

Seroprevalence of Leptospirosis among Shabramant Village Inhabitants, Giza, Egypt

Amgad ElZahaby¹, Samy Zaky¹, Nawal Hassanain, A.², El Sayed Radwan³, Mohey Hassanain, A.^{2*}, Mohammed Hegazy¹ & Ahmed Maher, M.²

¹Tropical Medicine Department, Faculty of Medicine Al-Azhar University, Cairo, Egypt

²Department of Zoonotic Diseases, National Research Centre, 33 EL Bohouth Street, Dokki, 12622, Giza, Egypt

³Leptospirosis Unit- Animal Reproduction Research Institute (ARRI), 5 Hadaek Al-Abram Street, Giza, Egypt

***Correspondence to:** Dr. Mohey Hassanain, A., Department of Zoonotic Diseases, National, Research Center, 33 EL Bohouth Street, Dokki, 12622, Giza, Egypt.

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Received: 13 September 2018

Published: 30 October 2018

Keywords: *Leptospira*; Prevalence; Risk Exposure; Rodents; Egypt

Abstract

Leptospirosis is considered as the most common zoonosis in the world. This study was designed to be a cross section study conducted at the period extending from June 2016 to May 2017 with the aims of determining leptospiral infection prevalence among people living in Shabramant village at El-Giza governorate and identifying associated environmental and behavioral risk factors for the infection. We operated our study on 400 subjects; a structured questionnaire was carried out to collect information on person's potential risk factors for leptospiral infection and MAT (Micro Agglutination Test) was done using nine *Leptospira* serovars to determine the presence of leptospiral antibodies and their titers in the sera of investigated subjects. Our results showed that leptospirosis disease was found to be common among studied cases (44%) and *L. Icterohemorrhagiae* was the only serovar detected among the studied cases.

Age and sex can be regarded as risk factors of leptospirosis as middle age female patients were predominating (52.6%). Co-infection was noted in this study; 22.2% of cases had positivity for HCV Abs which can be regarded as a risk factor for acquiring this disease. Also living close to places where rodents and animals inhabit was considered as important risk factor for exposure to such infection. It can be concluded that high prevalence (44%) of leptospirosis was detected among the studied subjects at Shabramant village. Many of the risk factors and environmental drivers identified in our study provide a significant cause for concern about future risk of leptospirosis.

Introduction

Leptospirosis is one of the world's most wide-spread zoonoses, caused by pathogenic spirochaetes of the genus *Leptospira* [1]. Being spread by the urine of animals, the bacteria enter body via abraded skin, conjunctivae or mucous membranes, after which they disseminate throughout the body. The infection is common in regions characterized by high rainfall, close contact with livestock or wild animals. Due to increased rainfall and global warming, leptospirosis is re-emerging, especially in urban areas, where slums are rapidly growing [2].

Incidence of leptospirosis is variable and marked under-reporting is very common. Generally there is a higher incidence in tropical areas. *Leptospira* is also encountered in a variety of wild and domestic animals but rodents, cattle, dogs and pigs are the predominant hosts in many countries [3]. Different serogroups predominate in different countries but *icterohaemorrhagiae* is encountered in the majority of countries [4].

The usual presentations of leptospirosis are highly variable [5]. Leptospirosis has been misdiagnosed including other diseases as dengue fever disease, typhoid fever, influenza, encephalitis, yellow fever disease and malaria. But asymptomatic disease is also common (up 60-70%) in endemic regions. Most patients develop a mild febrile illness with rather non-specific symptoms like fever, myalgia and headache. A proportion of patients, however, develop rapidly progressing and severe complications, with a fatality rate up 70% in patients with severe pulmonary complications [6]. Other presentations include pulmonary hemorrhage, iritis, non calculus cholecystitis and myocarditis [7].

Due to the diverse symptoms similar to various other diseases leptospirosis is difficult to be diagnosed clinically alone. So laboratory investigations are required for confirmation. The reference serological one is micro agglutination test (MAT). The used MAT antigens reflect the most prevalent serotypes in the country. Other tests include ELISA and the dipstick test [8].

The available data on the incidence and prevalence of leptospirosis in the Middle East is few [5]. Therefore the purposes of this study were estimating leptospiral infection prevalence among people living in Shabramant village at El-Giza governorate and identifying associated environmental and behavioral risk factors for the infection.

Subjects and Methods

Study Design and Area

This cross-sectional survey was carried out in Shabramant village in El-Giza governorate, from June 2016 to May 2017. Shabramant with a population of about 60,000 population, located about 20 km southwest of the Cairo capital city, 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.

Sample Size Determination and Sampling Technique

Statistical formula for sample size calculation was considered. The average seroprevalence of *T. gondii* was considered as 50% from literature and sample size was calculated as 384 we increased the number of studied individuals to 400 so we operated our study on 400 subjects after written consent from 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.

Lab Investigations Which Include

Complete blood picture, PT, liver function tests (ALT, AST, serum albumin, and serum bilirubin; total and direct), renal function tests (Urea nitrogen and serum creatinine) and viral markers (HCV Ab and HBs Ag).

Microscopic Agglutination Test (MAT): MAT was done 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.* [9]. Antigens were 4 to 7 days' old live cultures of the nine *Leptospira* serovars; *L. int.* Icterohaemorrhagiae, *L. int.* Copenhagen, *L. int.* Canicola, *L. int.* Pomona, *L. int.* Wolffi, *L. int.* Pyrogenes, *L. int.* Bataviae, *L. int.* Grippotyphosa and *L. int.* Hebdomadis 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 leptospire were agglutinated, while negative results were seen as free motile leptospire with no clumps. Any reactions that were positive on the screening assay would have to be titrated. A reactive MAT was determined by titers \geq 1:200 [10]. 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 [11].

Statistical Analysis

The data were processed and analyzed using the statistical package for social sciences (SPSS) program. Statistical tests used were description of quantitative variables in the form of mean and standard deviation (mean \pm SD) and description of qualitative variables by frequency and percentage. Student's t-test of two independent samples was used to compare quantitative variables. One way ANOVA test was used to compare more than two groups' quantitative variables was carried out by Chi-square test used to compare qualitative variables and Correlation co-efficient test (r-test) used to rank different variables against each other either directly or indirectly. Significance level (P) value was > 0.05 .

Results

Table (1) shows the socio-demographic characteristics of the studied subjects; mean age of subjects is in late of thirties, the majority of participants are women (57%). As regard to occupation of the studied subjects most of them are farmers and house wives. Table (2) represents ecological characteristics among the studied subjects; 36% have non-concrete ceiling of their houses, 37% are exposed to rodents, 29% show presence of barns & pens in their houses and 37% are in contact with nearby drain/ canal. Table 3 displays prevalence of leptospirosis among the studied subjects which is found to be 44% and *L. int. Icterohemorrhagiae* is the only serovar detected. Analysis of the positive leptospirosis cases regarding socio-demographic and ecological characteristics of the studied subjects showed the following; Leptospira infection occurred in subjects in their late thirties (38.0 ± 14.7) and female patients are predominating (52.6%), most of infected persons are farmers and house wives, positive leptospirosis cases are significantly more frequent among non-concrete ceiling, exposure to rodents, presence of barns and nearby drain/canal. Table 4 illustrates the impact of positive leptospirosis on laboratory abnormalities. By studying different risk factors among the studied samples in regression models, only exposure to rodents was the significant risk factor for leptospiral infection (Table 5).

Table 1: Socio-demographic characteristics of the studied subjects

Variable		Mean \pm SD	Range
		N	%
		12.0-60.0 %	
Age (years)	12.0 -	64	16.0
	20.0 -	72	18.0
	30.0 -	144	36.0
	40.0 -	60	15.0
	50.0 - 60.0	60	15.0
Sex (n, %)	Male	172	43.0
	Female	228	57.0

Occupation (n, %)	Housewife	108	27.0
	Farmer	108	27.0
	Worker	36	9.0
	Official	88	22.0
	Student	60	15.0

Total=400, **Farmer** includes all farming activities and farm products sellers, **Worker** includes all manual workers, **Official** includes all office-related works, **Student** include all non-working students.

Table 2: Ecological characteristics among the studied subjects

Variable	Measure	N	%
Ceiling of houses	Non-concrete	144	36.0
	Concrete	256	64.0
Exposure to Rodents	Yes	148	37.0
	No	252	63.0
Presence of Barns & pens	Yes	116	29.0
	No	284	71.0
Nearby drain/Canal	Near	148	37.0
	Far	252	63.0

Total=400, Non-concrete includes: floor and walls of wood or bamboo, Roofs are made of thatch and leaves, **Rodent exposure** is exposure to rodents or its excreta anywhere, **Barns & pens** include places connected to house to grow up livestock and birds.

Table 3: Serovar and titre of *Leptospira* in positive cases among the studied subjects

Variable	Measure	N	%
Serovar (N, %)	<i>L. int. Icterohaemorrhagiae</i>	176	100.0
	<i>L. int. Canicola</i>	0	0.0
	<i>L. int. Pomona</i>	0	0.0
	<i>L. int. Grippityphosa</i>	0	0.0
	<i>L. int. Wolffi</i>	0	0.0
Titre	1:200	84	47.7
	1:400	48	27.3
	1:800	28	15.9
	1:1600	16	9.1

Table 4: Impact of positive leptospirosis on laboratory abnormalities & Fisher's Exact test

Variable	Positive (N=44)	Negative (N=56)	&P
Anemia	12 (27.3%)	11 (19.6%)	0.368
Leukopenia	9 (20.5%)	11 (19.6%)	0.920
Thrombocytopenia	3 (6.8%)	8 (14.3%)	0.236
High creatinine	0 (0.0%)	4 (7.1%)	0.070
High ALT	10 (22.7%)	9 (16.1%)	0.400
High AST	14 (31.8%)	17 (30.4%)	0.875
High total bilirubin	9 (20.5%)	9 (16.1%)	0.571
High PT	8 (18.2%)	8 (14.3%)	0.598
HCV Ab	10 (22.7%)	14 (25.0%)	0.792
HBVs Ag	0 (0.0%)	1 (1.8%)	0.373

Table 5: Regression model for factors influencing infection with *Leptospira*

Lab	B	SE	P
Exposure to rodents	1.288	0.399	<0.001*

Total=100, β : Regression coefficient, SE: Standard error, *highly Significant

Discussion

Leptospirosis is a worldwide emerging infectious disease of zoonotic importance and large epidemics and epizootics have been reported all over the globe [12]. In our study, 44% of the studied subjects were seropositive for *Leptospira* antibodies. Seroprevalence nearly similar to concurrent work was reported by many researchers; Laras and Bounlu [13] about 40.9% in Thailand, Al-Robasi *et al.* [14] 42% in Yemen and Sohail *et al.* [15] 40.83% in Pakistan. On the other hand, lower incidence was reported; Kuriakose *et al.* [16], 29.6% in a midland rural area of Kerala State, Swai *et al.* [17], 15% in Tanga City, Tanzania and Reis *et al.* [18], 15.4% in an urban slum in Brazil and 37% in Indonesia [13]. However Higher findings were demonstrated in studies conduct-ed in Ethiopia about 48% [19], in Tamil Nadu, India about 58% [20], in

Mazandaran Province, Iran was 58% [21] and in Alto Mayo Valley in the Peruvian region of San Martín was 64.6% [22]. Regarding Egypt; lower results were recorded by Maronpot and Barsoum [23] 5.6%, Ismail *et al.* [24] 24% among patients with hepatitis and Barakat *et al.* [25] 25.9%. While higher result was reported by Samir *et al.* [26] 49.7% in Egypt.

In the present study, *L. Icterohaemorrhagiae* was the only *Leptospira* serovar observed and 27.3% of the studied leptospirosis cases had a titer of 1:400 and more (1:800 and 1:1600) reflective of probable recent leptospirosis and further emphasizes the potential public health relevance of serogroup Icterohaemorrhagiae [3]. In Egypt, Ismail *et al.* [24] reported that *L. Icterohaemorrhagiae* was the major serotype present among the studied patients. Also several investigators recorded that *Icterohaemorrhagiae* was the most prevalent infecting serovar; Juarez *et al.* [27] in Salvador, Brazil, and Picardeau [28] in mainland, France and French Atlantic and Pacific territories and Alarcón-Villaverde *et al.* [22] in Alto Mayo Valley in the Peruvian region of San Martín, Peru. On the other hand, the finding of the present study is in contrast with several researches who reported other serovars are most common; Jansen *et al.* [29] in Germany, serovar Grippotyphosa, Nazrie *et al.* [30] in Kelantan serovar Betaviae, Khalil *et al.* [31], in southeast Iran serovar *Grippotyphosa* and Samsudin *et al.* [32] in Selangor serovar Sarawak.

In the present study, the demographic factors (Age, Sex and occupation) could be considered as potential risk factors of leptospiral infection; higher prevalence was noted among age group of late of thirties. This finding is similar to results of studies conducted by Yanagihara *et al.* [33] and Desakorn *et al.* [34] revealed that most of leptospirosis cases occurred between ages 20-50 years. On the other hand, Anna and Tzimoula [35] recorded that most of leptospirosis cases were among ages of 50-69 years and Kanimozhi *et al.* [36] suggested that senior age group people (age more than 60 years) are highly proven to this disease probably due to the less immunity factor.

In the concurrent work, our findings showed a significant association between female sex with the seroprevalence of leptospiral antibodies among the studied subjects, as (52.6%) of females were seropositive for leptospiral antibodies while (32.6%) of males were seropositive for leptospiral antibodies ($p = 0.045$). This is in agreement Padma and Mohammed [37] and Kanimozhi *et al.* [36]. Our result concerning sex contradicts with other researches who concluded that males are infected with *Leptospira* than females; Desakorn *et al.* [34], Al-Robasi *et al.* [14] and Sakinah *et al.* [38]. Much more, Vimala *et al.* [39] found that, the male to female ratio was 1:1, concluded that leptospirosis is prevalent in men and also in women, both are equally affected because of the fact that nowadays both of them are more exposed to the contaminated environment.

In the present work, people in occupations dealing with animals or its products (farmers) are more exposed to *Leptospira*, and there was statistically significant association among positive cases ($p < 0.001$). This is in acceptance with findings of Alavi and Khoshkho [40], Lau *et al.* [41] who concluded that occupational exposure in the agricultural industry is likely to be an important source of leptospiral infection. On the other hand our study is inconsistent with a study conducted by Swapna *et al.* [42] in Calicut, India, reported that the highest seroprevalence was in hospital sanitary workers (46%), construction workers (28%), and sewage workers (26%). Much more, Alvarado-Esquivel *et al.* [43] found that seroprevalence of *Leptospira* exposure was significantly higher in meat workers (17.7%) than the 4.4% seroprevalence found in waste pickers in Durango City, Mexico.

Concerning the other risk factors among the studied sample in regression models, we found that having non-concrete ceiling, presence of barns or pens and nearby drain/canal are considered risk factors and exposure to rodents is considered the significant one ($p < 0.001$).

A systematic review from a selection of 2723 unique publications containing information on leptospirosis, 428 papers dealing with risk factors were identified by Mwanajaa *et al.* [44], twenty-five studies evaluated exposure to rodents, A majority of the studies were done in South America (10 studies) and on islands (8 studies), concluded that rodents play a major role in transmission of leptospirosis. Lau *et al.* [41] stated that variables linked with the presence of *Leptospira* antibodies involved living in villages, working outdoors, living in rural areas, living <100m from a major river and high cattle density in the district.

in our study nonuse of PPE and lack of previous knowledge of leptospirosis collectively were found to be risk factors for acquiring infection. Sakinah *et al.* [38] reported that the prevalence of leptospiral antibodies was higher (41.2%) among respondents who did not use any protective equipment and they found that respondents in their study who had poor knowledge regarding leptospirosis are higher than respondents who had good knowledge. However, Awosanya *et al.* [45] found that, wearing of hand gloves, or protective clothing, and boots was not found to be protective against leptospirosis. While De Araújo *et al.* [46], who revealed that, of the 257 people interviewed, 232 (90.3%) had previously heard about leptospirosis. Much more, Samarakoon and Gunawardena [47], conducted a descriptive study among 460 school students in Sri Lanka, the awareness of leptospirosis was (100%).

In the present study concerning laboratory findings of the studied subjects, our study showed that anemia was present in (27.3%), leukopenia in (20.5%), thrombocytopenia in (6.8%), high ALT in (22.7%), high AST in (31.8%), high total Bilirubin in (20.5%), high P.T in (18.2%) of *Leptospira* seropositive cases, and no significant differences between leptospirosis positive and negative cases regarding laboratory abnormalities were recorded.

Greene *et al.* [48] stated that hepatic involvement in leptospirosis can vary from mild liver enzyme elevations with or without hyperbilirubinemia to severe liver failure with signs of hepatic encephalopathy. Das *et al.* [49] showed that, hemoglobin <10 g/dl in 26.3%, leukocytosis >12000/mm³ in 68.4% and 34.2% of the cases had elevated levels of both serum creatinine and ALT/AST. However, Dutta and Christopher [50] stated that, most of the routine laboratory tests showed nonspecific findings.

In the present study, 24% of patients were HCV-Ab positive with insignificant differences between positive or negative *Leptospira* Ab group. Angnani *et al.* [51] reported serological evidence of both leptospirosis and hepatitis in 39.4% of their patients. Much more, Das *et al.* [49] revealed that cases of leptospirosis co-infected with HCV were 5.2% of his patients.

Conclusions

It can be concluded that leptospirosis is an important neglected zoonotic disease despite its high prevalence, as 44% of the studied subjects at Shabramant village of EL-Giza Governorate were seropositive for leptospiral antibodies by MAT. *L. Icterohemorrhagiae* was only detected serovar among the studied subjects. Symptoms of leptospirosis are not specific, leading to under-reporting of this disease. The main risk factors of leptospiral infection were exposure to rats, farming, non-concrete ceiling of houses, presence of barns, pens at houses, presence of nearby drain or canal, lack of PPE, and no prior knowledge of leptospirosis. Many of the risk factors and environmental drivers identified in our study provide a significant cause for concern about future risk of leptospirosis. So there is a need to set up reference laboratories for leptospirosis in specialized hospitals and awareness of the clinicians especially of fever hospital should be increased about leptospiral infection.

Funding

No funding sources.

Ethical Approval

We operated our study on 400 subjects after written consent form them was taken. Blood samples were drawn after signing a consent form approved by the Ethics Committees of the National Research Centre.

Conflicts of Interests

The authors declare that they have no conflict of interest.

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