Original article

Seroepidemiological investigations on typhus in Mekele, Dessie and the nearby towns

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Abstract: A seroepidemiological study on typhus was conducted in Dessie, Mekele and the nearby towns. A total of 792 serum specimens were collected from patients with acute febrile illness who came to seek treatment in hospitals, health centres and malaria control centres. Also, 246 blood specimens were collected from apparently healthy individuals who donated blood to the blood banks of Dessie and Mekele. The specimen collection was performed during the rainy (July 31 - Sept. 3, 1993) and during the dry (May 21 - June 21, 1994) seasons. All specimens were tested by the WeilFelix test and the Enzyme Linked Immunosorbent Assay (ELISA) for IgM antibodies specific to *Rickettsia prowazekii*. The disease prevalence was significantly higher during the rainy season than the dry season. A Weil-Felix test positivity of 5.3% and 13.5% in Mekele (OR=2.78, P<0.05); 6% and 18.7% in Dessie (OR=3.36, P<0.0001) were obtained for the dry and the rainy seasons, respectively. Using the IgM ELISA: the rates for Mekele of 8.7% and 31.1% (OR=4.75, P<0.001) and for Dessie, 21.6% and 28.4% (OR=1.44, P>0.05) were found during the dry and rainy seasons, respectively. Among the various occupational groups, higher prevalence was observed in the student population (up to 36%). A general prevalence which ragned from 6% to 9% and 10% to 22% was observed in blood donors from the two towns by the Weil-Felix test and IgM ELISA respectively. The seroprevalence observed in the various groups, the seasonality of the disease as well as the importance of laboratory diagnostic methods have been discussed in relation to possible future outbreaks of epidemic typhus. [*Ethiop. J. Health Dev.* 1998;12(1):9-16]

Introduction

Rickettsial infections are prevalent throughout the world and cause serious diseases in humans. The genus Rickettsia usually are divided into the typhus, spotted fever and scrub typhus groups. The typhus group includes *Rickettsia prowazekii* which is the etiologic agent of epidemic typhus, *Rickettsia typhi* which is the cause of murine typhus or endemic typhus and *Rickettsia canada* which is a tick-borne species of unknown pathogenecity (1-4). In the past, different investigators have reported the presence of epidemic typhus, endemic typhus, Q-fever, trench fever and tick-borne typhus in Ethiopia (5-8).

Epidemic typhus, which is a human body louse-transmitted rickettsioses, is caused by *Rickettsia prowazekii*. The human body louse, Pediculus humanus, is the vector of the disease. Transmission of the disease does not occur due to the louse bite but rather by contamination of the bite sites with feces or crushed bodies of infected lice or by inhalation or innoculation of the conjunctivae with aerosolized feces. Since the lice die following infection with *R. prowazekii*, they can not serve as the reservoir of the disease. Humans serve as the reservoir of the disease which may relapse as Brill-Zinsser disease many years after the first infection (9-11). More recently, in the United States flying squirrels, *Glaucomys volans*, were found to be animal reservoir of a different variant of *Rickettsia prowazekii* (12).

Louse-borne typhus has been a significant cause of morbidity and mortality throughout ages especially during times of war or natural disaster. Historically, louse borne epidemic typhus was

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distributed world wide. But today, because of improved public health measures in most developed countries, the major foci of the disease remain in less developed countries in Africa, among native Americans and in the middle east and Asia.

In Ethiopia, the first typhus epidemic was reported in 1866 in army camps and prisons (13). A six-month survey in 1964 and 1965 on typhus in patients referred from various hospitals in Addis Ababa and the surroundings showed that 24% of the patients tested were positive for antibodies against *Rickettsia prowazekii, Rickettsia typhi* or *Rickettsia conori*. The prevalence of positivity was more or less evenly distributed in all the six months of the survey. Most of these results were based on convalescent sera on which micro agglutination test was used (14). The laboratory diagnosis of typhus is mainly done serologically. In Ethiopia, presently the diagnosis of typhus is made either using the Weil-Felix test which is based on the use of antigen suspension from *Proteus vulgaris* or only using clinical pictures after excluding malaria. However, in countries where alternative techniques are available, the Weil-Felix test is not recommended because of its inadiquate sensitivity and specificity (15). Currently available techniques for the diagnosis of typhus include Immunoflourescent Assay (IFA), Enzyme linked immunosorbent Assay (ELISA), Complement Fixation, Micro agglutination, DOT ELISA and also the recently developed Polymerase Chain Reaction (PCR) (16). A technique of shell vial culture centrifugation followed by monoclonal antibody staining has also been described recently for the rapid detection of the spotted fever rickettsia in blood culture (17).

According to two different publications of the World Health Organization on rickettsial diseases, Ethiopia is one of the three African countries where most cases of epidemic typhus were reported in the world during the period 1981 to 1984 (18, 19). However, there are only few Studies conducted on typhus in Ethiopia, and almost all of these studies were conducted in Addis Ababa.

We report here a seroepidemiological investigation on typhus conducted in Mekele, Dessie and the nearby towns. **Methods**

Serum specimens: Blood samples were collected, using evacuated blood collection tubes (Sherwood Medical, Bally money, North Ireland) and venoject needles, (Terumo Corporation, Tokyo, Japan) from acute febrile patients who came to seek treatment at the Mekele Hospital and health centre, Wukro Hospital and health centre, Quiha zonal Hospital, Lachi Clinic Mekele Malaria Control Centre, Dessie Hospital and health centre, Boru-Meda Hospital, Haik Health Centre and Kombolcha Malaria Control centre between July 31 - Sept. 3, 1993 (rainy season) and between May 21 and June 21, 1994 (dry season). Serum samples were separated the same day and stored in liquid nitrogen until analysis. A total of 792 specimens were collected from acute febrile patients from all the study participants during the dry and rainy seasons. In addition, 246 specimens were collected from those who donated blood to individuals at Mekele and Dessie Blood Banks during the study period. The sites in the nearby towns are located in a range of 10km to 48km away from the central towns. Patients from the nearby towns usually come to the centres in Mekele and Dessie for medical purposes and also in some cases patients living in the central towns go to the health institutions in the nearby towns. The distance of the nearby towns from the central towns is as follows: From Mekele, Wukro 48km, Quiha 10km, Lachi 5km; From Dessie: Haik 30km, Kombolcha 23km, BoruMeda 10km.

A questionnaire, which includes age, sex, address, duration of illness, housing condition and clinical history, was filled based on information obtained from each patient who is included in the study and with the help of medical personnel on site.

Serological analyses: All the specimens were tested by the Weil-Felix and enzyme Linked Immunosorbent Assay (ELISA) for IgM class of antibodies to *Rickettsia prowazekii*.

Weil-Felix test: a suspension of proteus OX-19 antigen, Omega diagnostics, UK., was used for the test. The test was run according to the instruction of the manufacturer. A two fold dilution of the specimen using saline (0.85% NaCl) was made in a micro titration plate wells and 5 μul of the antigen was added to each well. The plates were incubated at 37ºC overnight.

Agglutination was observed visually and the titer of the last agglutination was recorded. 1:40 and 1:80 were taken as indeterminate titers. Titers greater than or equal to 1:160 were considered reactive.

Enzyme linked Immunosorbent Assay (ELISA): Immunoglobulin M (IgM) capture ELISA: 96 well micro titration plates (Dynatech laboratories, Inc., Virginia, USA) were coated by adding 100

µul of 1:1000 dilution of anti human IgM (Kirkegaard & Perry laboratories, Inc., Maryland, USA) diluted in Phosphate buffered saline (PBS) pH 7.4 to each well and keeping the plates overnight at 4ºC. The plates were washed five times with PBS + 0.1% tween-20 and then blocked with PBS + 0.1% tween-20 + 5% skim milk for 30 minutes at 37ºC to avoid non-specific reactions. Then the following were added stepwise with incubation at 37ºC for one hour and similar washing between each step. A two-fold dilution of serum specimens; 1:500 dilution of Rickettsia typhi antigen; 1:1000 dilution of rabbit anti R. Typhi; 1:1000 dilution of horseradish peroxidase conjugated Goat anti-rabbit antibody and ABTS (2,2'-azino-di(3-ethyl-benzthiazoline sulfonate)) substrate. The optical density (OD) of each well was determined using a Multiskan spectrophotometer (Flow Laboratories, U.K.) at 405 nm against air as a blank. The cut off value was calculated by adding the mean of the ODS of the three negative controls plus 3 SD (20). Specimens with OD reading above the cut-off value were considered positive.

Data analysis: statistical analysis of results was performed by using EPI-INFO version 6 statistical package.

Meteorological information was obtained from the National Meteorological Service Agency.

Results

Duration of fever in the study population was recorded and it was found out that 67% of them had fever for seven or less days; 416 of the specimens from the febrile patients were collected during the rainy season, and the remaining 376 specimens during the dry season.

Table 1: **Results of Weil-Felix (OC-19) and IgM ELISA serology in patients with acute febrile illness.**

Town/Season		Weil-Felix test	IgM ELISA
Mekele and near by towns (NT)			
Dry season	No. tested	150	150
	Negative	107(71.3%)	137(91,3%)
	Suspected	35(23.4%)	
	Positive	8(5.3%)	13(8.7%)
Rainy season	No. tested	133	132
	Negative	80(60.2%)	91(68.9%)
	Suspected	35(26.3)	
	Positive	18(13.5)	41(31.1%)
Dessie and nearby towns (NT)			
Dry season	No. tested	218	218
	Negative	160(73.4%)	171(78.4%)
	Suspected	45(20.6%)	
	Positive	13(6.0%)	47(21.6%)
Rainy season	No. tested	283	284
	Negative	145(51.2%)	202(71.6%)
	Suspected	85(30.0%)	
	Positive	53(18.7%)	
Total		784	784

Climatological information for the study period

	Rainy season		Dry season	
	(July 31 - Sept. 3, 1993)		May 21 - June 21, 1994)	
	Mekele & NT	Dessie & NT	Mekele & Nt	Dessie & NT
MRF	106-107	151-229	0.8-68	37-86
MMₓT	22.9-26	26.7-27.1	26-30	28-31
MMT	12.6-13.1	13.6-14.6	11-13	12-16

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MRH	69	44-80	39	28-34

MRF, Monthly rain fall in millimetre; MmxT, Monthly mean maximum temperature in degree centigrade; MMT, Monthly mean minimum temperature in degree centigrade; MRH, mean relative humidity in percent.

   Patients with serological evidence for typhus presented to the hospitals within an average of 9.7 days (range 1-30 days) following the first onset of distinct symptoms. The clinical symptoms of the sero positive patients include headache (90%), generalized ache (80%), weakness (90%), chilly sensation (76.2%), malaise (51%), rash (6%) and others (vomiting 1, cough 2). Percentage of males among the acute febrile patients in the study sites ranged between 55% and 66% and the percentage of females ranged between 34% and 45%.

   Table 1 shows percentage positivity distribution by season obtained by the WF test and IgM ELISA among the febrile patients. Climatological information specific for the investigation period are also shown. Higher positivity rate was obtained during the rainy season than the dry season in both towns. A Weil-Felix positivity of 5.3% and 13.5% in Mekele (OR=3.36, P<0.05) and 6.0% and 18.7% in Dessie (OR=4.75, P<0.0001) was detected for the dry and rainy seasons, respectively. The IgM ELISA positivity of the dry and rainy seasons were 8.7% and 31.1% in Mekele (OR=4.75, P<0.001); 21.6% and 28.4% (OR=1.44, P>0.05) in Dessie, respectively. We were able to get residential addresses of 272 and 215 of the people tested during the rainy and dry seasons, respectively in Dessie and the nearby towns. IgM ELISA positivity was correlated with addresses (Table 2). As shown in Table 2, among the people residing in the nearby towns outside Dessie we

Table 2: **Distribution of IgM ELISA positive acute febrile patients by residence**

Season/Residence	No. tested	No. and % positives
Rainy season		
Dessie	69	17 (24.6%)
Outside Dessie	180	44 (24.4%)
Mekele	79	29 (36.7%)
Outside Mekele	36	8 (22.2%)
Dry season		
Dessie	92	31 (33.6%)
Outside Dessie	146	30 (20.5%)
Mekele	95	7 (7.4%)
Outside Mekele	94	4 (8.2%)

found a prevalence rate of 24.4% (44/180) during the rainy season and 20.5 (30/146) during the dry season whereas the prevalence in the people living in Dessie was 33.6% (31/92) during the dry season and 24.6% (17/69) during the rainy season (OR=1.01, P>0.05) for the rainy season; OR=1.97, P=0.034 for the dry season). Similarly, among the people tested in Mekele and the nearby towns, the prevalence rate during the rainy season was 36.7% (29/79) and 22.2% (8/36) respectively (OR=2.03, P>0.05). During the dry season the prevalence in these

areas was 7.4% (7/95) for Mekele and 8.2% (4/49) for the nearby towns (OR=0.89, P>0.05).   The association of antibodies to rickettsial antigen with gender, age and occupation was examined. Table 3 shows the distribution of IgM ELISA positivity by gender. The proportion of males in the study population was higher but there was no significant difference in the positivity rate between males and females.

Table 3: **IgM positivity by gender among patients with acute febrile illness**

Town/ Season	Males	Females	Total
Mekele and nearby town (NT)			
Dry season No. tested	80	70	150

Positive	6	7	
% Positive	7.5	10	
Rainy season No. tested	74	58	132
Positive	27	14	
% Positive	36.4	24.1	
Dessie and Nearby towns (NT)			
No. tested	30	88	218
Positive	28	19	
% Positive	21.5	21.5	
Rainy season No. tested	84	98	284
Positive	54	26	
% Positive	29.3	26.5	
Total	468	314	782

Table 4 shows the distribution of IgM ELISA patients by age and season. The number of acute febrile patients increased with age. Although it is not statistically significant the highest prevalence in Dessie and the nearby towns was observed during the rainy season in the 6-14 age group (37.5%) followed by the 15-20 age group (32.2%) (OR=1.25, P>0.05). Similarly percentage positivity was also higher in the 15-20 age group (40.9%) followed by 21-30 age group (37.5%) during the rainy season in Mekele and the nearby towns (OR=1.15, P>0.05).

Table 5 shows the distribution of patients by occupation. Students represented 22-35% (26.8%) of the patient population. This occupation group also showed the highest percentage of positivity. Although few in number are tested, high prevalence was also observed in the factory workers tested in Dessie during the rainy season.

Table 4: **Number and percentage IgM ELISA positivity by age, study area and season in patients with acute febrile illness.**

Age group(Years)	Mekele		Dessie		
	Dry	Rainy	Dry	Rainy	Total
2 - 5 No. tested	8	8	15	23	54
No. +ve	0	0	5	6	
% +ve	0	0	33.3	26	
6 - 14 No. tested	31	19	39	48	137
No. +ve	2	3	9	18	
% + ve	6.4	15.7	23	37.5	
15 - 20 No. tested	35	22	44	59	160
No. +ve	3	9	8	19	
% + ve	8.5	40.9	18.1	32.2	
21 - 30 No. tested	30	32	70	73	205
No. + ve	4	12	15	20	
% + ve	13.3	37.5	21.4	27.3	
31+ No. tested	46	51	50	79	220
No. + ve	4	17	10	17	
% +ve	8.7	33.3	20	21.5	
Total					782

Table 6 shows the seroprevalence in blood donors from Dessie and Mekele during the rainy and dry seasons. The Weil Felix positivity ranged from 6% to 9% whereas the IgM ELISA positivity,

ranging from 10% to 22%, was higher and comparable to the prevalence observed in acute febrile patients during the dry season. Among the blood donors 89% were males and 11% females (data not shown).

Table 5: **Occupation of IgM ELISA positive patients with acute febrile illness by season and town.**

Occupation	Mekele		Dessie		Total
	Dry	Rainy	Dry	Rainy	
Civil servant					
No. tested	8	13	23	15	59
No. Positive	1	1	2	7	
% positive	12.5	7.7	8.7	46.6	
House wife					
No. tested	30	33	18	19	100
No Positive	6	7	2	5	
% Positive	20	21.2	11.1	33.3	
Student					
No. tested	52	72	48	25	197
No. Positive	11	26	4	8	
% positive	21.2	36.1	8.3	32	
Others					
No. tested	96	101	27	29	253
No. Positive	25	26	3	8	
% positive	26%	25.7	11.1	27.5	
Total					735

Discussion

   In this study two different techniques (a Weil-Felix test which is based on a proteus antigen and an IgM capture enzyme immunoassay which uses a rickettsial antigen) were used. The prevalence detected is relatively higher by the IgM ELISA than the Weil-Felix test. This is in agreement with previous studies which have indicated that the Weil Felix test is less sensitive and can miss many cases (21). The same types of discrepancies were also observed in a study which compared the WeilFelix test and mico immuno fluorescence test in the serodiagnosis of rocky mountain spotted fever (22).

Table 6: **Seropositivity among blood donors in Dessie and Mekele.**

Town/Season	No. tested	Weil-Felix		IgM ELISA Positives
		Suspected	Positives	
Dessie				
Dry Season	49	15 (31%)	3 (6%)	11 (22%)

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Rainy Season	100	14 (14%)	7 (7%)	20 (20%)
Mekele				
Dry Season	21	7 (33%)	1 (5%)	2 (10%)
Rainy Season	69	12 (17%)	6 (9%)	13 (19%)

Higher prevalence of typhus was detected during the rainy season than during the dry season in all the places investigated. This agrees with other previous studies. Tesfayohannes (23) in his study in different altitudinal zones observed higher prevalence of the disease in cold areas with higher altitudes. In another study increase in percentage prevalence of body lice, the vector of the disease, was detected during the rainy season and the prevalence increased with increase in elevation, irrespective of the season indicating that prevalence of the disease is associated with the prevalence of the human body lice (24).

Although sholdt et al. (24) reported that in their study population males had significantly higher body louse infestation compared to females we did not detect significant difference in seroprevalence rate between males and females for both seasons. There was a significant difference in sero-prevalence of typhus among people living in Dessie and the nearby towns only during the dry season. No significant difference was detected in the sero-prevalence of typhus between the people living in Mekele and those living in the nearby towns outside Mekele during both the rainy and dry seasons.

Although it was not statistically significant , the student population, among the various occupational group in the various places, showed the highest percentage of positivity (up to 36%). This was also reflected in the comparison of prevalence by age group. In a study conducted in 1989 in three altitudinal zones of Ethiopia, a large percentage (66.8%) of school children (age 6-25) were observed to harbour body lice in various numbers irrespective of altitudinal zones (23). In our study a lower sero-prevalence was observed in the younger and older age groups. This may be because of the fact that special attention is given by the parents to the younger ones and there is lower infestation rate in this group and also mechanical delousing which is a risk to infection is not exercised in this very young age group. Also the older age group is aware of the stigma attached to lice infestation and may probably change their clothes and bath more frequently, and hence minimizing the risk of infection.

A substantial percentage of sero-prevalence was detected among blood donors both in Dessie and Mekele (Table 6). This is in agreement with a study conducted in Gondar in 1961 by Yoseph (25) where he reported antibody detection in apparently healthy prisoners and among selected groups in the general population. Gebreselassie et al (21) also reported sero-prevalence rate of 6% among blood donors. The sero-positivity among blood donors could be due to a recent past infection, a mild acute infection on a background of acquired immunity from past infection or due to false positive results of the test. Thus the diagnosis of typhus should be based on a combination of clinical examination and specific laboratory tests.

A reliable serological confirmation of typhus depends on a four fold or greater rise in antibody titer from the acute phase to convalescent phase specimen. Since this procedure takes time it lacks clinical relevance. In our previous studies we compared the diagnostic efficacy of the Weil-Felix test, IgM and IgG ELISA with the IFA as a gold standard when only a single serum from a patient is available. It was found that the IgM ELISA is a sensitive test for the laboratory confirmation of suspected typhus (26, 27). More recently, Silpapojakul et. al. (28) compared the dot blot ELISA to latex agglutination and the Weil-Felix test for the diagnosis of typhus. Both the dot blot ELISA and latex agglutination were more sensitive and specific and also more rapid than the Weil-Felix test. Moreover the results form these two tests are available within one hour of testing.

In conclusion, more studies are needed involving more areas of the country to better understand the extent of the problem, evaluate the WF and ELISA based serological tests, and also assess how long IgM antibodies against typhus persist after infection. Moreover, considering the potential for the occurrence of epidemics in the study areas, there is a need for improved clinical diagnosis,

introduction of more specific laboratory tests and timely reporting to appropriate health authorities for effective prevention and/or control measures.

Acknowledgements

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