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Nationwide seroprevalence, spatial distribution and risk factors of *Leishmania* in Jordan

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ABSTRACT

Objective: To explore the seroprevalence, spatial distribution and risk factors for *Leishmania* seropositivity in Jordan.

Methods: Blood samples from 872 apparently healthy participants were randomly selected from 11 governorates in Jordan and tested for anti-*Leishmania* K39 IgG. Risk factors (animal ownership and agriculture practices) and demographic data were also collected using pre-tested and validated questionnaire.

Results: Overall, 2.52% of participants were seropositive for *Leishmania* spp. Participants living in the Jordan Valley plateau had significantly greater odds (adjusted odds ratio = 3.70, 95% *CI* 1.37-9.93) of seropositivity than those living in the Highlands after adjustment for age.

Conclusions: This study supports the intermittent reports of cutaneous leishmaniasis outbreaks in the Jordan Valley. Vector control measures in the Jordan Valley should be considered, including insecticide treated bed nets, sugar baits and using flowering plants to attract and trap *Phlebotomus papatasi* sand flies. Active surveillance in the Jordan Valley is also recommended in light of this and other reports.

1. Introduction

Leishmania spp. are sandfly-transmitted protozoal pathogens, which are endemic in Latin America, South East Asia, Africa and the Middle East[1]. These pathogens cause two main forms of leishmaniasis; visceral and cutaneous. Visceral leishmaniasis is the most severe form of the disease and manifests as hepatomegaly, severe wasting, and death in 95% of untreated cases[1,2]. Cutaneous leishmaniasis typically appears as localized lesions at the sandfly bite location, starting with a pink papule, and then progressing to a nodule or plaque and eventually an ulcer[3]. The ulcer heals spontaneously over a period of several months, but permanent scar may occur[3].

Approximately 20 species of *Leishmania* genus can cause leishmaniasis with differences in the distribution and clinical signs of the disease by the causative species[4]. In addition, several sandfly species can transmit the *Leishmania* parasites; where each

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sandfly species transmits one *Leishmania* species. Predominantly, *Phlebotomus* sandflies act as the biological vector for transmitting *Leishmania* parasites to humans in Europe, Africa and Asia[5,6]. Sandflies become infected from biting an infected mammal. The infected mammals, which become the parasite reservoir, are either animals (zoonotic transmission: such as rodents, foxes, wolves, and dogs) or humans (anthroponotic transmission)[5]. In Jordan, *Leishmania major* causes the most reported cutaneous leishmaniasis, and is transmitted by *Phlebotomus papatasi* sandfly[4]. Other infections in Jordan are caused by *Leishmania tropica*, which is transmitted by *Phlebotomus sergenti*[4]. Visceral leishmaniasis is rare in Jordan and caused by *Leishmania infantum*[4]. It is believed that the primary reservoirs of *Leishmania major* are rodents, of *Leishmania infantum* are dogs and of *Leishmania tropica* are humans[1,4,7,8].

Cutaneous leishmaniasis is endemic in several countries around the world. A recent review reported the occurrence of approximately 75% of the worldwide cutaneous leishmaniasis in Syria, Algeria, Ethiopia, Sudan, Iran, Afghanistan, Brazil, Colombia, Peru and Costa Rica[9]. Jordan neighbors cutaneous leishmaniasis endemic countries such as Syria and Iraq and has been hosting hundreds of thousand refugees from endemic countries such as Syria in recent years[10]. In addition, given the presence of known leishmaniasis risk factors in Jordan (poverty, agriculture, contact with animals, sporadic outbreaks, and livestock use), it is important to examine the seroprevalence of infection throughout the country. Though leishmaniasis is endemic in Jordan, there is a lack of data on the seroprevalence, spatial distribution and risk factors specific to the country. Thus, the aims of this study were to determine the seroprevalence of *Leishmania* K39 antibody among humans in different regions of the country and to identify current risk factors for *Leishmania* spp. seropositivity in order to ultimately provide recommendations for controlling leishmaniasis in Jordan.

2. Materials and methods

2.1. Sample size calculation

The seroprevalence for *Leishmania* spp. is unknown in Jordan. Based on reports from neighboring countries, we conservatively assumed a prevalence of 2% and calculated a sample size of 872 to detect a (2±1)% prevalence[11].

2.2. Sampling and description of participants

Sampling the participants was conducted in different regions/ governorates in Jordan between November 2015 and May 2016. Using a list obtained from the Jordanian Ministry of Health, two to six government health centers were selected randomly from each of 11 governorates based on the size of the population in that governorate. There are 12 governorates in Jordan and one, the governorate of Aqaba, was excluded because it is sparsely populated. Sera from healthy relatives of individuals seeking health care at the selected centers were obtained. The study population did not include any Syrian refugees.

2.3. Samples analysis

The SERION classic *Leishmania* IgG ELISA test (Virion\Serion GmbH, Würzburg, Germany) was used to determine the presence of *Leishmania* spp. antibodies in the sera samples. The test used inactivated preparation of the kinesin-like protein (K39) for detecting and quantifying *Leishmania*-IgG antibodies in sera samples. This test has a sensitivity and specificity of >99%, according to the manufacturer, based on testing 203 serum samples from healthy blood donors and patients with suspected leishmaniasis. In addition, assays that use K39 showed high sensitivity and specificity for detecting leishmaniasis[12–14]. The test results were validated and interpreted as recommended by the manufacturer. Briefly, antibody activities of >15 U/mL were considered positive.

2.4. Risk factors data collection

Risk factors (including animal ownership, agricultural practices, drinking water sources and consumption of raw or undercooked foods) and demographic data from each participant were collected using a self-administered, validated and pre-tested Arabic questionnaire. Data collection was granted approval by the Institutional Research Bioethics Committee of Jordan University of Science and Technology (Policy # 7601) and by the Jordanian Ministry of Health that allows sampling at the government health centers.

2.5. Statistical analysis

The questionnaire and laboratory data were entered in Excel sheet (Microsoft, Redmond, WA, USA). Maps were generated using Tableau 10.4 software (Seattle, WA, USA). Descriptive statistics, *chi*-square tests and unadjusted odds ratios were calculated by Stata version 14.2 (College Station, TX, USA). A poisson regression was also fitted to assess the association between exposures and seropositivity status. The final model included all variables associated with seropositivity at a P-value < 0.05 in the bivariate analysis and variables found to be associated with seropositivity in the literature.

3. Results

Most participants live in a house (68.5%) (as opposed to an apartment), consume traditional wild herbs (50.9%), and use filtered water (63.6%). One third of participants has a garden, one quarter

grows vegetables, and about a quarter practices agriculture. Animal ownership in the study sample was highest for goats (15.0%), sheep (13.0%), and dogs (9.6%).

Table 1 shows the unadjusted (univariate analysis) and adjusted odds ratios (AOR) (multivariate analysis) for factors associated with anti-*Leishmania* spp. K39 seropositivity. Overall, 2.52% (95% *CI*, 1.67%-3.29%) of tested participants were seropositive for anti-*Leishmania* IgG. The seroprevalence was higher among females (2.9%) compared to males (2.0%) and among participants with an income less than $750 USD, but these differences were not statistically significant ($P > 0.05$). Similarly, there were no significant associations between seropositivity and age, education, and living abroad. Animal ownership, household water source, owning a garden were not associated with seropositivity. Among

Table 1. Seroprevalence, unadjusted and adjusted odds ratios for anti-*Leishmania* spp. K39 IgG in Jordan, 2015-2016.

Variables	No. leishmaniasis /No. tested	% Positive	Unadjusted *OR*	*P*-value	AOR (95%*CI*)
Seropositive	22/872	2.52	NA		NA
Age, mean (range), years	38.3 (6-91)	NA	NA		NA
Age, years					
<30	6/332	1.8	Reference		Reference
30–49	8/311	2.6	1.42	0.513	0.96 (0.32-2.83)
50+	8/229	3.5	1.93	0.222	1.24 (0.42-3.70)
Gender					
Female	15/521	2.9	Reference		NA
Male	7/351	2.0	0.69	0.422	NA
Housing					
Apartment	6/275	2.2	Reference		NA
House	16/597	2.7	1.23	0.663	NA
Education					
Elementary	10/241	4.1	Reference		NA
High school	7/322	2.2	0.51	0.183	NA
College	2/119	1.7	0.40	0.235	NA
University degree	3/190	1.6	0.37	0.136	NA
Household income					
<750 USD	17/635	2.7	Reference		NA
>750 USD	5/237	2.1	0.78	0.635	NA
Residence					
Village and Badia	12/482	2.5	Reference		NA
City	10/390	2.6	1.03	0.944	NA
Travel History					
Never lived abroad	17/733	2.3	Reference		NA
Ever lived abroad	5/139	3.6	1.57	0.382	NA
Region					
N. Highlands	8/260	3.1	Reference		Reference
N. Desert	1/77	1.3	0.42	0.416	0.45 (0.05-3.63)
Middle Area	2/244	0.8	0.27	0.094	0.28 (0.06-1.33)
Southern Area	3/219	1.4	0.45	0.232	0.47 (0.12-1.79)
Jordan Valley Plateau	8/72	11.1	3.61	0.010	3.70 (1.37-9.93)
Animal ownership[a]					
Goat	2/131	1.5	0.57	0.442	NA
Cow	0/19	0	NA		NA
Sheep	1/113	0.9	0.32	0.265	NA
Camel	0/11	0	NA		NA
Cats	1/53	1.9	0.73	0.762	NA
Dogs	0/84	0	NA		NA
Food consumption[b]					
Raw milk	1/97	1.0	0.37	0.339	NA
Undercooked meat	1/93	1.1	0.39	0.363	NA
Traditional wild herbs	10/444	2.3	0.80	0.604	NA
Water source[c]					
Rain water	0/105	0			NA
Filtered water	14/555	2.5	0.99	0.999	NA
Municipality Water	8/237	3.4	1.53	0.337	NA
Spring water	0/49	0			NA
Has Garden[d]	8/296	2.7	1.11	0.811	NA
Grows vegetables[e]	4/219	1.8	0.66	0.457	NA
Practice Agriculture[f]	2/199	1.0	0.34	0.144	NA

[a]Compared to not owning each animal; [b]Compared to not consuming each food; [c]Compared to not using each water source; [d]Compared to not having a garden; [e]Compared to not growing vegetables; [f]Compared to not practicing agriculture. *OR:* odds ratios. AOR: adjusted odds ratios.

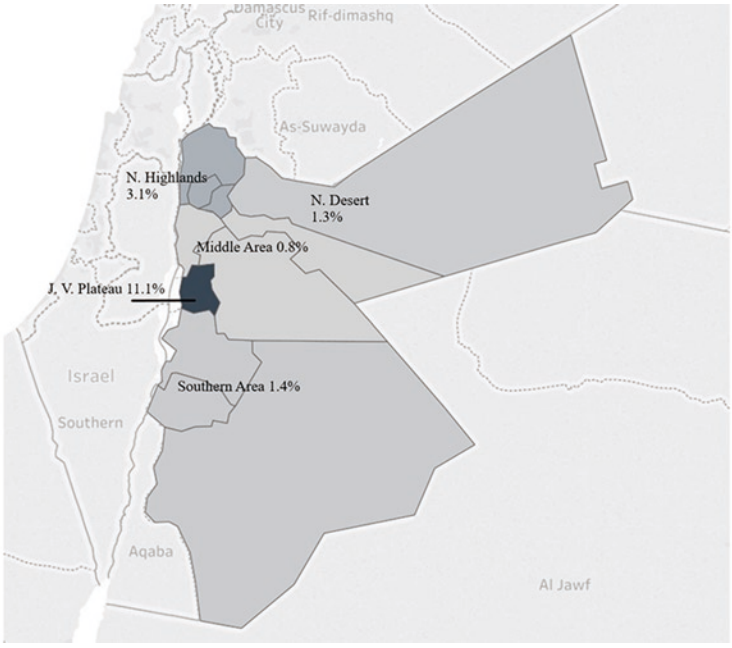

Figure 1. Seroprevalence of anti-*Leishmania* spp. K39 IgG by region of Jordan, 2015-2016 (Tableau version 10.4).

the study sample, those living in the Jordan Valley plateau had significantly higher odds of *Leishmania* spp. seropositivity (AOR = 3.70, 95% *CI* 1.37-9.93) compared with those participants living in the Highland governorates (Figure 1).

4. Discussion

This study reports that the national seroprevalence of *Leishmania* K39 antibody in Jordan is 2.52% (95% *CI*, 1.67-3.29%). This low seroprevalence supports a recent finding of a falling burden of cutaneous leishmaniasis in the Middle East and Africa[3].

Several risk factors for leishmaniasis reported in the literature were not associated with seropositivity in this study. For example, previous studies reported higher prevalence of leishmaniasis among males compared to females[6,15], but our study did not find any significant difference in seropositivity between males and females. The association between age and leishmaniasis has been debated in literature, with some studies reporting higher prevalence/cases among adults (15-40 years old)[15,16] and others reported higher prevalence/cases among children (<15 years)[6,17]. Our study found higher seroprevalence as age increases, but this increase was not significant.

Zoonotic and environmental risk factors for contracting leishmaniasis, have been reported such as contact with animals (particularly ruminants and dogs)[17], and traveling to leishmaniasis-endemic areas[18]. Our study found greater odds of seropositivity with traveling abroad, owning goats and cats, but none of these were statistically significant.

There was significant difference in leishmaniasis seropositivity by region of residence in our study, with the highest seroprevalence among participants living in the Jordan Valley plateau. The climate of the Jordan Valley is warm and humid[19]. This humidity is essential for the eggs of sandflies and for the feeding activities of the adult sandflies[20]. Previous studies indicated the association between leishmaniasis incidence with humidity, rainfall and warm temperatures[21,22]. Reports also indicated that the primary causative species of leishmaniasis in the Jordan Valley is *Leishmania major*, the primary reservoir is the desert rodent and the primary vector is *Phlebotomus papatasi*[4,23].

Iraq, Saudi Arabia, and Syria share borders with Jordan and have high cutaneous leishmaniasis cases. With the Syrian refugee crisis worsening, the trend of cutaneous leishmaniasis cases in Jordan is likely to increase given recent reports of leishmaniasis among Syrian refugee in Turkey and Lebanon[10,24-26].

This is the first study to report seroprevalence, spatial distribution, and risk factors for leishmaniasis in Jordan. In addition, this was a nationally representative study, with participants sampled from throughout the entire country. Based on this and other reports, it is likely the Jordan valley and its neighboring regions that are the foci of leishmaniasis in Jordan. Thus, interventions and public health awareness programs should specifically target this region of the country. Active surveillance of humans and vectors in the Jordan Valley is recommended, along with active surveillance among refugees.

Previous interventions such as sugar baits and local flowering plants were successful at catching *Phlebotomus papatasi* in Iran[27] and in the Western side of the Dead Sea[28], so it could be considered to control leishmaniasis in the Jordan Valley area. Insecticide-treated bed nets have been effective at reducing cutaneous leishmaniasis incidence in some countries the Middle East[29] and may also be worth considering in the Jordan Valley.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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