org/nr/climpag/pub/en3_051002_en.asp).

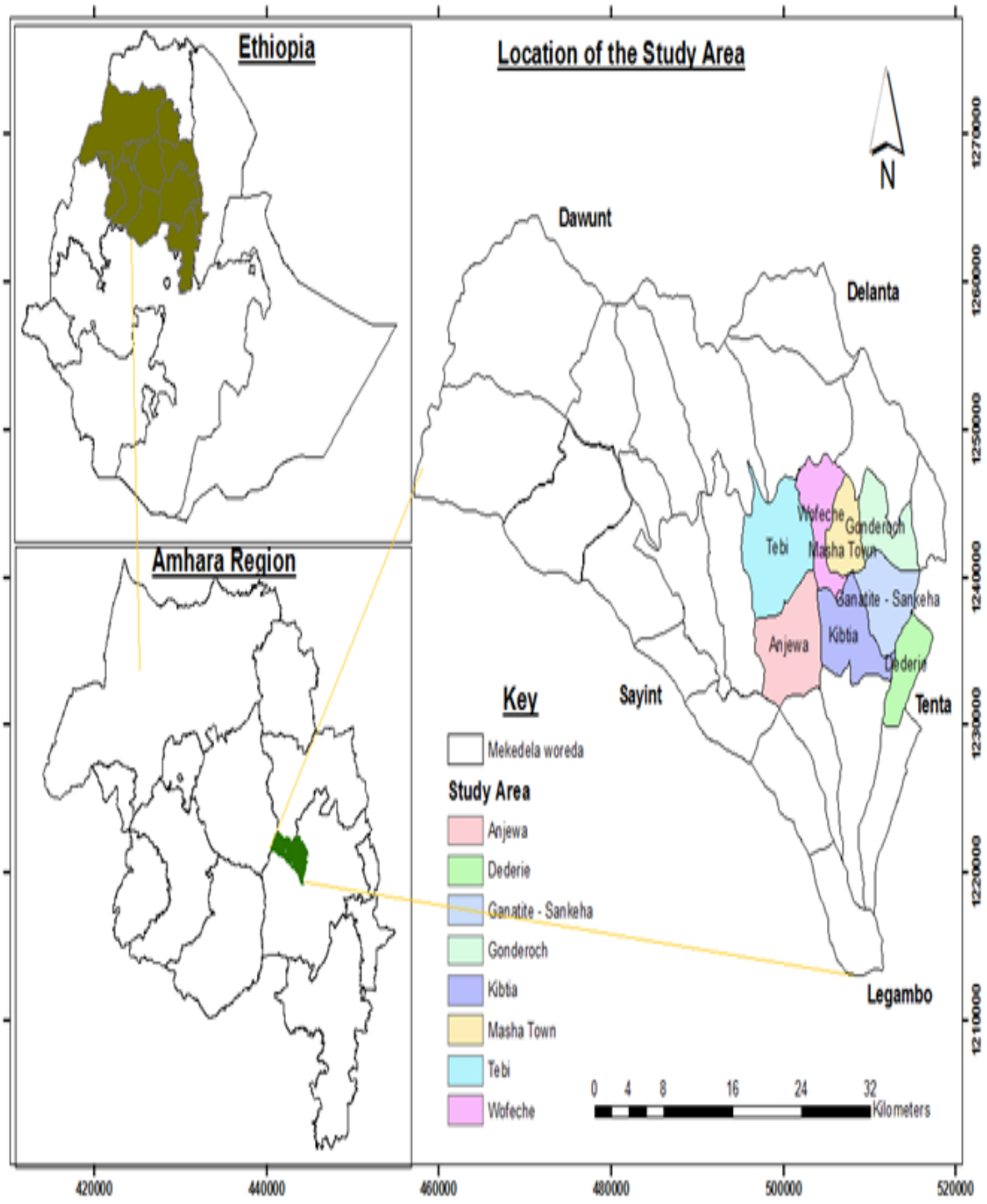



Figure 2 Map of the study site (the shaded area in different color as shown above were the study sites). (Source: GPS Coordination of Mekdela Agricultural Center).

## 4.2 Study design and population

All patients attending MHC from October 2016 to February 2017 formed the source population. In a cross-sectional study patients suspected of TF and who consented to participate in the study were requested to respond to questionnaire for socio-demographic and risk factors related to exposure to *S.typhi*. Patients who had been in the town for less than past six months and those who were residing out of the cluster were excluded. From the source population those suspected of TF and prescribed for blood test were prospectively recruited.

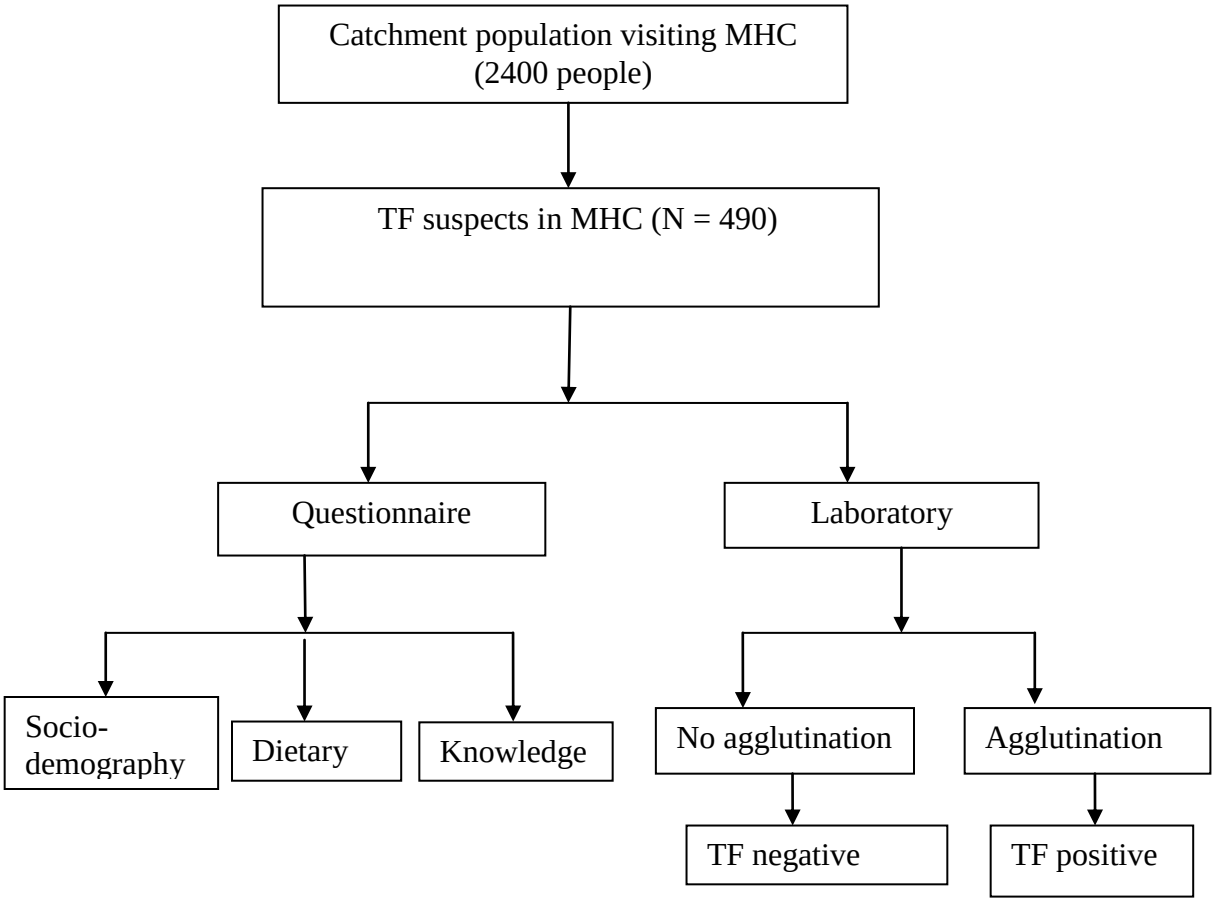



Figure 3 Study framework

## 4.3 Patient recruitment and socio-demography

Following patient recruitment, questionnaire/interview was used to collect individual and household socio-demographic data such as age, sex, family size, occupation, education, residence, marital status, dietary habits, safe water source and toilet access. In addition, treatment-seeking behavior and application of health package practices, knowledge of TF

transmission, symptoms/signs and prevention. The questionnaire was prepared in accordance with the World Health Organization (WHO) steps instrument for chronic/acute disease risk surveillance and the Global Physical Activity Questionnaire (GPAQ) Analysis Guide. (http://www.who.int/chp/steps/GPAQ/en/index.html).

### 4.4 TF serology

Blood samples (1-3 ml) were drawn by puncturing superficial veins (mainly from median cubital, cephalic or basalic veins) of the upper limb using 3/5 cc needle. Sera serum/plasma separated following centrifugation. Widal test kits (supplier lot catalog 21374) containing the O and H antigens of *S. typhi* obtained from SPINREACT (Barcelona, Spain) was used. The slide-agglutination test was performed as per the manufacturer's instruction. Briefly, the antigen vial was gently mixed saline by an aspirate dropper multiple times to make a thorough mixture. A 50 µl serum sample was added into a row of circles on the test card. Drops (one drop each) of positive and negative control sera were dispensed into respective circles. A drop of the appropriate well-shaken suspension of the antigen was added to each circle next to each sample to be tested and mixed the content of each circle with a disposable stirrer and spread over the entire area enclosed by the ring with separate applicators for each mixture. Afterwards, the slides were shaken gently by hand or by means of a mechanical rotator (100 rpm) for 1 minute. Finally, the test was observed immediately under a suitable light source for any degree of agglutination and qualitative results recorded (Fig 3).

### 4.5 Data analysis

Data entered into Statistical Package for Social Sciences (SPSS) Version 20.0 software (IBM-SPSS, USA). Univariate and multivariate logistic regression models were used to test the association between the dependent variable (TF serology) and independent variables. Odds ratios (OR), crude and adjusted were calculated at 95% confidence interval (CI) and a p-value $\leq 0.05$ taken as statistically significant.

## 4.6 Data quality control

Prior to data collection the questionnaire which was prepared in English was translated into the patients' vernacular language (Amharic) to ensure reliable information. Standard operating procedures were used for sample collection and processing. The result of laboratory examination was recorded on well-prepared format carefully. The collected information was reviewed and any errors cross-checked on daily basis.

Serological techniques and standardizations vary from laboratory to laboratory. Differences in titres could be expected. Under standard procedures care is taken not to use turbid and contaminated controls and after usage the controls were immediately recapped and replaced at 2-8°C. Reagent vials with leakage or breakage were discarded and only qualified well-trained health personnel used the reagents and performed all tests. The performance of the positive control was validated periodically using widal antigen suspensions. The reagents were obtained from trustworthy companies via the Ethiopian Federal Ministry of Health.

## 4.7 Ethics

The study was supported by letters from Department of Zoological Sciences, Addis Ababa University (ref. № SF/ZS/2241/08/2016). Permission was obtained from Mekedela Health Office. Local administrators were consulted and approval granted. Informed consent was obtained from each participant and for minors parents/guardians gave their consent. The confidentiality of collected information was ensured throughout the process. Blood samples were collected by qualified laboratory technicians and TF seropositives were treated as per the health center's routine service to its patients.

## 5. Results

### 5.1 Study population

During the study period, MHC was visited by a total of 2,400 patients for any health problem. From this catchment population, 1749 individuals came from 7 rural *kebeles* and 650 from urban *kebele* (Masha *kebele* 01). The number of TF suspects visited the health center daily during the 5-month period ranged from 0-28 and on average 8.9 patients and the highest numbers on a single day was in October (28(5.7%) and December (18(3.7%) (Table 1). Overall, the largest number of patients was in October (235(48%) and December (194(39.6%) (Table 1).

Table 1 Monthly number of patients examined during the study period

| Diagnostic result | Months | | | | | Total |
|---|---|---|---|---|---|---|
| | October | November | December | January | February | |
| | 02-30, 2016 | 03-25, 2016 | 01-30, 2016 | 05-23, 2017 | 01-25, 2017 | |
| Negative | 71 | 2 | 59 | 12 | 0 | 144 (29.4%) |
| Positive | 164 | 9 | 135 | 36 | 2 | 346 (70.6%) |
| Total | 235 | 11 | 194 | 48 | 2 | 490 (100%) |

A total of 490 individuals suspected of TF and who fulfilled the inclusion criteria were recruited and diagnosed for TF (329 males and 161 females) (Table 2). Among the TF suspected and examined patients; 192, 194 and 104 belonged to age groups (years) 1-15, 16-45 and over 45 respectively (Table 2, 3). The majority of the participants (304) were rural dwellers and only 186 were urban.

#### 5.1.1 Socio-demographic and dietary related risk factors

TF positives (74.5%), (72.2%) and (60.6%) belonged to age groups 1-15 (n=143), 16-45 (n=140) and over 45 (n=63) respectively and male individuals covered more number (74.8% n=246) (Table 2, 3). Concerning occupation of these TF positive, the highest number was recorded from farmer (77.2%, n=173), student (75.6%, n=62), merchant (67.6% n=25), civil servant (50.4% n=64) (Table 2, 3). Most TF positive were illiterate (77.2% n=173) and the family size of the participants with ≥5 was (75.2% n=215), 2-4 (63.2% n=67) and single (65.3% n=64). A large number of TF positive individuals had no safe toilet access (74.8% n=202) (Table 2, 3). A significant number of unmarried individuals were TF positive (65.4% n=136) while Out of the 490 TF suspects, (74.8% n=202) were not obtained safe water source, 289(60.0%) reported that they consume uncooked green vegetables (Table 2, 3).

### 5.1.2 Other related risk factors

Among TF suspected individuals (74.9% n=197) did not obtain health education, (67.5% n=208) had no awareness about TF transmission, (68% n=225) did not know TF prevention mechanism and 66.8% (n=195) did not seek medication (Tables 2, 3).

## 5.2 TF serology

Out of the 490 patients tested, 346(70.6%) were positive and the rest 144(29.4%) negative (Table 1). Seropositive males were 246(74.8%: 246/329) and females 100(62.0%: 100/161) with male-to-female ratio of 2.46:1 for seropositivity. Univariate analysis results showed that TF prevalence was significantly higher among individuals who did not obtain safe water source (COR 1.591, 95% CI 1.074-2.357, p 0.020), uncooked green vegetable eaters (COR 1.492, 95% CI 1.007-2.208, p 0.046), male individuals (COR 1.790, 95% CI 1.606-2.066, p 0.004), age group 1-15 years (COR 1.899, 95% CI 1.141-3.162 p 0.014), illiterate (COR 3.997, 95% CI 2.096-7.622, p<0.0001) and farmers (COR 4.219, 95% CI 1.727-10.306, p<0.0001). In summary, all socio-demographic variables except residence and education had significant association with TF in the univariate analysis (tables 2).

Table 2 Univariate logistic regression analysis of socio-demographic variables in TF suspected and seropositive patients at MHC, October 2016 – February 2017 (N=490)

| Variable | Alternatives | N | Positive,n(%) | COR | 95% CI | P-value |
|---|---|---|---|---|---|---|
| Age (years) | 1-15 | 192 | 143(74.5) | 1.899 | 1.141-3.162 | 0.014* |
| | 16-45 | 194 | 140(72.2) | 1.687 | 1.020-2.791 | 0.042* |
| | >45 | 104 | 63(60.6) | 1.00 | | |
| Sex | Male | 329 | 246(74.8) | 1.790 | 1.606-2.066 | 0.004* |
| | Female | 161 | 100(62.1) | 1.00 | | |
| Residence | Urban | 186 | 131(70.4) | 1.00 | | |
| | Rural | 304 | 215(70.7) | 0.986 | 0.661-1.471 | 0,945 |
| Family size | Single | 98 | 64(65.3) | 1.00 | | |
| | 2-4 | 106 | 67(63.2) | 0.898 | 0.721-1.301 | 0.060 |
| | ≥5 | 286 | 215(75.2) | 1.700 | 1.652-4.914 | 0.022* |
| Safe toilet access | Yes | 220 | 144(65.4) | 1.00 | | |
| | No | 270 | 202(74.8) | 1.567 | 1.061-2.317 | 0.024* |
| Eat uncooked vegetables | Yes | 289 | 214(74) | 1.492 | 1.007-2.208 | 0.046* |
| | No | 201 | 132(65.7) | 1.00 | | |
| Safe water source | Yes | 220 | 144(65.5) | 1.00 | | |
| | No | 270 | 202(74.8) | 1.591 | 1.074-2.357 | 0.020* |
| Occupation (N=470) | Farmer | 224 | 173(77.2) | 4.219 | 1.727-10.306 | 0.000* |
| | Merchant | 37 | 25(67.6) | 3.997 | 2.125-7.759 | 0.002* |
| | Student | 82 | 62(75.6) | 4.061 | 2.092-5.329 | 0.000* |
| | Civil servant | 127 | 64(50.4) | 1.00 | | |
| Educational status (N=470) | Illiterate | 224 | 173(77,2) | 3.238 | 2.033-5.158 | 0.000* |
| | Read and write | 34 | 23(67.7) | 3.682 | 1.497-9.057 | 0.005* |
| | ≤Secondary school | 83 | 57(68.7) | 3.997 | 2.096-7.622 | 0.000* |
| | ≥Grade 12 complete | 129 | 66(51.2) | 1.00 | | |
| Health education (N=470) | Yes | 207 | 136(65.7) | 1.00 | | |
| | No | 263 | 197(74.9) | 0.642 | 0.430-0.957 | 0.080 |
| Aware of TF transmission (N=470) | Yes | 162 | 97(59.9) | 1.00 | | |
| | No | 308 | 208(67.5) | 1.624 | 1.049-2.516 | 0.030* |
| Know TF prevention (N=470) | Yes | 139 | 84(60.4) | | | |
| | No | 331 | 225(68) | 1.641 | 1.035-2.603 | 0.035* |
| Seek medication (N=470) | Yes | 178 | 111(62.5) | 1.00 | | |
| | No | 292 | 195(66.8) | 1.716 | 1.119-2.633 | 0.013* |
| Marital status (N=278) | Married | 70 | 40(57.1) | 1.00 | | |
| | Unmarried | 208 | 136(65.4) | 2.118 | 1.104-4.063 | 0.024* |

COR: crude odds ratio, CI: confidence interval, n: number of people, N: total participants, %: percent, MHC: Masha Health Center, *: Statistically significant, TF: typhoid fever, N=470 participants eligible for some.

Multivariate analysis revealed that age 1-15 (AOR 2.259, 95% CI 1.227-4.161, p 0.009) and 16-45 (AOR 2.329, 95% CI 1.270-4.270, p 0.006) years old patients were twice at higher risk of getting TF compared to over 45 patients (tables 4 and 5). Using the female sex as a reference, maleness was twice at higher risk for TF (AOR 2.064, 95% CI 1.301-3.275 p 0.002). Illiterate patients were almost four times at increased risk of TF than those who got some kind of education (AOR 3.940, 95% CI 1.926-8.063, p<0.0001). Members belonging to a household with size 5 and more were twice at odds of having TF than those with lower family size (AOR 2.103 95% CI 1.213-3.171 p 0.029). Lack of toilet was also found to be a significant contributor of TF seropositivity (AOR 1.713, 95% CI 1.331-2.906 p 0.037) (Table 3).

Table 3 Multivariate logistic regression analysis of socio-demographic variables in TF suspected and seropositive patients at MHC, October 2016 – February 2017 (N=490)

| Variable | Alternative | N | Positive, n(%) | AOR | 95% CI | P- value |
|---|---|---|---|---|---|---|
| Age (years) | 1-15 | 192 | 143(74.5) | 2.259 | 1.227-4.161 | 0.009* |
| | 16-45 | 194 | 140(72.2) | 2.329 | 1.270-4.270 | 0.006* |
| | >45 | 104 | 63(60.6) | 1.00 | | |
| Sex | Male | 329 | 246(74.8) | 2.064 | 1.301-3.275 | 0.002* |
| | Female | 161 | 100(62.1) | 1.00 | | |
| Family size | Single | 98 | 64(65.3) | 1.00 | | |
| | 2-4 | 106 | 67(63.2) | 0.604 | 0.345-1.059 | 0.079 |
| | ≥5 | 286 | 215(75.2) | 2.103 | 1.213-3.171 | 0.029* |
| Safe toilet access | Yes | 220 | 144(65.4) | 1.00 | | |
| | No | 270 | 202(74.8) | 1.713 | 1.331-2.906 | 0.037* |
| Eat uncooked vegetables | Yes | 289 | 214(74) | 1.223 | 0.771-1.941 | 0.392 |
| | No | 201 | 132(65.7) | 1.00 | | |
| Safe water source | Yes | 220 | 144(65.5) | 1.00 | | |
| | No | 270 | 202(74.8) | 1.793 | 0.481-1.197 | 0.235 |
| Occupation (N=470) | Farmer | 224 | 173(77.2) | 1.783 | 0.995-3.197 | 0.053 |
| | Merchant | 37 | 25(67.6) | 0.750 | 0.851-2.191 | 0.091 |
| | Student | 82 | 62(75.6) | 0.867 | 0.945-0.690 | 0.055 |
| | Civil servant | 127 | 64(50.4) | 1.00 | | |
| Educational status (N=470) | Illiterate | 224 | 173(77,2) | 3.940 | 1.926-8.063 | 0.000* |
| | Read and write | 34 | 23(67.7) | 3.540 | 1.307-9.588 | 0.000* |
| | ≤Secondary school | 83 | 57(68.7) | 3.093 | 1.671-5.728 | 0.013* |
| | ≥Grade 12 complete | 129 | 66(51.2) | 1.00 | | |
| Health education(N=470) | Yes | 207 | 136(65.7) | 1.00 | | |
| | No | 263 | 197(74.9) | 0.724 | 0.463-1.131 | 0.156 |
| Aware of TF transmission (N=470) | Yes | 162 | 97(59.9) | 1.00 | | |
| | No | 308 | 208(67.5) | 1.572 | 0.976-2.530 | 0.063 |
| Know TF prevention (N=470) | Yes | 139 | 84(60.4) | 1.00 | | |
| | No | 331 | 225(68) | 1.273 | 0.768-2.110 | 0.349 |
| Seek medication (N=470) | Yes | 178 | 111(62.5) | 1.00 | | |
| | No | 292 | 195(66.8) | 1.597 | 0.996-2.562 | 0.052 |

AOR: adjusted odds ratio, CI: confidence interval, n: number of people, N: total participants, %: percent, MHC: Masha Health Center, *: statistically significant, TF: typhoid fever, N=470 participants eligible for some.

## 6. Discussion

Out of 490 samples 346(70.6%) were seropositive for TF. This figure is much higher than other results from other parts of Ethiopia (Andualem et al. 2014 (2.6%), Abera et al. 2010 (1.6%), Beyene et al. 2011 (11.5%) and Reda et al. 2011 (11.5%). Similarly a lower prevalence (24.4%) was reported by a study from Nigeria (Ibegbulam-Njoku et al. 2014). On the other hand, relatively closer finding (57.5%) was recorded from Cameroon (Nsutebu et al. 2003). But compared to other reports outside Africa a high prevalence (83.0%) was recorded (Prajapati et al. 2008). Participants in the age group 1-15 years old carried the highest proportion (74.5%) although it is comparable with age group 16-45 (72.2%) and a slightly lower than that for the over 45 (60.6%). This finding is similar to other reports (Weyesa 2012; Soomro et al. 2014).

The discrepancy in the prevalence estimates between different studies can be due to differences in the study design (community-based versus health facility-based), sample size, socio-economic-demographic or environmental and associated sanitary practices, seasonality of the study period, TF diagnostic methods and sample type used among others. Therefore, just comparing numbers from such varying studies may not prove much useful.

This study found a male-to-female ratio of 2.46:1 for the study population as whole and 2.04:1 for seropositive patients. The proportion of TF seropositive males (74.8%) to females (65.2%) showed variability from other studies conducted in Ethiopia. For instance, in a study from north Ethiopia, the proportion of TF seropositive females was 52.3% which was nearly comparable (47.7%) to that of males (Wasihun et al. 2015) in Mekele. Similarly, Kefelew et al. 2014 found 58.6% of female and 41.4% male TF seropositives in Gondar town, north Ethiopia. Furthermore, a retrospective study in a tertiary care center in Addis Ababa showed that there were 38% female and 62% male TF positive patients in 2007 and 42% females and 58% males in 2010 (Weyesa 2012). Outside Ethiopia, there are certain studies that reported a male-to-female ratio of up to 6.41:1(Ayaz et al. 2006) for TF seropositive cases. On the other hand, some other authors reported nearly equal or only slightly different male-female TF cases (Sudharshan 2014). Similarly, a study from Pakistan revealed 52.0% male and 48.0% female TF seropositive patients

(Kalsoom et al. 2014). Another similar study in Pakistan recorded the proportion of seropositive TF male and female patients 32.9% and 31.4%, respectively (Soomro 2014).

Apparent sex-based differences (males more implicated) among TF patients and suspects might be due to poor hygienic practice, habit of eating hawked food, patronizing restaurants and cafeterias where basic hygienic practices are most times compromised during food preparation, exposure of traditional agricultural practice than females. In addition unmarried males are highly vulnerable than married. In contrast, still there are some other reports that revealed females experiencing a higher TF attack rate than males accounting for 55.6% of the patients in Harare, Zimbabwe (Polonsky 2014).

Although risk factors vary for TF patients and the relative impact of each factor varies from setting to setting several studies reported different socioeconomic, demographic and knowledge and attitude that may affect the magnitude of TF prevalence. This study demonstrated that the most common factors that facilitate TF dissemination are highly prevailing in the study area. For example the proportions of participants being illiterates, students, farmers, greater family size, without a toilet, and without safe potable water source were 77.2, 75.6, 77.2, 75.2 and 74.8% respectively. All of these factors were significantly associated with TF in this study and several other similar ones (Allen and Honest 2010, Abera et al. 2010, Wen et al. 2010, Ibegbulam-Njoku et al. 2014). Nevertheless, the figure found in this study may be higher than expected and it may not show a more accurate picture for various reasons. The diagnostic technique used has its own limitations and is not definitive test of the target bacteria and is not supported by tube-agglutination, stool culture, blood culture and biochemical tests. Although the widal test has negative predictive value it is widely criticized for its less specificity and cross-reaction with several other enteric bacterial antigens is common (Borg 1991). This study was an indicator for forwarding bench marks for further investigation and it is an alarm for the city government and health administrators.

## 7. Conclusion and recommendation

TF is an important public health concern in Masha town and its environs. Age, sex, education, family size, lack of toilet and illiteracy were statistically significant predictors of TF. Scaling-up of toilet coverage, provision of safe drinking water supply and health education including proper documentation of cases is required.

## 8. References


Abera B, Biadegelgn F, Bezabih B (2010). Prevalence of salmonella typhi and inestinal parasites among food handlers in Bahir Dar Town, North west Ethiopia. *Ethiop. J. Health Dev*. **24**:46-50.

Akhtar A, Indu Shukla I, Khan F, Parvez A (2016). Comparative evaluation of blood culture, immunochromatographic test, widal and polymerase chain reaction for rapid diagnosis of enteric fever. *BJMHR*. **4**: 2394-2967.

Allen M, Honest N (2010). Prevalence and constraints of typhoid fever and its control in an endemic area of singida in Tanzania: Lesson for effective control of the disease. *J. Public Health Epidemiol*. **2**: 93-99.

Andargie G, Kassu A, Moges F, Tiruneh M, Henry K (2008). Prevalence of bacteria and intestinal parasites among food handlers in Gonder town, North west Ethiopia. *J. Health Popul. Nutr.* **26**: 451-455

Andualem G, Abebe T, Kebede N, Gebre-Selassie S, Mihret A, Alemayehu H (2014). A comparative study of widal test with blood culture in the diagnose of typhoid fever in febrile patients. *BMC Research Notes*.**7**:653.

Animut A, Mekonnen Y, Shimelis D, Ephraim E (2009). Febrile illnesses of different etiology among outpatients in four health centers in Northwestern Ethiopia. *Jpn. Infect. Dis*.**62**: 107-110.

Ayaz A, Perviaz1 M, Azad M, and Perviaz G (2006). Risk factors of enteric fever in children less than 15 years of age. *J. statistics*. **13**:1684-1690.

Beyene G, Asrat D, Mengistu Y, Assefa A,Wain J (2008). Typhoid fever in Ethiopia. *J. Infect. Dev. Ctries*.**2**: 448-453.

Beyene G, Nasir S, Asrat D, Mengistu Y, Engers H, Wain J (2011). Multi-drug resistant salmonella concords are a major cause of salmonellosis in children in Ethiopia. *J. Infect. Dev. Ctries*. **5**:023-033.

Bhan M, Bahl R, Bhatnagar S (2005). Typhoid and Paratyphoid fever. **366:**749-762

Bhutta Z (1996). Impact of age and drug resistant on mortality in typhoid fever. *Arch. Dis. Child*. **75**: 214-217.

Borg M (1991). Serological diagnosis of typhoid fever: A review of the limitations of the Widal test. *Maltese Med. J*. **3:** 13.

Breiman R, Comas L, Njuguna H (2012). Population based –incidence of typhoid fever in an urban informal settlement and a rural area in Kenya: implication for typhoid vaccine use in Africa. *PLOS One 2012*. 7: e29119.

Crump J, Luby S, Mintz E (2004). The global burden of typhoid fever. *Bull. WHO*. **82**:346-353.

Denise M, Anne M, Stanley F (2004). Persistent bacterial infections: the interface of the pathogen and the host immune system. *Nat. Rev. Microbiol*. **2:**747-765.

Desta M (2010).‟ Prevalence of Salmonella and Shigella among Food Handlers in Catering Establishments in Hawassa University, Hawassa, Ethiopia, Addis Ababa University''. Unpublished.

DeRoeck D, Jodar L, Clemens J (2007). Putting typhoid vaccination on the global health agenda. *N. Engl. J. Med*. **357**:1069-1071.

New_LocClim:LocalClimateEstimator,[http://www.fao.org/nr/climpag/pub/en3_051002_en.asp] , retrived 15 march 2017

Garmony H, Brown K, Titball R (2002). Salmonella vaccines for use in humans: present and future perspectives. *FEMS. Microbiol. Rev*. **26**:339–53.

Getamesay M, Getenet B, Ahmed Z (2014). Prevalence of shigella, salmonella and campylobacter species and their susceptibility patterns among under five children with diarrheain Hawassa town, south Ethiopia. *Ethiop. J. Health Sci.* **24**: 107.

Global Physical Activity Questionnaire (GPAQ) Analysis Guide. (http://www.who.int/chp/steps/GPAQ/en/index.html), retrieved 25 may 2017.

Gupta A (1994). Multidrug-resistant typhoid fever in children: epidemiology and therapeutic approach. *Pediatr. Infect. Dis. J*. **13**: 134-140.

Holt J, Kreig N, Sneath P, Stanley J, Williams S (2008). Bergey's manual of determinative bacteriology. Baltimore: William and Wikins.

House D, Bishop A, Parry C, Dougan G, Wain J (2001). Typhoid fever: pathogenesis and disease. *Curr. Opin. Infect. Dis*.**14**:573–8.

Hornick R, Greisman S, Woodward T, DuPont H, Dawkins A, Snyder M (1970). Typhoid fever: pathogenesis and immunologic control. *N. Engl. J. Med.* **283**:686–691.

Ibegubulam-Njoku P , Chijioke-osuji C, Duru F (2014). Prevalence of antibody titre in healthy individual and enteric fever patient in Owerri, Nigeria. *J. Public health Epidimol*. **6:**192-196.

Kalsoom, Akbar F, Younas M, Tasneem U, Suleman M, Ali S, Ali S, Roohi A (2014). Prevalence of typhoid fever in five Southern district of Khyber pakhtunkhwa, Pakistan: A preliminary study. *Int. J Biosci*. **4**:375-330.

Kariuki S (2008).Typhoid fever in sub-Saharan Africa: Challenges of diagnosis and management of infections. *J. Infect. Developing Countries.* **2**: 443 – 447.

Keddy KH, Sooka A, Letsalo ME, Hoyland G, Chaignate CL, Morrissey AB (2011). Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-saharan African sites. *Bull. World Health Organ*. **89**: 640-647.

Kifelew L, Wondafrash N, Feleke A (2014). Identification of drug-resistant Salmonella from food handlers at the University of Gondar, Ethiopia. *BMC Research Notes.* **7**:545.

Langrige, Gemma C, Fookes, Maria (2014). Pattern of genome evolution that have accompanied host adaptation in salmonella. *PNAS*. **112**: 863-868.

Le Minor L (1984). Genus III. Salmonella. In Krieg N, Holt J (eds): Bergey's Manual of Systematic Bacteriology. Baltimore, Williams & Wilkins. 427-458

Levantesi C, Bonadonna L, Briancesco R, Grohmann E, Toze S, Tandoi V (2012). Salmonella in surface and drinking water: Occurrence and water-mediated transmission. *Food Res. Int.* **45**: 587–602.

Marks F, Adu-sarkodie Y, Hunger F (2010) High incidence typhoid fever among Ghanaian children. *Emerg. Infec. Dis*. **16**:1796-1797.

Nsutebu E, Martine P, and Adiogod D (2003). Prevalence of typhoid fever in fibrile patients with symptoms clinically compatable with typhoid fever in cameroon. *Trop. Med. Int health*. **8**:575-578.

Pang T, Bhutta Z, Finlay B, Altwegg M (1995). Typhoid fever and other salmonellosis: a continuing challenge. *Trends Microbiol*. **3**: 253-255.

Park S, Kim H, Cho W, Kim J, Oh M, Kim S (2009). Identification of *Salmonella enterica* subspecies I, *Salmonella enterica* serovars Typhimurium, Enteritidis and Typhi using multiplex PCR. *FEMS. Microbiol. Lett.* **301**:137-46.

Pirisi M, Salvador E, Bisoffi Z, Gobbo M, Smirne C, Gigli C (2006). Unsuspected strongyloidiasis in hospitalised elderly patients with and without eosinophilia. Clin. *Microbiol. Infect.* **12**:787–92

Polonsky J, Martinez-Pino I, Nacker F, Chonzi P, Manangazira P (2014). Descriptive epidemologyof typhoid fever during an epidemic in Harar, Zimbabwe 2012. *PLOS ONE*. **9**: e114702.

Prajapati B, Rai G, Rai S, Upreti H, Thapa M, Singh G, Shrestha R (2008). Prevalence of *Salmonella* typhi and paratyphi infection in children: a hospital based study. *Nepal Med. Coll. J.* **10:** 238-241.

Public Health England (2015). https://www.gov.uk/uk-standards-for-microbiology, retrieved 13 July 2017.

Reda A, Seyoum B, Yimam J, Andualem G, Fiseha S, Vandeweerd J (2011). Antibiotic susceptibility patterns of salmonella- shigella isolates in Harar, Eastern Ethiopia. *J. Infect Dis. immun*. **3**:134-139.

Richard A, Pamela C, Bruce D (2007). Microbiology. 2nd edition, Lppincott Williams and Wilkins. 59-65.

Roumagnac P, Weill F, Dolecek C, Baker S, Briss S (2006). Evolutionary history of *Salmonella typhi*. *Sci*. **314**:1301-1304.

Soomro S, Baig S, Naseem S, Sharafat S (2014). Seasonal variation and recent status of typhoid fever in a tertiary care hospital. *IJEHSR.* **2:** 2310-3841**.**

Siddiquia F, Rabbania F, Hasanb R, Nizamic S, Bhuttac Z (2006). Typhoid fever in children: some epidemiological considerations from Karanchi, Pakistan. *Int. J. Infect Dis*. **10**: 215-222.

Sudharshan R (2014). Clinical profile and antibiotic sensitivity pattorn of typhoid fever patients admitted to padiatric ward in rural teaching Hospital. *Int. J. Med. Res. Health Sci*. **3**: 245-249.

Tadesse G (2014). Prevalence of human salmonellosis in Ethiopia: a systematic review and meta-analysis. *BMC Infect. Dis*. **14**: 88.

Teh C, Chua K, Thong K (2014). Paratyphoid fever: Splicing the Global Analyses. *Int. J. Med. Sci*. **11(7)**: 732-741.

Threlfall E, Ward L, Skinner J (1999). Ciprofloxacin-resistant Salmonella typhi and treatment falure. *Lancent*. 353: 1590-1591.

Uneke C (2008). Concurrent malaria and typhoid fever in the tropics: the diagnostic challenges and public health implication. *J. Vector Borne Dis*., **45**:133-142.

Wasihun A, Welekidan L, Gebremariam S, Welderufael A, Muthupandian S, Haile T, Dejene T (2015). Diagnosis and treatment of typhoid fever and associated prevailing drug resistance in Northern Ethiopia. *Int. J. Infect. Dis*. **35**:96-102.

Wattiau P, Boland C, Bertrand S (2011). Methodologies for *Salmonella enterica* subsp. *enterica* subtyping: gold standards and alternatives. *Appl. Environ. Microbiol.* **77**:7877-7885.

Welch H, Lee and Mickle F (1936). A rapid slide test for serological diagnosis of typhoid and paratyphoid fevers. *Am. J. Public. Health. Nations.* **26**: 248-255.

Went X, Chongsuvivatwong V, Lu L, Fu X Q(2010). Financial barriers against access to diagnostic procedures among enteric fever suspects in highly endemic areas of china. *J. popul. Nut.* **28**:53-60.

Weyesa J (2012). Seroprevalence of typhoid fever among subjects with acute febrile manifestations at tertiary care center, Addis Ababa, Ethiopia. *Inter. J. Sci. Research.* **3(10):**2319-7064.

Wilke A, Ergonul O, Bayar B (2002). Widal test in diagnosis of typhoid fever in Turkey. *Clin. Diagn. Lab. Immunol*. **9**: 938-941.

WHO (2003a). Manual for Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in Developing World. Salmonella serotype. 103-118.

WHO (2003b). Communicable Disease Surveillance and Response Vaccines and Biologicals. World Health Organization, Geneva, Switzerland.

Worku B (2000). Typhoid fever in an Ethiopian children's hospital: 1984-1995. *Ethiop. J. Health Dev*. **14**: 311-315.

## 9. Annexes

### Annex 1 consent form

Code number_____

This was an agreement request to assess prevalence of typhoid fever in all individual. With this request those individuals who are attending typhoid fever suspected follow up at Mekedela health center and willing to participate in the study will be invited to assess determinants of typhoid fever. Based on the laboratory investigation typhoid fever individual would be treated by low cost health insurance.

You would be requested to give small amount of blood. Blood would be collected from your hand by using sterile 3-5cc needle. There will be some pain during pricking of your hand but not harmful to your health. If you are agree to give samples you will be requested to answer for questionnaire.

Are you willing to participate with the study by giving blood sample and answer to the request?

A Yes B No

THANK YOU

Name of health institution_____

Patient name_____ signature_____ date_____

Name of data collector_____ signature_____ date_____

## Annex 2 Amharic version of the consent form

የዉል ስምምነት ቅፅ

መለያ ኮድ_____

በአዲስ አበባ ዩኒቨረሲቲ የሳይንስ ፋኩሊቲ የዙዩሎጂካል ትምህርት ክፍል፣

በመጠይቁም በመቅደላ ጤና አጠባበቅ ጣቢያ አገልግሎት ከሚያገኙ ተቅማጥና ቁርጥማት (የታጥፎይድ በሽታ)ታማሚዎች ዉስጥ በጥናቱ መካፈል ፍቃደኛ የሆኑ እንድሁም መመዘኛዉን በሚያሟሉ ላይ ለታይፎይድ መንስኤዎችና ኢጋላጭ ሁኔታዎች ለታይፎይድ በሽታ ስርጭት ያላቸዉ እደዛ ምን እንደሚመስል ለማጥናት የተዘጋጀ ዉል ነዉ፡፡ የደም ናሙና ምርመራ በማድረግ በሚገኘዉ ዉጤት መሰረት የታይፎይድ በሽታ ችግር ካለበዎት ከጤና ጣቢያዉ ባለሙያዎች ጋር በመተባበር ተገቢዉን የህክምና አገልግሎት ያገኛሉ፡፡

የጥናቱ አላማ

ለታይፎድ በሽታ መንስኤና ኢጋላጭ በሆኑ ተፅኖዎች ዙሪያ ቃለመጠይቅ ይቀርብሎታል በመጠይቁም ላይ ስመዎትን ወይም የእርሰዎን ማንነት የሚገለፅ ማንኛዉም ነገር አይጠቀስም ወይም አይያያዝም፡፡ የሚሰጡትም መረጃ ሆነ ናሙና ከዚህ ጥናት ዉጭ ሆነ ከርሰዎ ጋር ለተገናኘ ለሌላ ጥቅም በፍፁም አይዉልም፡፡

በዚህ ጥናት የደም ናሙና ለመስጠት እንድሁም ለሚቀርብለዎት ቃለፐ-መጠይቅ ምላሽ በመስጠት ሙሉ ተሳታፊ ለመሆንፈቃደኛ ነዎት?

አዎ ከሆነ መልሰዎ

ስለ ትብብርዎ እናመሰግናለን

የጤና ተቋሙ ስም _____

የጥናቱ ተካፋይ ስም _____ ፊርማ _____ ቀን _____

የመረጃ ሰብሳቢዉ ስም_____ ፊርማ _____ ቀን _____

## Annex 3 interview/ questionnaire

English version of interview questions for typhoid fever patient

**Socio-demographic, dietary and related characteristics of respondents**

1. How old are you?
2. Sex A/ Male B/ Female
3. Occupation A/ Farmer B/ Civil servant C/ Merchant D/ Student
4. Educational status A/ Illiterate B/ Red and writes C/ secondary school and below D/ grade 12 complete and above
5. Where do you live? A/ Urban B/ Rural
6. What is your marital status? A/ Married B/ Unmarried
7. What is your family size? A/ single B/ 2-4 C/ ≥5
8. Do you eat uncooked green leafy vegetables? A/ Yes B/ No
9. Do you get safe toilet access? A/ Yes B/ No
10. Do you get safe water source? A/ Yes B/ No
11. Do you know typhoid fever prevention? A/ Yes B/ No
12. Are you aware of typhoid fever transmission mechanism? A/ Yes B/ No
13. Do you get medication when sick? A/ Yes B/ No
14. Do you get health education? A/ Yes B/ No

## Annex 4 Amharic version interview questionnaires

በአዲስ አበባ ዩኒቨርሲቲ የሳይንስ ፋኩሊቲ የዙዮሎጂካል ትምህርት ክፍል ሙሉ ፈቃደኛ በሆኑ የታይፎይድ ፌቨር ህመም መንስኤዎችና አጋላጭ ሁኔታዎችን በተመለከተ ለሚደረግ ጥናት የተዘጋጀ መጠይቅ፤ ይህን ጥናት ለማጀብ የደም ናሙና ለመስጠትና ለሚቀርብለዎት ቃለ-መጠይቅ ምላሽ በመስጠት ሙሉ ተሳታፊ ለመሆን ፈቃደኛ ነዎት?

አዎን---------  አይደለሁም----------  የጤና ተቀሙ ስም ----------

የጥናቱ ተካፋዩ መለያ ኮድ ----------

ስነ-ማህበራዊ ፣ ምግብ እና  ተዛማጅ ህዝባዊ  መጠይቆች

1. እድሜዎት ስንት ነዉ?
2. ፆታዎ  ሀ/ ወንድ  ለ/ ሴት
3. ስራዎ ምንድን ነዉ?  ሀ/ ገበሬ  ለ/ የመንግስት ሰራተኛ  ሐ/  ነጋዴ  መ/ ተማሪ
4. የትምህርት ደረጃዎ ስንት ነዉ?  ሀ/ ማንበብና መፃፍ አልችልም  ለ/ ማንበብ ና መፃፍ እችላለሁ  ሐ/ ከ 9-10ኛ ክፍል ና በታች  መ/  12ኛ ክፍል ያጠናቀቁና በላይ
5. የት ነዉ የሚኖሩት? ሀ/ ገጠር  ለ/ ከተማ
6. የጋብቻ ሁኔታዎት?  ሀ/ ያገባ  ለ/ ያላገባ
7. ስንት ቤተሰብ አለዎት?  ሀ/ አንድ ብቻ  ለ/ ከሁለት እስከ አራት  ሐ/ አምስት ና በላይ
8. ያልተቀቀሉ ና አረንጓዴ ቅጠል ያላቸዉን እፀዋት ይመገባሉ?  ሀ/ አዎን  ለ/አይደለም
9. ንፁህ ሽንት ቤት ያገኛሉ?  ሀ/ አዎን  ለ/ የለም
10. ንፁህ የዉሃ አገልግሎት  ያገኛሉ?  ሀ/ አዎን  ለ/ አይደለም
11. የታይፎድ ፌቨር በሽታ መከላከያ ዘዴ ያዉቃሉ?  ሀ/ አዎን  ለ/ አይደለም
12. የታይፎይድ ፌቨር በሽታ ግንዛቤ  ጠንቅቀዉ ያዉቃሉ?  ሀ/ አዎን  ለ/ አይደለም
13. የታይፎይድ ፌቨር በሽታ ሲይዘዎት ጤናዎትን በህክምና ይከታተላሉ?  ሀ/ አዎን  ለ/ አልከታተልም
14. ስለ ታይፎድ ፌቨር በሽታ የጤና ትምህርት አግኝተዉ  ያዉቃሉ?  ሀ/ አዎን  ለ/ አይደለም

## Annex 5: Operational definitions of variables in the Thesis

**Catchment kebeles** - Place where patients come to MHC for examination of typhoid fever

**Masha town** - Administrative town of the Mekdela woreda Woreda.

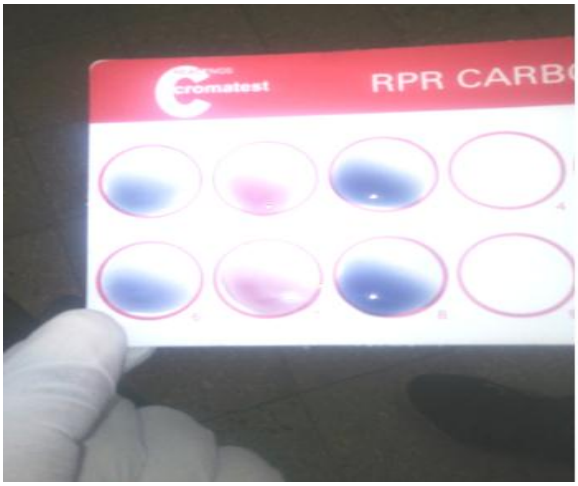
**Mekdela woreda** - Is segments of South Wollo Zone and compartmentalized into 29 kebeles.

***Salmonella enterica* subsp *enterica*, serovar typhi** -Causative agents of typhoid fever.

**Risk factors** -Any factors or conditions exposing individuals for typhoid fever positive

**Prevalence** -Is simply the proportion of individuals with typhoid fever positive in a population.

**Annex 6 picture of sample collection and facilities of the laboratory in MHC**




## Annex 7: Ethical clearance

ቀን 27/12/2008

ቁጥር SF/ZS/2241/08/2016

ለሚመለከተዉ ሁሉ

ጉዳዩ፡- ትብብር ስለመጠየቅ

ተማሪ _____ በአዲስ አበባ ዩኒቨርሲቲ የZoological Science ት/ክፍል የከረምት መርሀ ግብር የባዮሎጂ MSc ተማሪ ሲሆኑ በ2009 ዓ.ም. የMSc thesis ምርምር የሚያካሂዱ በመሆናቸዉ በመ/ቤትዎ በኩል አስፈላጊዉ ትብብር ይደረግላቸዉ ዘንድ በትህትና እንጠይቃለን፡፡

ከሰላምታ ጋር

የዞሎጂካል ሳይንስ ትምህርት ክፍል

## 10. Declaration

I, the undersigned, declare that this Thesis is my original work and all source materials used are duly acknowledged.

Name Alebachew Ali

Signature _____

Date _____

## 11. Statement of the supervisor(s)

This Thesis has been approved for submission to the Department of Zoological Sciences for public defense.

Name Hassen Mamo (PhD)

Signature _____

Date _____