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RESEARCH ARTICLE

SEROPREVALENCE OF VISCERAL LEISHMANIASIS AND ITS ASSOCIATED FACTORS AMONG ASYMPTOMATIC CHILDREN IN HADHRAMOUT VALLEY AND DESERT REGIONS, YEMEN

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Abstract

Background and Aims: Visceral leishmaniasis (VL) is zoonotic and human illness produced by *Leishmania* species. Protozoan parasite is spread to the host vertebrate via sand fly female bite (*Phlebotomus longipalpis*), in which the infected *promastigotes* convert into *amastigotes*; and VL is associated with high fatality if left untreated. The aims of the current study were to uncover the prevalence and potential risk factors for VL in children in selected districts of Hadhramout governorate, Yemen.
Subjects and methods: Six districts were randomly selected from 24 districts in Hadhramout governorate as follows: Al-Sum, Al-Qattan, Tarim, Thamud, Seiyun, and Shibam. Then a target sample size of 400 children were randomly selected, 66 children were selected from each district except 70 children in Seiyun and they were selected from the selected schools and health centers. Serum specimens were assemble from all children to determine the rate of anti-VL antibodies in human by immunochromatographic assay using recombinant K39 antigen.
Results: The age of the children from 1-15 years, by a mean of 8.4±2.7 years. The positivity rate of *Leishmania* species antibodies by immune-chromatographic dipstick strip (rK39) was 5.8%. There was statistically considerable association connecting male gender and VL infection (8.0%, *OR*=3, *CI*=1.1-8.2, *p*=0.02). There was statistically important association linking older children (11-15 years) and contracting VL (9.1%, *OR*=2.6, *CI*=1.1- 6.2, *p*=0.02). There was a considerable association (<0.05) connecting the presence of rats, dogs, and goats in or around live houses and positive VL antibodies with an *OR* come to to 2.6, 3.7 and 2.8, respectively. There was no significant association between displacement or district location and incidence of VL.
Conclusion: Visceral leishmaniasis was highly prevalent in the desert and the valley areas of Hadramout among children, and visceral leishmaniasis potential risk factors were males, older children, dogs, rats, goats, litter, open sewers, and the presence of sand flies in the household.
Keywords: children, Hadhramout, immune-chromatographic assay, potential risk factors, prevalence, recombinant antigen K39, visceral leishmaniasis, Yemen.

INTRODUCTION

In Hadhramout, Yemen, there are limited data on neglected tropical diseases (which are highly associated with war, poverty, and deteriorating health systems). Although recent studies have discussed trachoma, tuberculosis, leptospirosis, and brucellosis[1-11], but VL studies have been very old or limited and did not involved Hadhramout areas[12]. VL, is a protozoa disease caused by *Leishmania* species that is fatal if not treated. The parasites of VL are transmitted by the bite of a sand fly female (*Phlebotomus longipalpis*) into a vertebrate host, in which *promastigotes* convert into *amastigotes*. The *Leishmania* species that cause VL have been shown to be able to infect humans as well as wild animals and domestic animals worldwide[13,14]. The majority spread of *L. donovani* is understood to be human to human, and this is different with *L. infantum*

spread, from the canine host to humans, not just in the Mediterranean area as Yemen where it may have come from, however additionally in many more regions as in Latin America[15-17]. Humans, hyraxes, domestic dogs and rats are the most frequently hosts infected, and they can also be potential reservoirs. The most important reservoir of both *L. donovani* and *L. infantum* in Yemen might be domestic dogs[12]. Otherwise, the transmission of *L. donovani* is in general thought to be anthroponotic in the Mediterranean area as well as Yemen, it is thought to be partially zoonotic and anthroponotic, the nature of which varies with geographical areas[18]. Therefore, the decisive reservoir of VL in Arabia peninsula and Yemen continues to be ascertained and well studied[18]. Furthermore, multiple factors such as biological, ecological, seasonal migration and areas of low socioeconomic status are reasons that play a role to disease transmission in the region. Also, the presence of sand-flies and ravines in the area, presence of the reservoir host, open-air waste disposal and asymptomatic cases are essential risk factors for disease spread[18]. Leishmaniasis is a foremost health problem in Yemen and kala azar was firstly described from the northern part of Yemen in overindulgence of 90 years ago. The rare data 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*[19].

As in the case of laboratory analytical methods, a variety of techniques have currently been used to diagnose visceral leishmaniasis, established based on biopsy samples of tissues or aspiration (such as spleen, liver, bone marrow), where in bone marrow samples show lower sensitivity while samples of spleen are more specificity[20,21]. A recent method of diagnosis VL is the Immunochromatographic (ICT) assay of the *Leishmania* antibody by means of an extremely specific recombinant antigen, rK39, which is part of a kinesin-related gene. 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]. The purposes of the current research were to reveal the prevalence and potential risk factors for VL in children in selected districts of Hadhramout governorate, Yemen.

SUBJECTS AND METHODS

Study design and population: This cross-sectional study was conducted during the period from February 2022 to November 2022(Master's project time). Six districts were randomly selected from 24 districts in Hadhramout governorate as follows: Al-Sum, Al-Qattan, Tarim, Thamud, Seiyun, and Shibam.

Sample size: A target sample size of 400 has been empirically selected to determine the true prevalence and antibodies against VL by immune-chromatographic assay using the recombinant antigen K39, in which the children from 1-15 years population of Hadhramout was estimated to be 700,000[19]. Based on an expected prevalence of VL antibodies 6%[23]. At least 399 are needed out of a total estimated of 700 000 children in the site of the study at confidence level of 95% with SE equal to 2.33(2.33% acceptable error in estimation). The calculation was done by a computer calculation that depends on Epi Info 6 version software (CDC, Atlanta, USA).

Inclusion criteria: Age is less than 16 years in both sexes.

Exclusion criteria: Age over 15 years and pediatric patients who are in the last stages of serious diseases such as carcinomas and miliary tuberculosis.

Data collection: For this study a pre-designed standardized questionnaire was prepared to collected data from each child. The data collected includes demographic data such as name, age, and gender as well as potential risk factors for contracting VL infection.

Blood sample collection: From each child; five mL of whole blood aseptically by venipuncture was collected. After clotting of the blood serum was separated by centrifugation. At –20°C sera specimens were kept 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: The data were analyses performing Epi Info statistical program version 6 (CDC, Atlanta, USA). Conveying the quantitative data like mean values, standard deviation (SD), as the data were normally distributed. The qualitative data were expressed as percentages; for comparison of two variables to determine the *p* value, the Chi square test was used. Odd ratio (OR) was used with 99% confidence interval. p value<0.05 was regarded as statistically significant.

Ethical consideration: Consents were taken from all the parents of the participants and the parents of the participants were informed that participation is voluntary and that they can refuse without giving any reason.

RESULTS

This cross-sectional study was conducted during the period from February 2022 to November 2022. Six districts were randomly selected from 24 districts in Hadhramout governorate as follows: Al-Sum, Al-Qattan, Tarim, Thamud, Seiyun, and Shibam. First 6 districts were randomly selected from 24 districts of

Hadhramout governorate. Then 400 children were selected randomly, 66 children from each distract except Seiyun 70 children were selected from the selected schools and health centers. The children's ages ranged from 1–15 years, with a mean age of 8.4 ± 2.7. Total 28% of the children were in the 1-5 year age group, 33.5% in the 6-10 year group and 38.5% in the 11-15 year age group. Looking at gender, the number of males was 225 (56.3%) and females 175 (43.7%) (Table 1). The positive K39 recombinant antigen for VL in the current study was 5.8%. Considering the associations between sexes, age groups and prevalence of VL antibodies among children tested (Table 2); there was a significant association between male sex and VL infection as the rate of positive VL for males was 8% with an associated odds ratio (*OR*) of 3, and the 95% *CI* was 1.1–8.2, *p*=0.02; While the female positive rate for VL was 2.9%.

Table 1: Demographic characteristics of children participated in the study of VL prevalence in Hadhramout the valley and desert districts, Hadhramout-Yemen.

Characters	Number (%)
Age groups(years)	
1-5	112 (28)
6 -10	134 (33.5)
11 – 15	154 (38.5)
Total	400 (100)
Sex	
Male	225 (56.3)
Female	175 (43.7)
Total	400 (100)

Table 2: The association between sexes, age groups and prevalence of VL among tested children.

Characters	Positive recombinant antigen K39		*OR*	*CI 95%*	X^2	*p*
	No	%				
Age groups(years)						
1-5 n=112	4	3.6	0.52	0.17-1.5	1.3	0.24
6 -10 n=134	5	3.7	0.53	0.29-1.8	1.4	0.22
11 – 15 n=154	14	9.1	2.6	1.1-6.2	5.1	0.02
Sex						
Male n=225	18	8	3.0	1.1 – 8.2	4.8	0.02
Female n=175	5	2.9	0.33	0.12-0.92	4.8	0.02
Total n=400	23	5.8				

There was a significant association between older children (group 11–15 years) and VL infection as the rate of positive VL for this group was 9.1% with an *OR* of 2.6, 95% *CI* was 1.1–6.2, *p*=0.02; while the other age groups had a lower positive rate for VL (3.6% and 3.7%) (Table 2). Considering the association between animal species in or around homes and VL infection among children tested; there was a significant association between dogs and VL infection as the positive rate of VL for children who own dogs or are around living homes was 8% with an associated *OR* of 2.6, 95% *CI* was 1.1–6.6, *p*=0.04.

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

Type of Animals		Recombinant antigen K39				Total (n=400)		χ^2	*p	OR	CI (95%)	
		+ve (n=23)		-ve (n=377)								
		No.	%	No.	%	No.	%				Lower	upper
Dogs	Yes	17	8	196	92	213	53.3	*4.2*	*0.04*	*2.6*	1.1	6.6
	No	6	3.2	181	96.8	187	46.7					
Rats	Yes	16	10.1	143	89.9	159	39.8	*9.1*	*0.002*	3.7	1.5	9.3
	No	7	2.9	234	97.1	241	60.2					
Goats	Yes	15	9	151	91	166	41.5	5.7	0.01	2.8	1.2	6.8
	No	8	3.4	226	96.6	234	58.5					
Sheep	Yes	5	6.4	74	93.6	79	19.8	*0.06*	*0.8*	1.1	0.4	3.1
	No	18	5.6	303	94.4	321	80.2					
Camels	Yes	3	12.5	21	87.5	24	6	*2.1*	*0.14*	2.5	0.7	9.2
	No	20	5.3	356	94.7	376	94					
Cattle	Yes	3	9.4	29	90.6	32	8	0.8	0.35	1.8	0.5	6.4
	No	20	5.4	348	94.6	368	92					
Donkey	Yes	1	10	9	90	10	2.5	0.34	0.55	1.8	0.22	15
	No	22	5.6	368	94.4	390	97.5					

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

Environmental hazards		Recombinant antigen K39				Total (n=400)		χ^2	*p	OR	CI (95%)	
		+ve (n=23)		-ve (n=377)		No.	%					
		No.	%	No.	%						Lower	Upper
Living in cracked wall houses	Yes	17	9.1	170	90.9	187	46.8	7.2	0.007	3.5	1.3	8.9
	No	6	2.8	207	97.2	213	53.2					
Living house window nets	Yes	4	3.1	124	96.9	128	32	2.3	0.12	0.4	0.14	1.2
	No	19	7	253	93	272	68					
Living house door nets	Yes	1	1.6	60	98.4	61	15.3	2.2	0.13	0.24	0.03	1.8
	No	22	6.5	317	93.5	339	84.7					
Open sewers system for living house	Yes	11	17.5	52	82.5	63	15.8	18.9	0.00001	5.7	2.4	13.7
	No	12	3.6	325	96.4	337	84.2					
Garbage around living house	Yes	18	9.3	175	90.7	193	48.3	8.8	0.003	4.2	1.5	11.4
	No	5	2.4	202	97.6	207	51.7					
Notice of sand fly in living house	Yes	9	19.1	38	80.9	47	11.8	17.6	0.00002	5.7	2.3	14.1
	No	14	4	339	96	353	88.2					
Sleep outside the living house	Yes	12	8.6	130	91.4	142	35.5	2.9	0.85	2.1	0.9	4.8
	No	11	4.3	247	95.7	258	64.5					
Sleep under bed net	Yes	5	4.5	106	95.5	111	27.8	0.43	*0.5*	*0.7*	*0.25*	*1.9*
	No	18	6.2	271	93.8	289	72.2					

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

There was a significant association between the presence of rats (mice) in the living home and VL infection as the rate of VL positive for children who had rats in or around the living home was 10.1% with an associated *OR* of 3.7, 95% *CI* was 1.5–9.3, *p*=0.002. There was a significant association between the presence of goats in the live home and VL infection as the rate of positive VL for children who had goats in or around the home was 9% with an associated *OR* of 5.7, and the 95% *CI* was 1.2–6.8, *p*=0.002. While there was no significant association of VL infection with the presence of other animal species in the living homes of the children participating in the study (Table 3). Considering the association between environmental hazards around the participants and VL infection (Table 4). There was a significant association between living in homes with cracked walls and VL infection as the positive rate for VL of children living in homes with cracked walls was 9.1% with an associated *OR* of 3.5, 95% *CI* was 1.3–8.9, *p*=0.007.

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

Displacement	Recombinant antigen K39				Total (400)		χ^2	*p	OR	CI (95%)	
	+ve (n=23)		-ve (n=377)								
	No.	%	No.	%	No.	%				Lower	Upper
Yes	5	11.1	40	88.9	45	11.2	3.1	0.07	2.5	0.9	7.1
No	17	4.8	338	95.2	355	88.8					

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

There was a significant association between an open sewers system in residential homes and VL infection as the positive rate for VL of children living in homes with an open sewers system was 17.5% with an associated *OR* of 5.7, 95% *CI* was 2.4–13.7, *p*= 0.00001. There was a significant association between peri-home littering and VL infection as the positive rate for VL of children living in the home with peri-home living littering was 9.3% with an associated *OR* of 4.2, 95% *CI* was 1.5–11.4, *p*=0.003. There was a significant association between the presence of sand-flies in the living home and VL infection as the positive rate for VL of children in whom sand-flies were present in the living home was 19.1% with an associated *OR* of 5.7, and the 95% *CI* was 2.3–14.1, *p*=0.00002 (Table 4).

Consider the association between displacement and VL infection (Table 5). There was no significant association between displacement and VL infection as the positive rate for VL of children with displacement was 11.1% with an associated *OR* of 2.5, 95% *CI* was 0.9–7.1, *p*=0.07.

Finally, when considering the association between the region and VL infection. There was no significant association between the region and the incidence of VL, while the highest incidence of the disease was 9% in the Qattan region and the Thamud district, followed by 7.6% in the Soum region. While, lower rates were recorded in Tarim (3%), Shibam (3%) and Seiyun (2.9%) (Table 6).

Table 6: The association between distracts and prevalence of VL among children in Hadhramout the valley and desert-Yemen.

Distracts	Positive recombinant antigen K39		*OR*	*CI 95%*	X^2	*p*
	No	%				
Al-Som n=66	5	7.6	1.4	0.51-4	0.43*	0.48
Al-Qattan n=66	6	9	1.8	0.7-4.9	1.6*	0.2
Tarim n=66	2	3	0.46	0.1 -2	1.07*	0.29
Seiyun n=70	2	2.9	0.43	0.1-1.8	1.3*	0.25
Shibam n=66	2	3	0.46	0.1 -2	1.07*	0.29
Thamud n=66	6	9	1.8	0.7-4.9	1.6*	0.2
Total n=400	23	5.8				

*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).

DISCUSSION

VL is a highly neglected and underreported infection in Yemen. The aim of this study was to provide evidence and describe the risk factors for the spread of VL among children in selected areas of the Hadhramout Valley and Desert. The prevalence of VL in this study was 5.8%. This finding was higher than the study done by Al-Kamel in Yemen in 2016[24] (3.3%), but lower than that reported by Al-Shamahy 1998[12] where the prevalence was (34.7%; 99/285) among school children from endemic areas of infantile visceral leishmaniasis in Sana'a and Hajjah governorates. Also, the current study rate (5.8%) is almost similar to the recently reported rate in Sana'a city among adults attending hospitals (6%)[23]. The current study rate is also lower than that reported in the Ethiopian Somali region (15.8%)[25], Ethiopia (21%)[26] and eastern Sudan (32%)[27]. Children's age was a dependent factor for VL in the current study as there was a significant association between older children (11–15 years) and VL infection with an *odds ratio* of 2.6, 95% *CI*=1.1–6.2, *p*=0.02. This finding is consistent with other studies reported from India[28] and Italy[29] where the incidence of VL increases with age. Also consistent with Ethiopian studies of VL which confirmed that there is an upward trend of VL with age. This may be due to increased sand-fly exposure during daily activities, such as outdoor pet care[30,31]. On the other hand, this result differs from that recorded by other studies such as Yousif *et al.*,[32]; Dereure *et al.*,[33] where age was an independent factor for VL infection[34]. Concerning the sex in this study, VL was higher among males than females with the percentage (8%) *versus* (2.8%) with associated *OR* for male equal to 3.0, *CI* 95% =1.1-8.2, *p*=0.02. Similarly, this result agreed with other studies, in which the highest prevalence rates were found in males[28,32,35]. Also obtained result is similar to that reported by Altaf *et al.*,[36]; Rahim *et al.*,[37]; and WHO[19]. However, obtained result is different from that reported in Aden, Yemen by Hamid *et al.*,[38] where both sexes were approximately equally affected by VL[38]. The elevated prevalence among males is not yet fully understood. It has been proposed that there may be a hormonal factor connected to sex or exposure[39]. What's more, this may be clarified by the fact that women traditionally shielded their bodies – including the face – entirely with clothing when out of doors, while men are less covered and could be attacked by sand flies easily. 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[40]. The risk factors for dogs, rats, and goats in current study were *OR* equal to 2.1, 3.7 and 2.8, respectively. Both dogs and rats are known as reservoir to leishmaniasis taking into consideration that is mostly stray dogs. Environmental factors may have contributed to the increase in VL in the area in this period due to the war which lead to poverty, Yemen is one of the poorest countries in the world with over 45% of the population living on less than 2 USD a day[19], decrease in public health services and an increase in refuse and solid waste which provided that good habitat to the vector sand fly and also attracting stray dogs and rats. This in agreement with a study conducted in India and Ethiopian Somali Region in which dogs and rats in or around living houses where significant risk factors for VL infections[23,25,41].

In the present study, association between environmental risk and positive VL antibodies, there were important risk factors for open sewers system for living house (*OR*=5.7, *CI*=2.4–13.7), living in cracked wall houses (*OR*=3.5, *CI*=1.3–8.9), garbage surrounding the living house (*OR*=4.2, *CI*=1.5-11.4), also a significant risk of occurrence and observation of sand flies in the living house (*p*<0.001) (*OR*=5.7, *CI*=2.3–14.1). These findings are consistent with a study conducted in India[42], and the Ethiopian Somali region[25]. This is attributable to the reduction in public health services in the these areas and the escalate in solid waste and other types of waste, that make available of good environment for sand fly vectors and attracted rats and stray dogs that are *Leishmania* reservoirs and vectors. In current study findings, there was a non-significant association between displacement and predominance of VL (*p*=0.07) (*OR*=2.5). It is well known that human immigration is one of the known risk factors that rise the spread of the VL disease[28]. Preceding studies approved that migration plays a role in the epidemic of kala azar in India, Nepal and among Somali refugees and Kenyan herders in Africa[41,43].

Limitation of the study

The study should include all districts in Hadramout, with a larger sample size, and the diagnosis of VL

should be based on more than one marker. VL screening can be done among patients who have presented to hospitals with clinical signs such as fever, anemia and splenomegaly to give a more accurate result. The gold standard for diagnosis is visualization of amastigotes in splenic aspirate or bone marrow aspirate and it could have been performed to confirm positive immunochromatography results for rK39, although this is a technically difficult procedure and often not available in regions of the world where visceral leishmaniasis is endemic.

CONCLUSIONS AND RECOMMENDATIONS

These outcomes discovered the existence and prevalence of VL among children in Hadhramout the valley and desert, and VL may develop into a critical problem intimidating the health care system in this area. Consequently, awareness programs should be afforded to clinicians and the population concerning VL infection and its risk factors. The potential risk factors for visceral leishmaniasis were present in males, older children, and present of dogs, rats, goats, litter, open sewers, and the sand flies in the household. Additional studies of *Leishmania* species are desirable to determine the local and clonal dominant type of *Leishmania* species genes, and partnership between researchers and public health professionals in terms of research and extension of diagnostic services for VL.

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AUTHOR'S CONTRIBUTIONS

This research is part of a master's degree in the Department of Medical Microbiology. **Al-Dwailah HMAM:** field work, laboratory work. **Al-Moyed KA:** data analysis, drafting. **Al-Shamahy HA:** review, supervision. **Al-Haddad AM:** methodology, investigation. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

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

CONFLICT OF INTEREST

None to declare.

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