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Seroprevalence and Risk Factors of Leptospirosis Infection in Rural Community, Egypt

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Abstract

Background: *Leptospirosis is an emerging zoonotic disease caused by bacteria of the genus Leptospira. Despite its relatively common frequency, mild/moderate leptospirosis often goes unrecognized, due to its usually non-specific symptoms of fever, vomiting, and malaise. Knowledge of The burden of human leptospirosis in Egypt is unknown, leptospirosis in Egypt is limited, and the disease may often be misdiagnosed as Typhoid fever.*

Methodology and Principal Findings: *This study was designed to be a cross section study conducted at the period extending from June 2013 to May 2014 with the aim of determining leptospiral infection prevalence among people living in Monshaat Abbas village, Kafr El-Sheikh governorate and identifying associated environmental and behavioral risk factors for the infection. We operated our study on 120 subjects based on history taking and laboratory investigations, a structured questionnaire was carried out to collect information on person's potential risk factors for leptospiral infection. MAT (Micro Agglutination Test) was done using five Leptospira serovars; L.int. Icterohaemorrhagiae, L. int. Canicola, L. int. Pomona, L. int. Grippotyphosa and L. int. Wolffito determine the presence of leptospiral antibodies and their titers in the sera of investigated subjects. Results of our study showed that leptospirosis disease was found to be common among studied cases (45%) defined as MAT ≥ 200 and L. Icterohemorrhagiae was the only serovar detected*

among the studied cases. Middle age male patients were predominating (47.6%) and so sex can be regarded as risk factors of leptospirosis. Also living close to places where rodents and animals inhabit was considered as important risk factor for exposure to such infection and measures should be developed to minimize exposure to these animals. Rice field farming was the most common profession with high risk factors 44 (61.8%) 95% C.I. (61.1-62.4) P value < 0.001 which is highly significant then fishermen 7 (22.6%) 95% C.I. (21.5-23.7%) with significant P value 0.004. also walk barefoot was activity of significant risk factors 45(52.3%) 95% C.I.(51.7-52.9) P value 0.010.

Conclusion and Significance: *The 45% prevalence of Leptospira antibodies suggests that exposure of leptospirosis is common a rural village in the delta region in Egypt, in particular Leptospira serovars; L.int. Icterohaemorrhagiae, (sero group), Many risk factors were revealed to be minded.*

We recommend adding Leptospira infection to the list of possible diseases among febrile patients and increasing the awareness of the fever hospital clinicians about it.

Keywords*: Leptospirosis, seroprevalence, Risk factors, MAT.*

Introduction

Leptospirosis is a neglected zoonotic disease of worldwide public health importance which affects humans, domestic animals and wildlife (*Assenga et al.; 2015*). Human leptospirosis is a waterborne infectious disease caused by pathogenic bacteria called leptospira. Currently, it is one of the most common widespread spirochetal zoonosis and a growing worldwide public health concern *(Levett 2001)*. The disease is caused by different Leptospira serovars, which belong to the order of Spirochaetales, family Leptospiraceae, genus Leptospira *(Balamurugan et al.; 2013)*.

There are 20 species of leptospires, consisting of over 200 serovars, circulating in a wide range of animal reservoir hosts including rats, other rodents, livestock and domestic pets (*Ko Al et al.; 2009)*. Many of these serovars are known to be pathogenic to humans, who can acquire infection through direct contact with animals or through an environment contaminated by animal urine. Infection might occur through ingestion of contaminated food or water, through mucosal surfaces or through skin contact, particularly if there are breaks in the skin. Risk factors for infection therefore include occupational exposure (farming, slaughtering), recreational activities (swimming, canoeing), cultural factors (bathing in rivers, animal rearing, pets) and socioeconomic circumstances (sanitation, poverty) (*Bharti et al; 2003).*

The emergence of leptospirosis has been linked to many environmental and ecological drivers of disease transmission *(Lau et al.; 2012)* such as heavy rainfall, consequent floods, temperature, exposure to animals, etc. The ecology of leptospirosis involves very complex interactions among humans, animal reservoirs, leptospires and their surrounding environments (*Lau et al.; 2010)*. After an incubation period of 2–30 days, leptospirosis produces a biphasic illness with an acute or leptospiraemic phase lasting approximately 7–10 days followed by an immune phase in which immunoglobulins are produced to eliminate the organism from the host. (*Bharti et al; 2003), (WHO 2010).* Leptospirosis causes direct economic burdens to humans such as loss of productivity due to illness, increased treatment costs due to ill health and contributes to poverty among the population in the affected countries (*De Vries et al.; 2014)*. In recent years, leptospirosis has gained increased attention because of many outbreaks of leptospirosis around the world (*Vega-Corredor et al.; 2014).* Those outbreaks pose a great burden on health systems and cause significant economic and social disruption (*Sejvar et al.; 2003)*. Leptospirosis can produce a wide range of clinical syndromes, including non-specific febrile illnesses, renal failure, liver failure, pulmonary haemorrhage and meningoencephalitis. It causes severe disease in at least hundreds of thousands of people each year,

with fatality rates of up to 30% in some areas (*WHO 2010),*The disease is often mistaken for other febrile illnesses such as dengue, malaria, rickettsiosis and enteric fever (*Crump et al; 2013).* Diagnosing leptospirosis in humans is challenging; for example diagnosing using culture technique is not only time consuming but it is also not recommended for clinical diagnosis of leptospirosis in humans *(WHO 2010).* Similarly, the use of antibiotics limits the isolation of the organism in clinical samples. For these reasons, serological testing, specifically the microscopic agglutination test (MAT) remains the test of choice for identification and characterization of the prevalence of Leptospirasero groups (*WHO 2010; Mgode et al.; 2015)* However, MAT is mainly performed in limited laboratories this is because the test requires considerable expertise (*Thaipadungpanit et al.; 2011).*

Treatment options include antibiotics such as doxycycline (*McClain et al.,1984),*] penicillin G (*Watt et al., 1988*), ceftriaxone *(Panaphut et al., 2003)* and cefotaxime (*Suputtamongkol et al., 2004),*and patients with severe illness might require respiratory, renal or haemodynamic support *(C.L. Lau et al 2010*). Owing to its variable presentation, leptospirosis is often unrecognized or misdiagnosed and the true incidence is likely to be underestimated and underreported. The WHO has identified leptospirosis as a neglected tropical disease of global importance, requiring further research to understand its epidemiology, ecology and the disease burden that it causes around the world (*WHO 2010).*

Africa is the world's most rapidly urbanising region and also has the highest slum growth rates, with environmental refugees migrating from rural to urban areas as a result of droughts and floods. (*Moreno and Warah 2010).* Over 70% of inhabitants in African cities (accounting for at least 100 million people) currently live in slums that are prone to flooding (*Costello et al., 2009).* The average number of people in Africa impacted by flooding could increase from 1 million per year in 1990 to 70 million in 2080. (*Nicolls et al., 1999*)

One-half of the urban population in developing countries currently lack piped water, waste collection, paved roads, sewers and stormwater drains. (*Costello et al., 2009).*

Egypt; the situation is still under estimation; The above predictions in human demographics will therefore have significant implications for the future epidemiology of infectious diseases, including leptospirosis. In seroservey study on 886 patients with acute febrile illness; concluded that leptospirosis had almost never been diagnosed in Egypt; therefore, leptospirosis had not been recognized as an important public health problem. (*Ismail et al., 2006).*

Although these studies document a highly endemic state of leptospirosis in Egypt, they did not assess risk factors and morbidity and mortality rates among the confirmed cases in which *L. icterohemorrhagiae* was predominant (the major serotype associated with fatal human leptospirosis). (*Azza et al., 2007*)

Risk factors for human infection include occupational exposure, recreational activities, cultural factors and socioeconomic circumstances (*Bharti et al., 2003).*

Risk factors Analysis

Leptospirosis is a multi-factorial infectious disease that is highly associated with both environmental and socioeconomic factors (*Gracie et al., 2014).*

Climatic variables

Although occurs worldwide, leptospirosis is most prevalent in tropical and subtropical areas with high rainfall (*WHO 2002).* Heavy rainfall events and floods increase the risk of leptospirosis by bringing leptospira and their animal hosts including rat and pig into closer contact with humans (*Lau et al., 2010).*

Leptospires are able to survive for prolonged periods of time in higher temperatures and humid environments (*Victoriano et al, 2009,Levett 2014).*

Animal host density variable

Leptospirosis is found mainly in area where humans come into contact with the urine of infected animals or urine- contaminated environments *(WHO 2002)*.

Rodents and some domestic animals such as pigs and dogs are considered to be the most dangerous for transmitting leptospires to humans (*Yan et al., 2006)*. Leptospirosis is considered the only epidemic-prone infection that can be transmitted to humans directly from water, soil and food contaminated urine from infected animal hosts (*Levett 2001)*.

Water reservoir variable

Pathogenic leptospires was reported to be associated with the presence of water bodies including streams, lakes and springs. Therefore, areas with high density of water bodies might be at higher risk of leptospirosis occurrence (*Vegacorredor and Opadeyi 2014)*.

Economic variable

Leptospirosis cases have been reported in a variety of settings ranging from large urban centers after heavy rainfall events to remote rural areas with limited access to clean drinking water. Leptospirosis is often considered a disease of poverty in middle and low income countries because it affects mostly vulnerable populations *(Schneider et al. 2013*

Subject and Methods

A random cross-sectional survey was carried out *in Monshaat Abbas* village, Kafr El-Sheikh governorate., from June 2013 to May 2014, *Monshaat Abbas* village located ~20 km northwest of the Kafr El-Sheikh city with a population of ~ 20,000, this village was selected because it is located near streams of water, many villagers are engaged in work such as vegetable and fruit gardening, livestock farming, fishing, and weaving. Houses are usually built high-floored on high wooden or concrete poles, with floor and walls of wood or bamboo. Roofing is of thatch, leaves, and recently of corrugated tinplate. Cattle and buffalo are reared both in sheds and free range around the village. Livestock, goats, and chickens are also kept by many households, and they are usually reared free around the houses. We operated our study on 120 subjects based on history taking and laboratory investigations after written consent form them was taken. For all enrolled subjects, who agreed to participate, one individual face-to-face interview was carried out with each person included in the sample, using a structured questionnaire to collect information on that person's potential risk factors for leptospiral infection, such as occupation, ownership of different kinds of animals, activities associated with water and livestock, and the environmental conditions of the house and the village. After the interview a blood sample was drawn after signing a consent form approved by the Ethics Committees of the National Research Centre. Frozen serum samples were sent to the laboratory of Animal Reproduction Researches Institute for serologic analysis of leptospiral antibodies.

All the studied cases were subjected to the following:

A. Thorough history taking stressing on: presenting symptoms as fever, abdominal pain and tenderness, jaundice, renal insufficiency, meningeal sings and severe pulmonary affection; history of exposure to risk factors including: sewer workers, builders, farmers, any contact with rodents especially rats or contact with domestic animals and history of other causes for fever and co-infections.

B. Lab investigations which include: Complete blood picture, liver function tests (ALT, AST, serum albumin, and serum bilirubin; total and direct), renal function tests (Urea nitrogen and serum creatinine).

C. Microscopic Agglutination Test (MAT): MAT was done in this study to determine the presence of leptospiral antibodies and their titers in the sera of investigated subjects. It was carried out according to Faine *et al.* (1999). Antigens were 4 to7 days' old live cultures of the five *Leptospira* serovars; *L.int. Icterohaemorrhagiae*, *L. int. Canicola*, *L. int. Pomona*, *L. int.*

Grippotyphosa and *L. int. Wolffi* in EMJH medium at 30°C. Agglutination was examined by dark field microscopy. The agglutination was considered positive if at least 50% of the screened leptospires were agglutinated, while negative results were seen as free motile leptospires with no clumps. Any reactions that were positive on the screening assay would have to be titrated. A reactive MAT was determined by titers ≥ 1:200 (Crowin et al., 1990). The reported end point titers were calculated as the reciprocal of highest serum dilutions that agglutinated at least 50% of the cells for each serovar used (Cole et al., 1973).

Statistical Analysis

The data were processed and analyzed using Collected data were entered in Epi Info version 6.4 (Centers for Disease Control and Prevention, Atlanta, GA). STATA release 9.2 (Stata Corp., College, TX) was used to derive descriptive statistics and in subsequent multivariate analyses. The considered risk factors were subjected to univariate analysis using Wald x^2 and Fisher's exact tests for the whole study population and for selected strata. Multivariate analysis using a logistic regression model was performed with the laboratory results, with seropositive or seronegative as dependent variables and with age, sex, and other behavioral, socioeconomic, and environmental variables as independent variables. The model was adjusted for the command in STATA program, setting village as the primary sampling unit. A hierarchical backward elimination approach was used to identify significant interaction terms and exposure variables that were strongly associated with seropositivity for leptospirosis. Variables and interaction terms with Wald $P \leq 0.05$ were considered significant.

Results

Table (1): Seroprevalence of leptospirosis in different groups (P value < 0.05 is significant)

Exposure variable	Prevelance MAT + ve		C I (95%)		P-value
	No.	%	Lower	Upper	
Sex					
Female (n=57)	24	42.1	41.27 %	42.90 %	0.544
Male (n=63)	30	47.6	46.80 %	48.30 %	
Total (n=120)	50	45.0	44.60 %	45.40 %	
Age group by decades					
< 28 year (n=25)	12	48.0	46.0 %	50.0 %	0.966
28- < 38 year (n=36)	15	41.7	40.3 %	42.9 %	
38-< 48 year (n=35)	16	45.7	44.3 %	47.8 %	
≥ 48 years (n=24)	11	45.8	43.8 %	47.8%	
Occupations					
Rice field farmer (n=68)	44	61.8	61.1 %	62.4 %	<0.001
Fishermen (n=31)	7	22.6	21.5 %	23.7 %	0.004
Butcher (n=18)	8	44.4	41.7 %	47.1 %	0.959
General (n=8)	0	00.0	00.0 %	00.0%	0.008
Activities					
See rodents (n=44)	35	79.5	78.8 %	80.2 %	<0.001
Swimming (n=31)	16	51.6	50.0 %	53.1 %	0.390
Walk barefoot (n=86)	45	52.3	51.7 %	52.9 %	0.010
Household animals					
Dogs (n=10)	4	40.0	35.2 %	44.7 %	1.000
Poultry (n=116)	53	45.7	45.3 %	46.1 %	1.000
House sanitary facilities					
Laterine (n=86)	43	50.0	49.4 %	50.6 %	0.080
Toilet (n=34)	10	29.4	28.2 %	30.6 %	0.013

Table (2): Serovar and titre of leptospira in positive cases among the studied village

Variable Measure (N=54)		MAT + ve
Serovar (N, %)	L. int. Icterohaemorrhagiae	54 (100.0%)
	L. int. canicola	0 (0.0%)
	L. int. Pomona	0 (0.0%)
	L. int. Grippotyphosa	0 (0.0%)
	L. int. Wolfi	0 (0.0%)
Titre	1:200	25 (46.29%)
	1:400	14 (25.93%)
	1:800	9 (16.66%)
	1:1600	6 (11.11%)

Also *L. int. Icterohaemorrhagiae* was the only Leptospira serovar observed about half of the studied leptospirosis cases had Leptospirosis titre 1: 200, 1:1600 titer was present only among 11%.

Figure (1) show that: *L. int. Icterohaemorrhagiae* was the only Leptospira serovar observed; about half of the studied leptospirosis cases had Leptospirosistitre1: 200, 1:1600 titer was present only among 11%

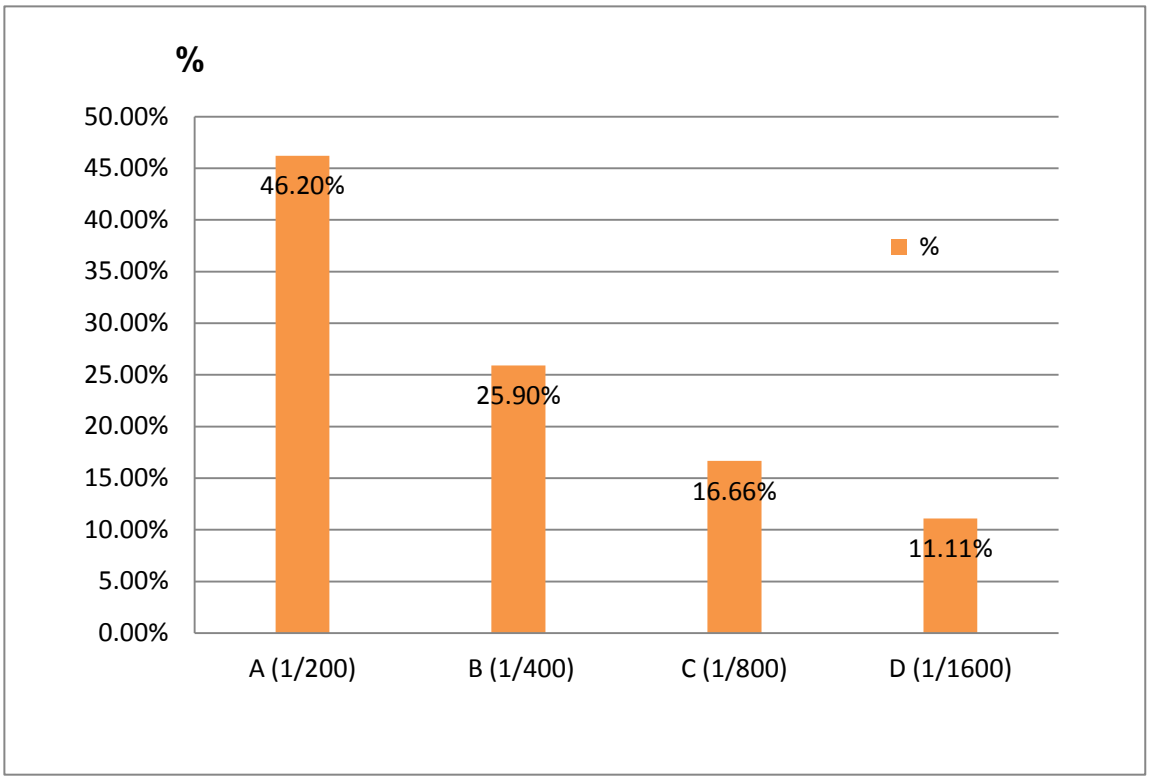

Table (3): Univariate results of potential risk factors among persons MAT + ve and MAT – ve for leptospirosis

	Leptospirosis				OR	C.I. (95%)		p value
	Positive (n=54)		Negative (n=66)			Lower	Upper	
Female (n=56)	24	44.44	33	50	1			
Male (n=64)	30	55.56	33	50	1.250	0.607	2.573	0.545
< 28 years (N=25)	12.0	22.2	13.0	19.7	1			
28 - < 38 years (N=36)	15.0	27.8	21.0	31.8	0.779	0.277	2.161	0.625
38 - < 48 years (N=35)	16.0	29.6	19.0	28.8	0.912	0.326	2.552	0.861
≥ 45 years (N=24)	11.0	20.4	13.0	19.7	0.917	0.298	2.817	0.879
Rice field farmer	42.0	77.8	26.0	39.4	5.385	2.396	12.099	< 0.001
Fishermen	7.0	13.0	24.0	80.3	0.261	0.102	0.667	0.005
Butcher	8.0	14.8	10.0	15.2	0.974	0.355	2.669	0.959
General	0	0	8.0	66.0	0.00	0.000	000	0.999
Swimming (s)	16.0	29.6	15.0	22.7	1.432	0.630	3.251	0.391
Walk barefoot (w)	45.0	83.3	41.0	62.1	3.049	1.275	7.288	0.012
Dogs (D)	4	7.4	6	9.1	0.800	0.214	2.994	0.740
Cattle (C)	51	94.4	53	80.3	4.16	1.12	15.49	0.031
Poultry (P)	53	98.1	63	96.9	1.683	0.148	19.075	0.674
Laterine (L)	43	79.6	43	65.2	2.091	0.908	4.812	0.083
Toilet (T)	11	18.5	26	39.4	0.350	0.150	0.815	0.015

Table (4): Univariate analysis study shows significant variable for risk groups

Significant variables	Leptospirosis				OR	C.I. (95%)		p value
	Positive (N=54)		Negative (N=66)			Lower	Upper	
Rice field farmer	42.0	77.8	26.0	39.4	5.385	2.396	12.099	< 0.001
Fishermen	7.0	13.0	24.0	80.3	0.261	0.102	0.667	0.005
Walk barefoot (w)	45.0	83.3	41.0	62.1	3.049	1.275	7.288	0.012
Toilet (T)	11	18.5	26	39.4	0.350	0.150	0.815	0.015

Table (5): Multivariate logistic regression study shows significant variants

Significant Variants	OR	C.I. (95%)		p value
		Lower	Upper	
Sex (female=0 &male=1)	7.570	2.072	27.658	0.002
Rice field farmer	4.527	1.148	17.853	0.031
Fishermen	0.181	0.042	0.786	0.022
Walk barefoot (w)	4.677	1.227	17.821	0.024

Of 120 serum samples tested 54 (45%)were seropositive for antibodies against Leptospira (95% confidence interval [C.I.]:44.6-45.4% with agglutination titres ranging from 1/200 to 1/1600 table (1) & (2) .Of the 5 most common serogoup tested only one Icterohaemorrhagiae was detected among the samples it is the only prevalent serogroup (100%). The prevalence among males (47.6%) was higher than females (45.0%). Seropositivity rates were almost uniformly distributed among all age groups. The median age of infected was 42 years (range,38-42) .The age group of 28-38 years which had the lowest seropositive rate (41.7%) was used as the reference group for statistical comparisons. Univarite analysis indicated that infected persons were significantly more likely to rice fild farmers (OR 5.385; 95% [C.I] 2.396-12.099% & P value < 0.001), Fishermen (OR 0.261; 95%[C.I] 0.102-0.667% & P value =0.005) also activities like walk barefoot was significant((OR 3.049; 95% [C.I] 1.275-7.288% & P value =0.012) regarding house hold animals the cattle were signifant (OR 4.16; 95%[C.I] 1.12-15.49% & P value =0.031) also rodents were significant since (OR 11.6; 95%[C.I] 4.4-30.7% & P value < 0.001). Tiolet was significant positive protecting factors (OR 0.350; 95% [C.I] 0.150-0.815% & P value

=0.015), regarding as parametters were not statiscally significant as butchers (P value = 0.959), swimming in water (P value =0.391), poultry at home (P value =0.674) and also dogs (P=0.740)

Discussion

Globally, reported leptospirosis seroprevalence vary significantly between and within countries, based on environmental settings, behavioural risk factors, and socio-demographics; our results corroborate these findings.

In our study; the total examined individuals were 120 and the positive for MAT test were 54 about 45% (95% CI ,44.6-45.4%) which was near to the international results; since *Laras et al; 2002* stated that a seroservay on leptospiral infection had been conducted among the general population in four different provinces in Lao PDR in 2000 and 2001 the four provinces ' prevalence rates ranged from 19% to 45% whereas across sectional survey by *Pharaisuwan et al. in 2002* of 315 persons involved in leptospirosis activities in Thailand reported a prevalence as high as 41% ; A study on 359 in western Uganda for seroprevalence by *Dryfus et al., in 2015* found that the total positive samples represented about 35%; *Sharma et al in 2006* discovered that in a large seroprevalence study on 611 individual from high risk groups the prevalence was about 52.7% .

As regard to the predominant serovar observed in our study L. int. Icterohaemorrhagiae was the only Leptospiraserovar observed about 25 (46.2%) of the studied leptospirosis cases had Leptospirosis titre 1: 200 and 1:400 represent 14 (25.93%) and fortunately recent leptospirosis with MAT ≥ 1:800 titer was present only in about 15 (27.8%) with 95% C.I (27.05-28.50%).lso, in Egypt, *Ismail et al. (2006)* reported that *Icterohemorrhagiae, Canicola, Djasiman, Grippotyphosa, Pyrogenes,* and *Pomona* were the most commonly reactive serovars among patients with AFI, *Djasiman, Grippotyphosa and Icterohemorrhagiae* were the most reactive among patients with acute hepatitis. Much more, *Ehsanollah and Gholam (2011)* conducted a study to evaluate prevalence of human leptospiral infections by MAT in three north-east provinces of Iran revealing that the most prevalent serovar was *Leptospira Icterohaemorrhagiae* and the least prevalent serovar was *Leptospira Ballum*. The finding of the current study is also in agreement with *Picardeau (2013),* who reported that *Icterohaemorrhagiae* is the most often implicated infectious serovar in mainland, France and French Atlantic and Pacific territories.

In our study; Regarding the sex; the male positive cases were more than female positive cases by 30 (47.6%) for male and 24 (42.1%) respectively by odds [OR], 1.250 and (95 % C.I : 46.8-48.3 %) which is not statistically significant after multivariate logistic regression odds [OR] was 7.570 and (95% C.I.: 2.07-27.65%) & P value was 0.002) which is significant and increase of males over females which suggests that males are more likely to have contact through their daily activities or occupational exposures; because there were differences in certain daily activities between males and females. This result was agree with the most international studies; *In 2008 Kawaguchi et al.* found that prevalence of leptospirosis among males (28.5%) and among females (19.4%); An association between prevalence of leptospirosis and gender has been reported in many studies. Particularly, men tend to present much higher incidence or prevalence of the disease than women and this fact was not attributed to more frequent exposure of men .*Anna & Tzimoula (2015)* presented a study that analyzes the demographic, epidemiological and clinical data of 168 leptospirosis cases laboratory diagnosed in northern Greece during 1998–2014.this study revealed that most patients were males. Also, *Megan et al. (2011)* mentioned that it was Interestingly, all patients with leptospirosis were boys. Moreover, our findings are agree with *Al Robasi, et al,(2015)* who conducted a study of seroprevalence of leptospirosis in Yemen and reported that males were the predominant of contracting leptospiral infection than females,

whereas Leptospira IgG antibodies detected among males were 42.6% compared with 34.4% in females, and this was consistent with previously reported studies in elsewhere.

In our study we found that no different age group was significant; the prevalence was higher in age group 38-48 years (29.6 %) then age group 28-38 years (27.8%) and the mean age was around 42 year old as theses age groups were the highest age of activities specially agriculture and exposed to water with no statistical significance ($p > 0.05$). In univariate study by *Kawaguchi et al. ; 2008* Found that the median age of infected was 35 years and none of age groups were found to be significantly associated with infection; Varunce Desakorn et al. stated that most cases occurred between age 20-50 years *(Desakorn V., et al., 2012). Yanagihara et al. (2007)* conducted a study In Cabatuan, concluded that The seropositive percentage was greater among males, The average age of male patients was younger than that of females, 24.4 years vs. 29.7 years, respectively. However, higher prevalence was noted among age group less than 50 years old (27.8%).

In our study we found that Rice field profession representing the most common profession in the under investigated area about 68 (56.7%) then fishing 31(25.8%) then butcher 18 (15%) then the other general group 8 (6.7%) the prevalence was high in rice field by 44 (61.8%) 95 % C.I (61.1-62.4%) which was highly significant($p < 0.001$) then butcher by 8 (44.4%) 95% C.I (41.7-47.1%) with no significance then fishermen 7 (22.6%) 95% C.I (21.5-23.7%) which prevalence was significant statistically (p 0.004). Agriculture is the major occupation in rural areas and the methods used for cultivation are purely conventional, utilizing cattle or buffaloes for farming. Our present study showed that farmers, in addition to the occupational environment, are risk factors for leptospiral infection .Also keeping animals such as cattle, sheep, and dog in addition to farm crops maintenance, are good places for rodent/rat entrance; therefore farmers are also directly or indirectly exposed to leptospir-alinfection sources at home. Also, *Picardeau (2013)* reported that the risk for leptospirosis is mainly occupational (individuals working in rice fields and sugar cane fields, etc.), affects preferentially some exposed occupational categories (farmers, animal raisers, slaughter house personnel, sewage workers, veterinarians, etc.) and outdoor sportsmen (fishing, water sports) by contact fresh water soiled by the urine of infected animals.

Rice is a crop that requires a lot of water; therefore farmers have more contact with the pooled water in the farm. Most of the required water for rice farms in the rural areas is supplied from rivers or streams that are likely contaminated with the infected rodents or animals Also, previous studies from rice producing countries such as Thailand, Bangladesh, Brazil and India have documented leptospirosis as an occupational infection in the rice farms *(Kliegman, 2011).*

In our study we found that walking barefoot was markedly significant and represented 83% of positive cases with OR. 3.049 &95% C.I.(1.275-7.288) and this agree with Studies that found a strong positive association between seroprevalence and the habit of walking barefoot, and also the greater risk observed in the present investigation among subjects with low levels of schooling, also strengthen this explanation. The fact that Leptospira interrogans Icterohaemo-rrhagiae was the most prevalent serogroup among the study subjects suggests that infection by leptospires that are known to cause severe disease forms may also produce a high proportion of subclinical or non-apparent infections. *(Vijayachari et al .,2008).*

During the rainy season, the populations in these poor areas experience frequent contact with environmental sources of contamination such as fooding and sewage water in the household or workplace setting without access to adequate protection such as shoes and boots. This exposure may produce infections with higher inoculum levels and, in turn, impart a greater risk of

developing severe clinical outcomes following infection. (*Galloway and Hoffmaster.,2015).*

In our study we found that Particular animals present in the home or workplace were found to be significant risk factors and this was agree with *Kamath et al. (2014)* who mentioned that rearing domestic animals at home , presence of rodents and seeing more than five rats per day at home or work place ,practices such as use of vegetables eaten by rats, contact with contaminated soil with rat's urine surrounding home could be a potential risk factor for the disease. Much more, *Lau et al.(2016)* stated that variables associated with the presence of *Leptospira* antibodies included living in villages, working outdoors ,living in rural areas, living <100m from a major river ,and high cattle density in the district.

It is known that infected enclosing domestic animals may be considered as source of contracting Leptospira. In our study we observed that there was an association between the seropositivity of Leptospira antibodies among studies groups who contacted animals. This fact, confirmed by study found to have the highest seropositivity for leptospira antibodies among population engaged in animals rising (Sharma et al.,2006).

Rodents are well recognized reservoirs of infection and are often assumed responsible for contamination of soil and water through shedding of Leptospira in the urine. (*Chetta et al ,2014).* Pathogenic leptospires are responsible for a worldwide zoonosis, leptospirosis, in which humans are occasional hosts in a cycle involving wild and domestic animals. The animal reserve includes mostly rodents; they excrete leptospires in their urine and thus contaminate hydric environment and transmit the disease to other animals or to humans. (*Roacha et al .,2010).*

In our study we found that fishermen appeared to be at higher risk for the disease with odds [OR] 0.261 C.I.(95%)10.2-66.7 P value 0.005 which is highly statistically significant which is agreed with most of studies and lectures

Angnani et al. declared that Certain occupational groups, including rice farmers, fishermen, sugarcane workers, sewer workers, and military personnel, are considered to be at increased risk of leptospirosis *(Angnani et al., 2003).* And this may be explained by that farmers, in addition to the occupational environment, are also exposed to other Leptospira spp. contaminated sources in their place of living. Iranian farm houses, because of keeping animals such as cattle, sheep, and dog in addition to farm crops maintenance, are good places for rodent/rat entrance; therefore farmers are also directly or indirectly exposed to leptospira infection sources at home. In the current study as well as other studies, living in villages and rural areas is associated with higher risk of contact with rodents and rats, cattle, dogs and other animals, exposure to the river and streams water with high probability of contamination with urine of rodents/rat or other animals suspected to infection with Leptospira species (*Tangkanakul et al. 2005, Levett 2003, Sarker et al.,2002).*

In multiple studies by *Alavi, Khodaverdi and Mosallanejad* reported that that Some occupations, including veterinarians, rice farmers, butchers, fishermen, sewer maintenance workers, slaughterhouse workers, and laboratory staff, appear to be at higher risk for the disease *(Khodaverdi et al 2013, Mosallanejad et al. 2013, Alavi and Khoshkho 2014*).

A new risk factor explaining fresh water fish farming as risk factor for leptospirosis is the transmission through fish infection. In a study by *Machng'u* reported that The prevalence of leptospirosis in fish is higher exceeding the prevalence of leptospirosis in terrestrial animals such as cattle and rodents in Morogoro, which range from 10 to 20% *(Machang'u, 2006).*

However, a study by *Georgies et al. 2014* reported that. *Leptospira* infection is high across freshwater fish types. Fish could be source of *Leptospira* infection to humans since tilapia and catfish are the common fish type widely consumed in Tanzania.

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Dedication: My parents, My Family.

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