

Seroprevalence of Typhoid Fever among Subjects with Acute Febrile Manifestations at Tertiary Care Center, Addis Ababa, Ethiopia

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Abstract: *Background: Typhoid fever is a life-threatening systemic disease characterized by stepwise rise of fever (38-41°C) and frequent abdominal manifestations. It continued to be a major endemic disease in developing countries where there are unsanitary practices. The reports of global burden of typhoid fever suggested that up to 16 to 22 million cases and 200 000 to 600, 000 deaths occur each year. Over past decades varying trend observed in developing countries, which reported of having an average incidence of 540 per 100, 000 populations. In Ethiopia, the definite diagnosis of typhoid fever by isolating the organisms is not normally done because of lack of bacteriological facilities and longer time required. However, the sensitive rapid slide agglutination tests alone, mostly without tube dilution method, routinely performed to detect presence of antibody production against typhoid fever. Here, we determined the seroprevalence of Salmonella Typhi from patients suspected of typhoid fever at the International Clinical Laboratories, Addis Ababa, Ethiopia. Result: Over five years, a total of 5, 029 patients with suspicion of typhoid fever were serologically diagnosed in the International Clinical Laboratories, Addis Ababa, Ethiopia. Of those patients, smaller numbers (43%) were females with 17% Widal positive and greater numbers (57%) were male with 12 %Widal positive. The age of patients distributed from less than a year to eight seven years and mean age was located at 33.39±14.72 years [95% CI]. Approximately 22% of patients had significant titer equal to 1 in 20 or more for H and/or O agglutinins corresponding antibody presence in serum. Of widal tests, 8.26% had significantly reactive antibody against H and O antigens whereas 6.7% and 5% were significantly reactive for only H and O antigens, respectively. The significant titer level indicative of typhoid diagnosis was equal to 1 in 80µl or more in both H and O agglutinins. Conclusion: Widal slide test is non-specific test that need additional confirmatory tube tests and bacteriological isolation of Salmonella Typhi. Hence, seroprevalence report may overstate or understate estimate of typhoid fever.*

Keywords: Seroprevalence, Typhoid fever, Salmonella typhi, febrile illness, Widal test, Bacteriological methods, cut values

1. Introduction

Typhoid fever continued to be global public health problem in developing countries from thousands of years back. It is estimated that upto 16-22 million cases of typhoid fever and 200, 000-600, 000 deaths reported globally each year.^{1, 2} In industrialized countries like the United States and UK, typhoid incidence is equal or lower than 1 in 100, 000 populations (\approx 300 clinical cases per year) and traveler-related cases comprise significant proportion of report.^{3, 4}

Unlike developing countries, the disease burden is still significant and typhoid fever remains one of the main concerns in developing countries.^{5, 6} According to health information report of 1994, approximately estimates of 2, 655, 000 typhoid cases and 130, 000 deaths with incidence rate of 500 cases per million populations were occurred in Africa whereas estimates of 13, 310, 000 typhoid cases and 440, 000 deaths with certain peak incidence of 1000 cases per million populations were occurred in Asia. In same year, estimates of 595, 000 typhoid cases and 10, 000 deaths with incidence of 150 cases per million populations were occurred in Latin America. In that report, the incidence estimates of typhoid fever in developing countries varied from 150 cases per million populations per year in Latin America to 1000 cases per million populations per year in some Asian countries. In 2000, an average incidence of Typhoid fever was 540 in 100, 000 populations in developing countries.⁷

The disease affects all age group with varying severity and incidence rate. However, the estimates of illness burden are highest among infant, children, and adolescent age categories in south central and Southeastern Asia.⁸ In global context, children easily develop typhoid fever infection compared to other age group, although the clinical course is generally milder in children than in adults. Typhoid fever has no race predilection but attacks males more than females. In certain literature, the Salmonella typhi case fatality rate vary in range of 10-30% but effective thepeutic measures can reduce to <4%.⁹

2. Methods and Materials

2.1. Study Design and Area

A retrospective data analysis was carried out to determine seroprevalence of typhoid fever at the International Clinical Laboratories. Electronic data of patients with suspicion of typhoid fever over the five years were retrieved from ICL databases in Polytech technology software and collected using standard format. The Headquarter of the International Clinical Laboratories, in which this study was carried out, is geographically located in Addis Ababa that provides more than 200 laboratory tests for across country clients, mainly from Addis Ababa and surroundings (Figure 1). The ICL is a private higher Laboratory facility established in 2004, now it has expanded its facilities to four regional towns of Ethiopia. It is running its full 24 hours services per day with about 90 employees working in three shifts. So far, it has

established only two big collaborative partners: Bioscientia-Germany and LabCorp-USA. Among East Africa Medical Laboratories, this is the only accredited by the Joint Commission International.

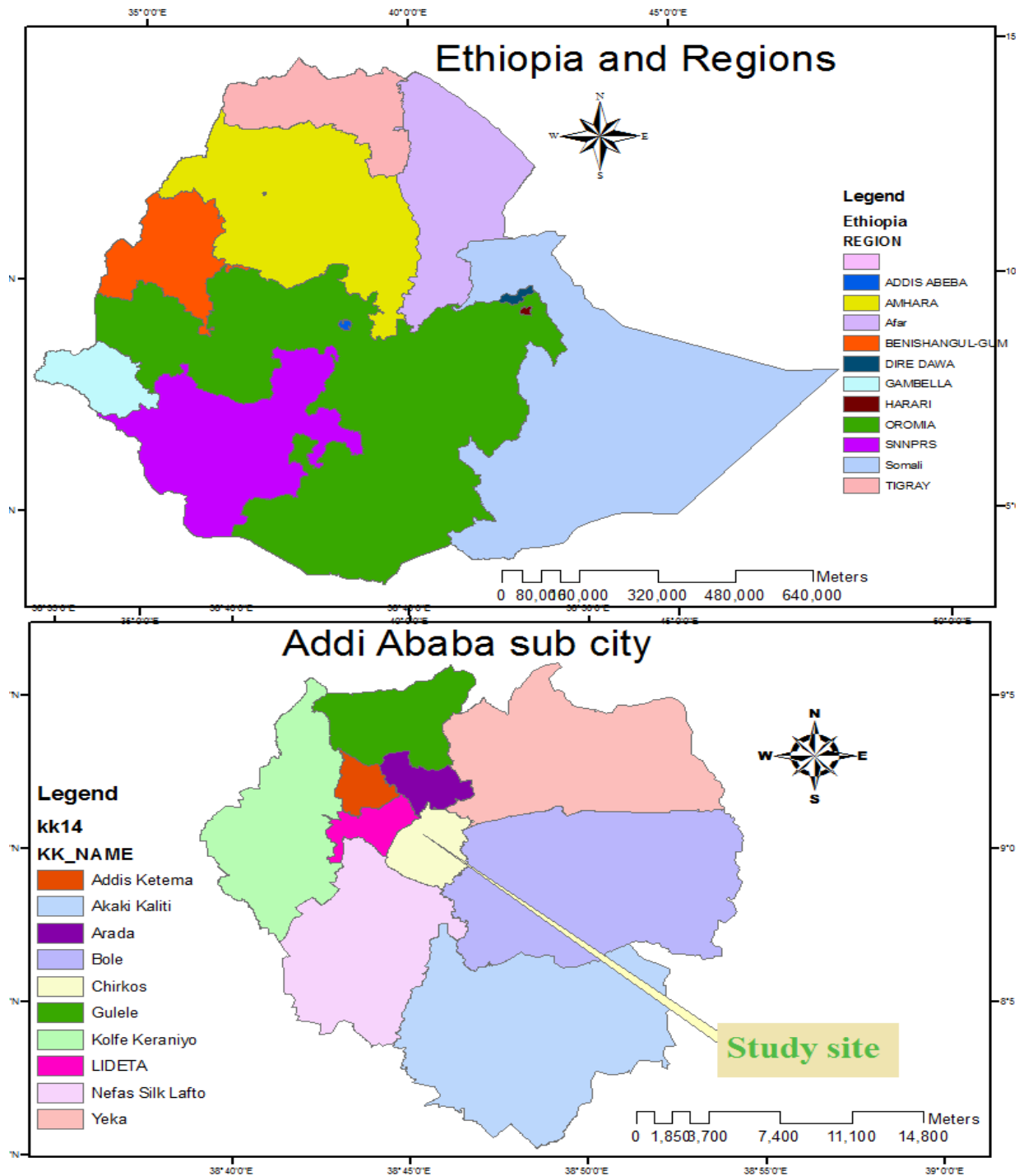


Figure 1: Geographical location of study site, Addis Ababa

2.2. Data Collection

The data collection activities were being run from Dec 29, 2011 to Feb 10, 2012. Data of all patients who serologically diagnosed from January 2007 to December 2011 was included in this study. Those populations in study area, Addis Ababa region, based on their choice or might be directly referred to make serological tests.

Necessary data from Polytech Technology retrieved and recoded into statistics that suggest epidemiological characteristics of typhoid fever. Widal test of patients with Age, Sex, Aetiologic agents, and seasonal distribution data was extensively employed in this study. Serum activity that produced an agglutinin titer greater or equal to 1 in 160 μ l was suggestive of typhoid diagnosis but all data from 1 in 20 μ l to 1 in 1280 μ l were collected.

According to national guideline, the same cut point value both for somatic O antigen and flagellar H antigen agglutinin was used frequently. Always slide screening test converted into equivalent tube dilution titer and only slide agglutination test result was collected.

2.3. Sample Collection and Materials

The blood sample was collected mainly in head branch but a few proportion transported from regional sites. The sample was collected in sterilized sample separator tube (SST) and allowed to clot. Then, fresh sera were separated from whole blood by centrifuging at 1000 rpm for 10 minutes at room temperature and kept at 2-8°C temperature away from direct sunlight until time of titration. Serum for shipping always stored at 2-8 °C temperature or frozen at -20 °C temperature away from direct sunlight and let these sera be attained to room temperature before tests. Samples collected and transported to laboratory should be evaluated according to established criteria before they were tested. It should be free of hemolytic reaction, clotting, lipemic (after high speed centrifugation), Icteric and so on. It should also be sufficient for test and loaded with proportional sample to anticoagulant ratio. Samples that didn't fulfill these criteria automatically rejected.

Bio-merieux technology product, standard HumaTex Febrile Antigens commercial kit (PH=8.2±2), was used for determination of antibodies against febrile illnesses in freshly processed serum or preserved serum with 0.095% sodium azide until time of tests. The salmonella kit 50301 with package size of 8×100 tests specifically designed for slide screening test and confirmatory tube titration of enteric fever-causing pathogens, usually Salmonella Typhi and Salmonella Paratyphi A, B and C. The kit contains salmonella species, vitally stained, inactivated with formaldehyde (0.5%) or phenol (0.5%) and standardized antigen suspensions. The blue stained antigens are specific to the somatic "O" antigens but the red stained antigens are specific to the flagellar "H" antigens. Polyvalent goat antiserum with red cap against antigens for positive control and non-reactive goat serum with blue cap were also included in the kit reagents (see details in Annex).

2.4. Data Analysis

The data were entered electronically into Epidata version 3.1 and statistical analyses were performed with STATA 11 for windows version. Independent continuous variables were statistically analyzed by using mean calculation with corresponding 95% confidence interval. Pearson correlation calculation also used to measure association of explanatory and outcome variables. Frequency analysis was appropriate statistics for most study variables which was applied extensively. In general, a vast majority of variables were analyzed descriptively and some were analyzed inferentially so that generalizable statistics could be drawn for study populations.

2.5. Ethical Statement

The ethical clearance was obtained from the Institutional Review Board of Aklilu Lemma Institute of Patho-biology before this study conducted and additional from the ICL. Only patient ID number was taken by anonymizing the data during collection activity. Since secondary data was collected, individual consent wasn't possibly made for but data were handled confidentially in processing. Also these data handled by researchers and never shared with third parties.

3. Results and Discussion

3.1. Epidemiological data

A total of 5, 029 patients were serologically diagnosed for typhoid fever between January 2007 to December 2011 in the International Clinical Laboratories. Greater proportion of the patients was residents of Addis Ababa and surrounding community. Young adults represented the largest number of patients and mean of age was 33.89±14.72years [95% CL]. The analysis also showed that of patients, adolescent and adult of age category between 20 to 30 years (32.08%) were the highest in number. Next, approximately 25% of patients were from age category of 30 to 40 years. Generally, the number of patients was consistently decreasing towards lower age and greater than fifty age group. So, data from the study suggested that the people of age category between 20-40 years were dominantly affected by typhoid infection, unlike school-aged children and older age people (See Figure 1& 3).

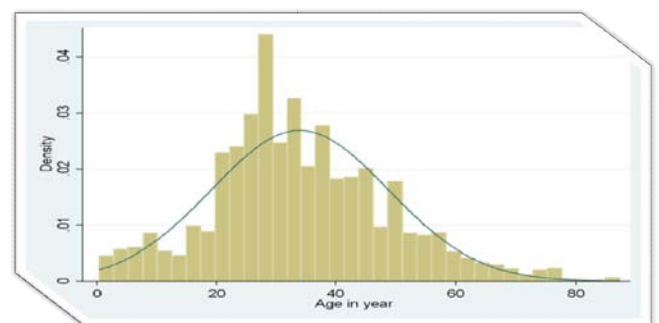


Figure2: Age distribution of patients from 2007 to 2011

Of typhoid suspected, greater numbers were male population (57%) and fewer numbers were female population (43%) even though a few sex data were not recorded. Throughout five years for all ages, male patients consistently remained higher in number than females and their number proportionally varied with respect to seasons of year. For instance, the sex-specific analysis showed that in patients of 2007 there were 38% females and 62% males but in 2010 there were 42% females and 58% males. The number of female patients was decreasing constantly at extreme age categories but form bell-shape at middle age category, peaking at 33 years. The spearman correlation analysis showed weak association of sex and seasons (Pearson chi2 (22) = 30.8527; P-value = 0.099 and 95% CL). Like female, the number of males was also constantly decreasing towards extreme age categories, corresponding to seasons of year that affect number of

patients. The number of male patients proportionally rose to peak points and fallen down to trough points in response of seasonal variation. Also no association of male sex and seasons each year was observed in spearman correlation calculation. However, the typhoid disease was strongly affected by sex distribution of patients in all ages (Pearsonchi2 (4) = 45.2151; P-value ≤ 0.001) (See Table 1, Figure 1& 2).

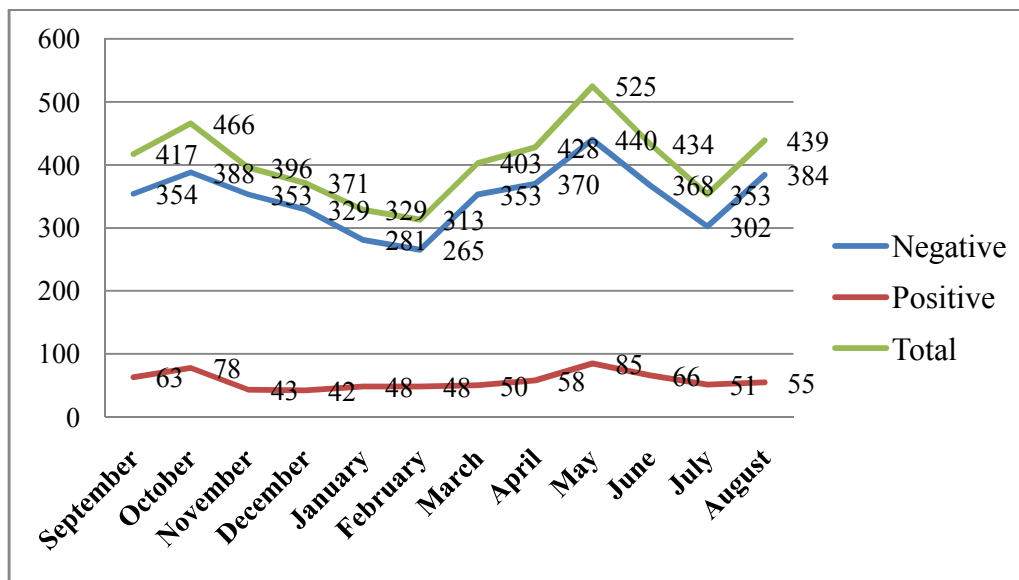


Figure 3: Typhoid fever diagnosis in each months from 2007 to 2011

Even though the occurrence of typhoid suspected patients remained high throughout each year, prominent seasonal pattern variation was detected. During that period, the distribution of the patients was being varied with respect to seasons of the year showing significant influence of weather of a day. Generally speaking, the number of patient for Widal test increases comparatively in autumn and spring seasons with peak incidence in May and October. Starting from late autumn, the typhoid suspected patients were decreased in number to trough point in middle of all winters, February (6.37%); the positivity rate was proportionally varying with that of number of patients. Gradually, before start of rainfall the patients number was rising that by far peaked in May (10.78%).Over the summers, the significant rise in number was observed after once again fall in July, which remained high in autumn with second larger peak in October. In addition, there was relatively significant change in number from 2007 to 2011, higher being observed in later years. Out of total populations, 21.6% of study populations were serologically diagnosed for typhoid fever in 2011 as compared to 12.5% populations in 2007(See Figure 2).

Table 1: Distribution of patients in five years stratified by gender

Year	Sex		
	Female (%)	Male (%)	Total (%)
2006	5(50)	5(50)	10(100)
2007	240(38)	390(62)	630(100)
2008	358(43)	478(57)	836(100)
2009	506(47)	570(53)	1,076(100)
2010	575(41)	813(59)	1,388(100)
2011	459(42)	627(58)	1,086(100)
Total (%)	2,143(43)	2,883(57)	5,026(100)

Table 2: Typhoid fever diagnosis against sex

Diagnosis	Sex		
	Female (%)	Male (%)	Total (%)
Positive	348(51)	338(49)	686(100)
Negative	1,731(421)	2,455(59)	4,186(100)
Total (%)	2,079(43)	2,793(57)	4,872(100)

3.2. Widal Test

Over five years, a total of 5, 029 patients tested serologically for typhoid fever. Generally, the data from typhoid serology showed that 1, 071(22%) sera had

agglutination reactions which was 3 fold higher compared to 3, 803(78%) sera that had no agglutination reaction. Of those, 1, 540(43%) represented female populations which were less in number but slightly found more widal positive 338(17%) whereas 2, 259(57%) represented male populations which were higher in number but found less widal positive 348(12%) (Pearson's chi-square (4) =45.2151;p-value ≤0.001). The age pattern analysis (Figure 3) showed that patients suspected of having

typhoid fever were highest among populations of age between 20 to 30 years 1, 403(32%) followed by 30 to 40 years 1, 078(25%). But populations older than 70 years, 61(36%) and 9(33%) of patients with positive sera were from age categories of 71-80 and 81-90 years, respectively, comparatively sera from these age categories tended to be more positive than others (Figure 3).

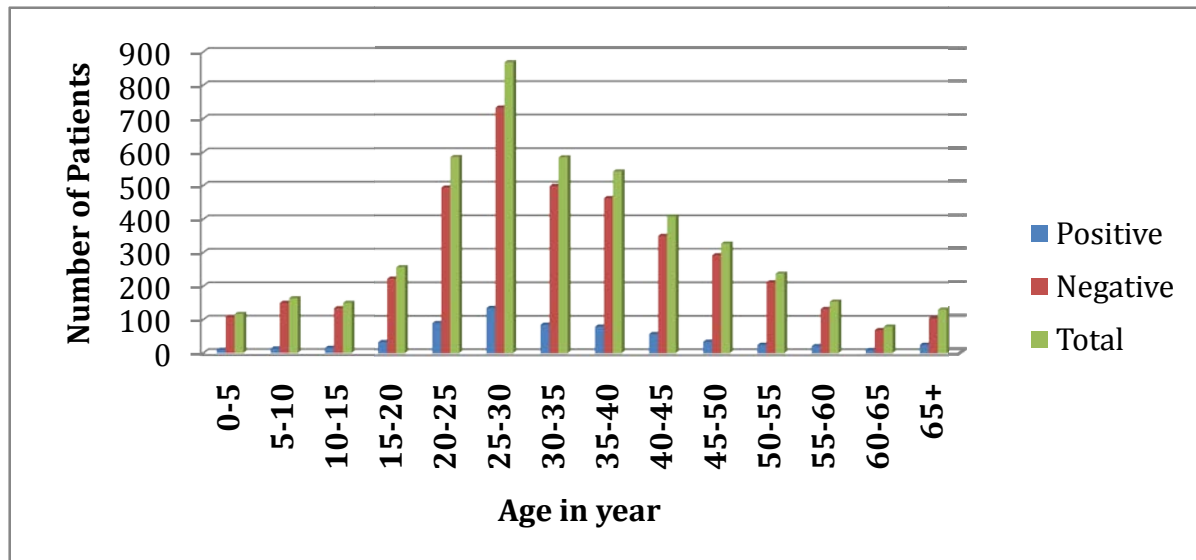


Figure 4: Age distribution of Widal positive and negative patients from 2007 to 2011

Throughout five years, sera from every age categories were found positive for typhoid fever with varying value and frequency. The sera examination produced positive result, either both positives or positive for only “H” or “O” agglutinin, subjected to serial dilution methods to determine antibody strength. At the end of titration, the level of agglutination reactions was not ordinarily categorized as low, medium, high, very high with corresponding percentage of 25%(+1 Grade), 50%(+2 Grade), 75%(+3 Grade) and 100%(+4 Grade). Both “H” agglutinin and “O” agglutinin titers ranged from 1 in 2µl for slide titration and 1 in 20µl for tube titration to 1 in 128µl for slide titration and 1 in 1280µl for tube titration against suspended antigens for Salmonella typhi. Relatively higher titration values were frequently recorded in titration against “H” agglutinin determination than titration against “O” agglutinin determination, being the highest value of H agglutinin titer was 1 in 1280µl but the most frequent record was 1 in 8µl for slide titration and 1 in 80µl for tube titration(Table 2&3).

The data from titration result showed that 389(8%) of “O” agglutinin and 336(7%) of “H” agglutinin titers were 1 in 80µl that was the most frequent titer. Next to this, larger proportion of the test results was 1 in 40µl which accounted for 279(5.7%) of “H” agglutinin and 209(4.4%) of “O” agglutinin tests. Significant proportion of tests was also 1 in 160µl that accounted for 98(2%) of “H” agglutinins and 83(1.7%) of “O” agglutinins tests. Patients’ sera were slightly more reacted to “H” agglutinin and immunologically responded than “O” agglutinin. With consideration of typhoid endemicity in Ethiopia, The

national cut value to determine evidence of antibody presence against Somatic and Flagellar antigens of Salmonella typhi was 1 in 80µl. of all, 4.37% of sera examination produced positive to “H” antigens were 1 in 160µl in 2.45% females and 1.65% males, 1 in 180µl in 0.05% females, 1 in 320µl in 0.1% females and 0.07% males and 1 in 1280µl in 0.05% females.

But for O antigens, significant titer were 1 in 160µl in 2.3% females and 1.3% males, 1 in 180µl in 0.05% males, and 1 in 320µl in 0.04% females and 0.05% males. The titers were almost found equivalent for the two antigens, 15.47% of sera titers were identical but highest titer was found in titration against ‘H’ agglutinin. Might be due to differing in clinical course of typhoid fever and past history of exposures, three categories of seropositive patients in Widal tests were diagnosed. Sera significantly reactive to ‘H’ and ‘O’ antigens, only H antigen reactive or only O antigen reactive were observed.

Data from titer analysis showed that 11.3% of sera contained significant antibodies reactive against H and O antigens, which suggested of typhoid fever diagnosis. None of these sera, 2.8% of sera which suggested presence of antibody against typhoid fever reported of having corresponding antibody against only H antigen or O antigen (see Table 2). Generally speaking, sera of females were found more positive to rapid slide agglutination throughout five years with highest titer was observed in young adult and lowest titer in extreme ages. In the study, the highest titer was observed in females with age category between 20 to 30 years. Most frequently,

females had higher titer than males did but no pronounced difference was encountered. The data of male sera showed no higher titer compared to female populations, greater proportion of titers was low. Generally, not only the titer

but also the reactivity of sera was higher in between 20 to 40 years but decreasing towards extreme ages.

Table 3: Cross-tabulation for ‘H’ and ‘O’ Agglutinin titers

Titer		‘O’ Agglutinin (%)								
		1:320	1:180	1:160	1:80	1:60	1:40	1:20	NG	Total
‘H’ Agglutinin	1:1280	0	0	0	1(100)	0	0	0	0	1(100)
	1:320	1(25)	0	1(25)	0	0	0	0	2(50)	4(100)
	1:180	0	0	0	0	0	0	0	1(100)	1(100)
	1:160	0	0	26(27)	30(31)	0	11(11)	0	30(31)	97(100)
	1:80	0	1(0.3)	24(7)	142(42)	1(0.3)	27(8)	2(0.6)	139(41)	336(100)
	1:60	0	0	0	0	3(33)	1(11)	0	5(56)	9(100)
	1:40	0	0	16(6)	79(28)	1(0.4)	82(29)	5(2)	96(34)	279(100)
	1:20	0	0	0	3(3)	0	9(10)	27(30)	52(57)	91(100)
	NG	1(0.02)	0	16(0.4)	134(3)	4(0.1)	79(2)	28(0.7)	3, 793(94)	4, 055(100)
	Total	2(0.04)	1(0.02)	83(2)	389(8)	9(0.2)	209(4.3)	62(1.3)	4, 118(84)	4, 877(100)

NB: NG = Negative Test

Table 4: Two way frequency distribution of ‘H’ Agglutinin titer by sex

Sex	H Agglutinin titer									
	1:1280	1:320	1:180	1:160	1:80	1:60	1:40	1:20	NG	Total
Female (%)	1(100)	2(50)	1(100)	50(52)	167(50)	6(67)	147(53)	45(49)	1660(41)	2, 081(43)
Male (%)	0	2(50)	0	47(48)	169(50)	3(33)	132(47)	46(51)	2395(59)	2, 796(57)
Total (%)	1(100)	4(100)	1(100)	97(100)	336(100)	9(100)	279(100)	91(100)	4, 055(100)	4877(100)

NB: NG=Negative test

Table 5: Two way frequency distribution of ‘O’ Agglutinintiter by sex

Sex	O' Agglutinin								
	1:320	1:180	1:160	1:80	1:60	1:40	1:20	NG	Total
Female (%)	1(50)	1(100)	47(57)	207(53)	4(44)	102(49)	28(45)	1, 688(41)	2, 080(43)
Male (%)	1(50)	0	36(43)	181(47)	5(56)	107(51)	34(56)	2, 429(59)	2, 796(57)
Total (%)	2(100)	1(100)	83(100)	388(100)	9(100)	209(100)	62(100)	4, 117(100)	4875(100)

NB: NG=Negative test

4. Discussion

Typhoid fever is endemically posing significant health problem in developing countries where there are high risk factors. Likewise; in Ethiopia, typhoid fever is considered to be highly endemic and represented by high morbidity and mortality each year. In this study, 14.1% of sera examinations were observed to have significant titre equal to or greater than 1 in 80µl that was considerable serological estimates. Serological estimates for the five years significantly increased from 2007 to 2011 years showing high seroprevalence of typhoid fever in study area. Many observations reported earlier from Ethiopia showed that typhoid fever estimates in bacteriological tests were much lower than our findings.¹⁰

The data from this retrospective study perfectly suggested that typhoid fever was still known to cause considerable health problem in the populations, especially highest at young adult age category. However, findings of institutional based prospective observation in 2010 from Nigeria showed significantly higher serological estimates compared to this study.¹¹ National infectious diseases surveillance system are not well coordinated and targeted to continuously monitor notifiable diseases in Ethiopia. In addition, retrospective and prospective research studies were not providing sufficient and consistent data in past years on typhoid fever, even few literatures conducted were published in local journals that created difficulty to review. As a result, sufficient data from all regions were not available to observe trend of serological and bacteriological estimates of typhoid fever. National center for diseases intervention programs information is also not accessible to have crude estimates; thus, why this

observation faced many limitations to compare and contrast findings.

Many reports suggested that no advancement in standard of health infrastructure was made in Ethiopia as effective diagnostic techniques have not been applied. So, the most practical diagnostic techniques for Salmonella Typhi in all facilities is single rapid slide agglutination tests from several years back. The wide use of and popularity of this single rapid agglutination tests result from its ease of performing (need no special skill and equipment supplies), cheap, availability, and shorter time to yield result. Despite of these benefits, Widal tests have inherent limitation because it is unable to identify acute infections and on top of that it cross-reacts with related antigenic determinants of Salmonella species.

People who live in typhoid endemic area supposed to have background antibody due to repeated infection with Salmonella species and/or Salmonella Typhi that can react with HamaTex febrile antigens package. In most literature, it has been reported that high sensitivity and low specificity of Widal test kits has resulted in high false positive and mistreatment, particularly in developing countries. As a result, most developing countries have made Widal test positive cut-off titre guidelines for O and H agglutinins level both in slide and tube agglutination techniques except a few relying on level of endemicity, immunization with typhi vaccine and commercial kits package standards.¹²This way of determining significant titer for Widal test differs from classical method where fourfold rise of antibody in paired sera from 7-10 days was sufficient indicative of typhoid diagnosis.¹³In case of single Widal tests reading also, fixed diagnostic significant level for O or H agglutinins were equal to 1 in 160 μ l or more and 1 in 180 μ l or more respectively in classic times. Based on set guidelines health professionals are able to determine significant titre objectively, deciding positive or not to Widal tests.¹³

In Ethiopia, however, cut points for minimum criteria for serodiagnosis of typhoid fever are not nationalized to have common understanding and interpretation of Widal test results conforming to manufacturer instructions. In Ethiopia, two studies reported that significant titre for sera examination for typhoid fever should be equal to or greater than 1 in 160 μ l and 1 in 320 μ l, respectively for H and O agglutinins.^{14, 15} Differently, these findings are not in agreement to our study in which the O and H agglutinins titre in patient serum, usually 1 in 80 μ l or greater, were considered as significant titre to suggest typhoid diagnosis positive. In present study, reports were being made on basis of identical cut-off titer both in single slide and tube agglutination techniques which might yield different titers and Widal reading was inter-translatable. The data from our study indicated that the titer significance should also be re-determined from clinical diagnosis compatibility with typhoid fever, history of antibiotics, and number of visit with same health problem. So, titers from minimum detectable evidence of antibody production 1 in 20 μ l through highest value were reported. As already stated above in our study, sera examination in which significant agglutination reaction produced titre

equal to or greater than 1 in 80 μ l for "H" and for "O" antigens were considered sufficient suggestive of typhoid fever diagnosis. These observations were almost consistent with what was suggested by many previous studies in Africa countries as well as in oversea countries. In most countries, the minimum significant titre for Widal test should be equal to 1 in 160 μ l or more both for H and O agglutinins.¹²However, fewer countries set lower minimum titre for serodiagnosis of typhoid fever by considering population background immunity.¹²

Few literatures also reported their observation on diagnostic significance of either raise in IgM flagellar H or IgG somatic O agglutinin, or presence of only H or O agglutinin in Widal serological tests. Some literatures give greater emphasis in raise in H agglutinin than in O agglutinin but other literatures give significant diagnostic value in reverse of that and no consensus as it differs from study to study (Pang, 1982).¹⁶However, my findings suggested that detection of only H and/or O agglutinins or raised level of either O or H agglutinin resulted from clinical courses of infection and time duration. In infection process, agglutinins usually appears in first few days of Salmonella Typhi infection and H agglutinin always appears in late course of infection, lasts longer and mostly observed in chronic typhoid patients in whom O agglutinin disappeared(Okonko et al., 2010).¹¹As far as we are concerned with which of H or O agglutinin gives high diagnostic value, we should look into serotype specific H agglutinin than serogroup specific O agglutinin and absence of phase II flagellar H antigens in Salmonella Typhi also narrows down false positive result.

In many developed and developing countries where bacteriological facilities are available, widal test positive serum are subjected to further definitive diagnosis of Salmonella Typhi by blood culture and molecular techniques. Whereas only single slide agglutination Widal test aids health professional to select antibiotics of choice that potentially lead to misdiagnosis in this study. Routinely further specific diagnosis is required because of the fact that only 18-30% Widal test positive patients will be confirmed of having true diseases(Dayrit, 1992).¹⁷Many professionals stated that large proportion of patients with sera positive to Widal test always bacteriologically proved to be free of diseases. That was still big downsides in tracing level of diagnostic value of Widal test commercial kits and trend of typhoid fever. Even though the test kits parameters are being gradually improved, the Widal test critics are growing more and more.

In most typhoid fever endemic countries, typhoid infection suspicion and isolation of organisms are observed throughout the year, peaking from July to September when heavy rain occurs in report of Okonko et al. (2010).¹¹Similarly, new incidence of Typhoidal Salmonellosis occurred year round in Ethiopia that contrast with many observations in peaking seasons. The level of occurrence of typhoid fever in every month is considered to be significant but peak incidence of typhoid fever and sera positive were observed in May and October. Typhoid fever equally affects both sexes

everywhere but occasionally males are more affected by infection due to travel. Eventhough both sexes are equally susceptible to typhoid fever infections, sometimes sera of males are found more positive than females are in widal examination (Okonko et al., 2010).¹¹Our observations contrast another in that sera of females were more positive to slide agglutination tests compared to males in this retrospective study. The difference in our findings might be resulted from behavioral factors of sexes and/or geographical variability (Figure 3).

Many previous studies reported incidence of typhoid fever in three countries where it was known to be endemic.¹⁸,¹⁹In their report Salmonella Typhi isolates were very common in age less than 10 years in three of them and in Indonesia and New Dehli, children less than five years were more susceptible to typhoid fever than adult populations. But typhoid fever incidence rise in school-aged children compared to lower and higher age categories throughout five years in Chile and Vietnam. Different from these, Widal seropositivity rate of this study rise in age between 20-40 years and falls otherwise. Eventhough tests with varying diagnostic value are correlated, our findings are not coinciding in peak positive result distribution in all age groups of different countries. Many studies result shows that age pattern of typhoid fever almost consistent with one another in worldwide and not attack only specific age group. In other words, general populations where underlying causes largely exist are at high risk of having typhoid fever out of age predilection (Singh, 2001).

5. Conclusion and recommendations

In conclusion, serological estimate of typhoid fever was considered to be significant among study populations involved and had prominent epidemiological profiles in Ethiopia. Widal agglutination test has long been used for diagnosis of typhoid fever from suspected febrile patients. Still it is widely used over all part of Ethiopia as it is most practical laboratory tool but reading of Widal test result varies greatly. Rapid slide quantitative test is non-specific type of serological tests in population with unknown baseline titer and superimposed by typhoid fever endemicity. The background antibody in Ethiopian populations has not been yet determined to know diagnostic reliability of Widal test and also non-typhoidal Salmonella species serogroup A, B, and D which have O antigenic determinants are very common. Attention should be given as in most patients immunity production impaired or delays due to host factors and history of antibiotic initiation that lead to false negative. As well, laboratory confirmation of rapid slide test positive by isolating diseases-producing organisms and/or Widal tube confirmatory test should be performed to definitely diagnosis typhoid fever.

Scientifically it is proved that in apparently health person serum titer can rise up to 1 in 60 μ l due to history of immunization with TAB vaccines, Malaria infection, and other febrile illnesses. Many reports of study involving slide semi-quantitative test agree with cut-off titre at equal to 1 in 80 μ l or more but cut-off titre equal to 1 in 160 μ l or

more used second to that titre. Titers of agglutination greater than 1 in 80 μ l to 1 in 160 μ l with tube test or 1 in 8 to 1 in 16 μ l with the rapid slide tests are usually significant indicative of an acute infection. The detection of specific antibodies produced against febrile antigens clearly suggests valuable diagnostic information on potential disease. In unpaired serum slide agglutination test, the final diagnosis whether widal positive or negative, however, should take into consideration the patient's history, antibiotic administration, and additional clinical investigations. Generally speaking, fourfold or more rise of H or O agglutinins in paired sera of 7-10 days apart is strongly indicative of typhoid fever and more meaningful than single slide agglutination test.

Data from this study showed that typhoid fever is still a major public health problem next to malaria among febrile illnesses in Ethiopia. The findings really inspire further studies to make sure the epidemiological patterns and level of occurrences of Typhoid fever are consistent throughout regions. Further studies also should focus largely on test validies by incorporating multiple laboratory tests. As it is highly affecting school-aged children and young adults who are not fully developed resistance against invasion of organisms and productive populations respectively, greater emphasis should be given to improve per capita water supply and sanitary standards. To fully protect community, it would be better if immunization programs are given as supplementary measures to sanitary standards all over regions. Other important measures to consider are regular control of food establishment and implementation of integrated sanitary control programs in the areas.

6. Acknowledgement

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7. Authors' Contribution

Author endeavored to this thesis research from proposal to manuscript preparation.

8. Conflict of Interest

The authors declare that there is no any conflict of interest.

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Annex: Serological Procedures

Bio-merieux technology product used for both slide agglutination and tube titration tests in this laboratory. The serum of patients that well processed is tested for detections of evidence for presence of antibody against febrile pathogens. The antisera of patients that mounted against somatic O antigen and flagellar H antigens are measured by their agglutination reactions. Each slide

screening test, and confirmatory tube titrations are performed in specific procedures as follows.

A. Slide qualitative test

One drop (50 µl) of Serum sample was placed into individual cells of slide with 6 reaction cells. Next, a drop of IgM somatic O antigen, IgG flagellar H antigen, and combined paratyphi A, B and C antigens were pipetted by micropipette into respective cells of slide and homogenized well with the serum by separate disposable applicator sticks. Finally, the glass slide placed on automated rotator with 80-100 rpm to rock kit reagent-serum complex solution. After approximately 1 minute of rotation, the agglutination pattern was observed under bright artificial light with naked eye. The semi-quantitative slide test resumed after observation of significant reactive antigens.

B. Semi-qualitative slide test

Serum dilution in physiological saline (9g NaCl/l) was performed to have serial dilution of 0.08ml, 0.04ml, 0.02ml, 0.01ml and 0.005ml. Against serial dilutions a drop of antigens was pipetted into six reaction cells slide and quantification process repeated for every reactive antigens. The reagents and serum were well homogenized with separate disposable applicator sticks. Finally, the glass slide placed on automated rotator with 80-100 rpm to rock kit reagent-serum complex solution. After approximately 1 minute of rotation, the agglutination pattern was read under bright artificial light with naked eye.

C. Interpretation

Within 1 minute, the characteristic pattern of H and O antigens agglutination for Salmonella Typhi and Salmonella Paratyphi were examined under bright artificial light with naked eye. Usually, the somatic O antigens reaction was identified as coarse, compact agglutination that don't easily be dispersed. In case of flagellar H antigen, the loose flocculent agglutination which suggests presence of corresponding antibody was reported. The titration reading was taken at highest serum dilution in which still reaction was visibly observed. The result was reported in tube equivalent dilution as 1 in 20, 1 in 40, 1 in 80, 1 in 160, and 1 in 320.