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RESEARCH ARTICLE

PREVALENCE OF VISCERAL LEISHMANIASIS AMONG ADULTS IN SANA'A CITY-YEMEN

Ameera Saleh Abu-Hurub[1], Amin Abdulkarem Okbah[2], Hassan Mohammed Ali Al-Dweelah[1], Khaled Abdulkareem Al-Moyed[1], Abdulwahab Ismail Mohamed Al-Kholani[3], Mohammed Mohammed Ali Al-Najhi[4], Hassan Abdulwahab Al-Shamahy[1,5]

[1]*Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University.*
[2]*Department of Histopathology, Faculty of Medicine and Health Sciences, Sana'a University, Republic of Yemen.*
[3]*Department of conservative dentistry, Faculty of Dentistry, Sana'a University, Republic of Yemen.*
[4]*Department of conservative dentistry, Faculty of Dentistry, Genius University for Sciences & Technology, Dhamar city.*
[5]*Medical Microbiology department, Faculty of Medicine, Genius University for Sciences & Technology, Dhamar city, Republic of Yemen.*

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***Address for Correspondence:**
Dr. Hassan A. Al-Shamahy, Faculty of Medicine and Heath Sciences, Sana'a University. Faculty of Medicine, Genius University for Sciences and Technology, Dhamar/Sana'a, Yemen. Tel- +967-1-239551;
E-mail: *shmahe@yemen.net.ye*

Abstract

Background and Aims: Visceral leishmaniasis (VL) is a zoonotic and human disease caused by species of *Leishmania*. Parasites are transmitted to the vertebrate host by the bite of a sand fly female (*Phlebotomus longipalpis*), where the infected *promastigotes* transform into *amastigotes*; and this is deadly if left untreated. The purposes of the current research were to reveal the prevalence and potential risk factors for VL in adults in Sana'a city, Yemen.

Subjects and methods: This cross-sectional study was performed during the period from January 2020 to November 2020. Individuals who come for a regular medical examination at Al-Zahrawi Medical Center, Althobhani Specialist Laboratories, Police Hospital, and University of Science and Technology Hospital. A target sample size of 300 was selected, and serum samples were collected from all subjects to determine the prevalence of anti-VL antibodies in human by immune-chromatographic assay using K39 recombinant antigen.

Results: The ages of the participants' ranged from 18-65 years, with a mean of 29.8±8.2 years. The positive rate of antibodies against *Leishmania* species by immune-chromatographic dipstick strip (rK39) was 6.0%. There was statistically important association linking male gender and contracting VL (8.8%, OR=4.1, CI=1.2-14.4, *P*=0.01). There was a significant association (<0.001) between the presence of dogs, rats, and goats in or around live houses and positive VL antibodies with an OR equal to 8.8.7.3 and 8.4, respectively. There were significant risk factors for garbage around the living house, there was also a significant association between displacement and the incidence of VL (*P*<0.001) (OR=8.6, CI=2.8–27.2).

Conclusion: Visceral leishmaniasis was highly prevalent in Sana'a city, and potential risk factors for VL were present with displacement, dogs, rats, goats, garbage, sleeping outside enclosed rooms, and sand flies in living houses. Further studies of human VL need to be conducted to clarify this issue in Yemen, to track and confirm potential reservoirs among canines and other animals, as well as to study vectors.

Keywords: adults, immune-chromatographic assay, prevalence, potential risk factors, recombinant antigen K39, Sana'a city, visceral leishmaniasis, Yemen.

INTRODUCTION

In Yemen, there is limited data of neglected tropical disease (NTD*)* that is greatly interrelated with war, poverty and fall down health systems. In spite of recent studies that discussed trachoma, tuberculosis, lepto-spirosis, brucellosis[1-11], but visceral leishmaniasis (VL) studies were very old or limited[12]. VL, as well famous as kala azar. Kala azar is a parasitic disease caused by *Leishmania* species that is lethal if left untreated. VL parasites are spread by bite of female sand flies (*Phlebotomus longipalpis*) to the vertebrate host, where the infected *promastigotes* transform into *amastigotes*. *Leishmania* species that cause VL have proven their

ability to infect humans as well as wild animals and domestic worldwide[13,14]. Most transmission of *L. donovani* is assumed to be human to human, and this differs with *L. infantum* transmission, from the canine depot host to humans, not just in the Mediterranean area where it may have originated, but as well in many more regions of the world as in Latin America[15,16,17]. Leishmaniasis is a major public health problem in Yemen and kala azar was first reported from the northern part of Yemen in excess of 90 years ago. The rare information on the epidemiology of the disease in Yemen showed that the causative organisms are the compound *Leishmania donovani* and the complex of *L. infantum*, and the vectors are *Phlebotomae orientalis* and *P. arbicus*. Adult cases were also reported from the southern part of Yemen[18,19].

As for laboratory diagnostic methods, a variety of methods have currently been used to diagnose VL, based on aspiration or biopsy samples of tissue (e.g. spleen, liver, bone marrow), where bone marrow samples show lower sensitivity while spleen samples show more specificity[18-21]. A recent diagnostic method of VL is the Immunochromatographic (ICT) assay of the *Leishmania* antibody using a highly specific recombinant antigen, rK39, which is part of a kinesin-related gene, includes 39 amino acid remains that has been developed and is extensively used for diagnosis while simultaneously conserving it among the *L. donovoni* complex. It asserts a sensitivity of 98% and a specificity of 90%. Like direct agglutination test (DAT), it is also simple, fast, and requires no tools (useful in field studies). In India, recombinant K39 has been widely used for the detection of visceral leishmaniasis[22]. Current study was carried out due to appearance of VL cases at the hospitals in Sana'a – Yemen, which is might be a result to the recent war and displacement in Yemen.

SUBJECTS AND METHODS

Study design and study area: From January 2020 to November 2020 (the time specified for the work of the second part of the master's degree in the Faculty of Medicine and Health Sciences for the first author), this cross sectional study was carried out. Individuals were selected from Al-Zahrowi Medical Centre, Althobhani Specialist Laboratories, Typical Police Hospital and University of Science and Technology Hospital in Sana'a city, Yemen. After randomly sampling of the medical centers, in which first hospital listed then selected by lottery method then from the list of patients systemic random selection was done for individual patients.

Inclusion criteria: All adults' patients of both gender attending selected hospitals in the period of the study.

Exclusion criteria: Pediatric patients and adult patients who are in the last stages of serious diseases such as cancers, miliary tuberculosis.

Sample size: A target sample size of 300 was chosen to determine true prevalence of VL by immune-chromatographic assay using K39 recombinant antigen. The population is estimated at 2,000,000. Based on the expected prevalence of anti-VL antibody 3.3%[24], the desired accuracy was chosen 0.021 (2.1% acceptable error in estimation). At least 294 of the total 200,000 were needed at the study site with a 95% confidence level. Calculation was done by a computer computation based on the Epi Info 6 version software (CDC, Atlanta, USA).

Data collection: Pre-designed standardized question-aire designed for this study was used to collected data from each patient. The collected data includes demographic data such as name, age, and gender as well as risk factors for contracting Kala-azar.

Blood sample collection: For each patient; aseptically, five mL of whole blood was collected by venipuncture. Then serum was separated by centrifugation after clotting. The sera samples were kept at –20°C until tested for the antibodies against VL by immune-chromatographic assay using the recombinant antigen K39.

Laboratory test: The rK-39 strip assay (Kala-azar Detect™, InBios Inc., and USA) was executed according to the manufacturer's protocol. In brief, one drop of serum samples was applied to the base of nitro-cellulose ribbons impregnated with recombinant rK-39 antigen. After air drying, 3 drops of test solution (phosphate-buffered saline, plus bovine serum albumin) were added, and the strip was held upright. The appearance of a lower red band (control) indicates proper performance of the test while the appearance of a top red band indicates the presence of anti-rK-39 IgG, indicative of a positive test. The strip was observed 10 minutes after for the test bands.

Statistical analysis: By using Epi Info statistical program version 6 (CDC, Atlanta, USA) the analysis of data was performed. Expressing the quantitative data as mean values, standard deviation (SD), when the data was normally distributed. Expressing the qualitative data as percentages; Chi square test was used for comparison of two variables to determine the *P* value. Odd ratio (OR) was used with 99% confidence interval. *P* value <0.05 was considered statistically significant.

Ethical consideration: From all participants, consents were taken and participants were informed that participation is voluntary and that they can reject this exclusive of stating any reason.

RESULTS

The age of participants ranged from 18-65 years, with mean equal to 29.8±8.2. The positive rate of antibodies against *Leishmania* species by immunechromato-graphic dipstick strip (rK39**)** was 6.0%. There was statistically significant association between male gender and contracting VL (8.8%, *OR*=4.1, *CI*=1.2-14.4, *P* was 0.01.The association between present of animals in or around the living houses of participants and positive antibodies there was risk factors for dogs, rats, and goats in which significant (<0.01) *OR* were 8.8,7.3 and 8.4 respectively. The association between distributions of the environmental hazards and positive antibodies of VL, there were significant risk factors of garbage around living house (*OR*=9.7, *CI*=2.2-43.3).

Table 1: Demographic characteristics of prevalence of VL antibodies among adult patients admitted to internal clinics in some health facilities in Sana'a city-Yemen.

Characters	N (%)
Age groups	29.8±8.2*
18-33 years	162 (54)
≥ 34 years	138 (46)
Sex	
Male	170 (56.7)
Female	130 (43.3)
Live in farm	
Yes	71 (23.7)
No	229 (76.3)
Animals	
Yes	231 (77)
No	69 (23)
Family	
≤ 6	197 (65.7)
> 6	103 (34.3)
Displacement	
Yes	95 (31.7)
No	205 (68.3)
Total	300 (100)

Also for a significant risk for notice sand fly in living house (*P*< 0.001) (OR=28.3, CI=8.7-91.7); and risk for sleeping outside the closed rooms of the home (*P*<0.001) (*OR*=16.8, *CI*=5.8-48). There was a significant association between displacement and prevalence of VL (*P*< 0.001) (*OR*=8.6, *CI*=2.8- 27.2).

DISCUSSION

In this study, the prevalence of visceral leishmaniasis was 6.0%. This result was higher than the study reported by Al-Kamel in Yemen in 2016[24] (3.3%), but lower than that reported by Al-Shamahy1998[12] where the prevalence was (34.7%; 99/285) among school children of the areas endemic with infantile VL in the governorates of Sana'a and Hajjah. Also, the current study rate is lower than the rate mentioned in the Ethiopian Somali region (15.8%)[25], Ethiopia (21%)[26] and Eastern Sudan (32%)[27]. The age of the patients was an independent factor for VL (*P*=0.89) in the current study.

Table 2: The association between age groups and gender with prevalence of VL among.

Age group (Years)	VL				Total		*OR(CI95%)*	χ^2	*p
	+ve		-ve						
	No.	%	No.	%	No.	%			
18-33	10	6.2	152	93.8	162	54	1.1(0.4-2.7)	0.01	0.89
>34	8	5.8	130	94.2	138	46	Reference		
Sex									
Male	15	8.8	155	91.2	170	56.7	4.1(1.2-14.4)	5.5	0.01*
Female	3	2.3	127	97.7	130	43.3	Reference		

*Fisher Exact, χ^2 Chi-square ≥ 3.9 (significant), *P* Probability value ≤ 0.05 (significant)

The association between the presence of animals in or around participants' living homes and positive antibodies where there were risk factors for dogs, rats, and goats (<0.01) with *OR* equal to 8.8, 7.3 and 8.4, respectively. This result differs from other studies conducted in India[28] and Italy[29] where more cases of VL were recorded at ages >35 years. There was a statistically significant association between male sex and contracting VL (8.8%, *OR*=4.1, *CI*=1.2-14.4, *P* was 0.01).

Table 3: The association between presence of animals in or around the living houses of participants and infections.

Type of Animals		VL +ve (n=18)		VL -ve (n=282)		Total (n=300)		χ^2	*P	OR	CI (95%)	
		No.	%	No.	%	No.	%				Lower	Upper
Dogs	Yes	17	8.4	186	91.6	203	67.7	*6.2*	*0.01*	*8.8*	1.2	66.9
	No	1	1.03	96	99	97	32.3					
Rats	Yes	15	11.6	114	88.4	129	43	12.7	<0.001	7.3	2.1	26
	No	3	1.8	168	98.2	171	57					
Goats	Yes	12	18.2	54	81.8	66	22	22.3	<0.001	8.4	3	23.5
	No	6	2.6	228	97.4	234	78					
Sheep	Yes	1	5.3	18	94.3	19	6.3	0.019	0.86	0.8	0.1	6
	No	17	6	264	94	281	95.7			6		
Camels	Yes	1	9.1	10	90.9	11	3.7	0.19	0.66	1.6	0.19	13
	No	17	5.9	272	94.1	289	94.3					
Cattle	Yes	1	8.3	11	91.7	12	4	0.12	0.72	1.4	0.17	11.4
	No	17	5.9	271	94.1	288	96					
Donkey	Yes	1	8.3	11	91.7	12	4	0.12	0.72	1.4	0.17	11.4
	No	17	5.9	271	94.1	288	96					

Table 4: The association between environmental hazards around participants and infection.

Environmental hazards		VL +ve (n=18)		VL -ve (n=282)		Total (n=300)		χ^2	*p	OR	CI (95%)	
		No.	%	No.	%	No.	%				Lower	Upper
Living in cracked wall houses	Yes	15	8.3	166	91.7	181	60.3	*0.2*	0.61	1.3	0.3	4.9
	No	3	2.5	116	97.5	119	39.7					
Living house window nets	Yes	4	3.4	114	96.6	118	39.3	*2.3*	0.1	0.4	0.13	1.3
	No	14	7.7	168	92.3	182	60.7					
Living house door nets	Yes	1	3.2	30	96.8	31	10.7	0.47	0.49	0.4	0.06	3.8
	No	17	6.3	252	93.7	269	89.3					
Open swage system for living house	Yes	4	13.3	26	86.7	30	10	*3.2*	0.07	2.8	0.86	9.1
	No	14	5.2	256	94.8	270	90					
Garbage around living house	Yes	16	11.2	127	88.8	143	47.7	13	<0.001	9.7	2.2	43.3
	No	2	1.3	155	98.7	157	52.3					
Notice of sand fly in living house	Yes	14	31.1	31	68.9	45	15	*59.1*	<0.001	28.3	8.7	91.7
	No	4	1.6	251	98.4	255	85					
Sleep outside the living house	Yes	12	28.6	30	71.4	42	14	44.1	<0.001	16.8	5.8	48
	No	6	2.3	252	97.7	258	86					
Sleep under bed net	Yes	1	9.1	10	90.9	11	3.7	*0.19*	0.66	1.6	0.19	*13*
	No	17	5.8	274	94.2	291	96.3					

*Fisher Exact, χ^2 Chi-square ≥ 3.9(significant), OR Odds ratio >1 (there is a risk), CI Confidence intervals, *P* Probability value ≤ 0.05 (significant)

This result is consistent with previous studies in Yemen also with those conducted in Bangladesh, Nepal, Iraq, Iran, India and Southeast Asia[12,24,28-38]. But the present result differs from that performed in Aden, Yemen by Hamid *et al*., both sexes were affected almost equally[37]. The higher prevalence in males is not yet fully understood, but it has been suggested that there may be a sex-related hormonal factor[12]. Also, this can be explained by the fact that women traditionally protect their bodies - including the face- completely with clothing when they are out of doors, while men are less covered and can be easily attacked by sand flies. This result confirmed the true association between leishmaniasis and dogs and rats as they are natural reservoirs of VL, as well as that goats could be potential reservoirs of VL in Yemen[39].

Table 5: The association between displacement and prevalence of VL among adults.

Displacement	VL +ve (n=18)		VL -ve (n=282)		Total (300)		χ^2	*p	OR	CI (95%)	
	No.	%	No.	%	No.	%				Lower	Upper
Yes	14	14.7	81	85.3	95	31.7	18.8	<0.001	8.6	2.8	27.2
No	4	2	201	98	205	68.3					

*Fisher Exact, χ^2 Chi-square ≥ 3.9 (significant), OR Odds ratio >1 (there is a risk)
CI Confidence intervals, *P* Probability value ≤ 0.05 (significant)

In the current study, correlations between distributions of environmental risk and positive VL antibodies, there were significant risk factors for garbage around the living house (*OR*=9.7, *CI*=2.2-43.3), also significant risk of presence and observation of sand flies in the living house (*P*<0.001) (*OR*=28.3, *CI*=8.7-91.7); and risk of sleeping outside enclosed rooms (*P*<0.001) (*OR*=16.8, *CI*=5.8-48). These outcomes are in agreement with a study performed in India[40], and the Ethiopian Somali region[25]. This is due to the decrease in public health services in the city of Sana'a and the increase in waste and solid waste, which provided a good environment for sand fly vectors and attracted stray dogs and rats that are *Leishmania* reservoirs and vectors. In this study, there was a significant association between displacement and prevalence of VL (*P*< 0.001) (*OR*=8.6, *CI*=2.8–27.2). This is due to migration from an endemic area to Sana'a. It is known that human migration is one of the known risk factors that increase the spread of the disease[28]. Previous studies confirmed that migration plays a role in the outbreak of kala azar in Nepal and in India and among Somali refugees and Kenyan herders in Africa[41,42].

CONCLUSIONS AND RECOMMENDATIONS

These results revealed the existence and high prevalence of human leishmaniasis among patients arriving at hospitals in Sana'a city, and this disease may become a serious problem threatening the health care system in Yemen. Therefore, awareness programs should be provided to clinicians and the population about VL infection and its risk factors. Awareness of VL transmission routes and the type of potential animal reservoirs can guide the community and prevent further infection. Further studies of serodiagnostics and

genetics of *Leishmania* species are needed to determine the local and clonal dominant type of *Leishmania* species genes, and collaboration between researchers and public health professionals in terms of research and expansion of diagnostic services for visceral leishmaniasis.

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AUTHOR'S CONTRIBUTIONS

Abu-Hurub AS: field work in hospitals, laboratory work writing original draft. **Okbah AA:** methodology, formal analysis, conceptualization. **Al-Dweelah HMA:** data curation, investigation. **Al-Moyed KA:** editing, methodology. **Al-Kholani AIM:** investigation, conceptualization. **Al-Najhi MMA:** data curation, investigation. **Al-Shamahy HA:** critical review. All authors revised the article and approved the final version.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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