Introduction

Visceral leishmaniasis, a vector-borne disease that is caused by obligate intra-macrophage protozoa, is endemic in large areas of the tropics, subtropics and Mediterranean basin. This disease is characterized by both diversity and complexity [1], it is caused by more than 20 leishmanial species and is transmitted to humans by about 30 different species of phlebotomine sandflies [2]. Leishmaniasis consists of four main clinical types: cutaneous leishmaniasis, mucocutaneous leishmaniasis, visceral leishmaniasis; and post-kala-azar dermal leishmaniasis (PKDL) [3].

Visceral leishmaniasis (VL) also known as black sickness or kala-azar in Asia is a life-threatening parasitic infection. There is evidence that in endemic areas of VL only about 20% of the subjects infected by Leishmania will develop symptomatic VL. The majority of the infected individuals have a sub-clinical infection that may remain completely asymptomatic. In Iraq, no data about asymptomatic VL are available although they are valuable to complete the epidemiological picture of the disease and help evaluating current control measures.

Methods: Screening of children of close family contacts and neighborhood contacts of 50 confirmed VL cases was done to determine the prevalence of asymptomatic infection among the contacts. Five children aged less than five years were selected from the same house or the immediately close neighborhood, making a total of 250 children. rk39 was used as a screening test; those with positive result were confirmed by Indirect Immunofluorescent Test (IFAT). All confirmed cases were followed for the maximum incubation period (six months); if symptoms developed during this period, the case was considered as “latent VL”, otherwise it was considered as “asymptomatic VL”.

Results: The prevalence of asymptomatic infection was 34.4%. All those tested positive for rk39 were tested positive for IFAT. The prevalence was significantly higher in household than neighborhood contacts, and in those living in mud-made houses than those living in block-made or mixed houses (p<0.05). None of seropositive contacts progressed into latent symptomatic case.

Conclusion: It was concluded that asymptomatic VL infections are frequent in Thiqar province and those living in the same case house and mud-made houses are at higher risk to contract infection.
Objectives

To study the asymptomatic part of the natural history of VL in Iraq, through determining the existence of asymptomatic VL among under 5 contacts of clinical VL cases, estimation the prevalence, and identify potential risk factors.

Patients and Methods

Study design: A screening cross sectional study.

Study Setting: The study was conducted in Thiqar governorate, south of Iraq which is of about 1,800,000 inhabitants.

Data collection time

The contacts were identified during Feb 15th to Mar, 31st, 2012, and then a follow-up period extended to Sep, 30th, 2012.

Study population and sample size

The study was conducted on 250 contacts of the last admitted 50 laboratory confirmed by IFAT kit VL cases to Al Nasiriya Pediatrics Hospital during the period of Jan, 1-Feb, 14, 2012. For each case, five contacts aged less than five years living either in the same house or in the nearest neighborhood were included. All contacts had no history of VL, and showed no sign or symptoms of VL on enrollment into the study.

Methods

All contacts were screened for VL using the dipstick rk39 rapid leishmaniasis test (CTK Biotech, Inc On Site Rapid Test”), a highly sensitive and specific test with sensitivity and specificity > 95%). The positive samples were confirmed by another serological test in Thiqar public health laboratory which is Indirect Immunoflurecent Agglutination Test (IFAT) kit, a test with sensitivity more than 95% and specificity about 100%, a cut off titer of 1:10 was used to determine the positive samples according to manufacture instructions. Positive samples are considered positive for VL. All positive subjects were followed for the maximum incubation period of VL (six months) for the appearance of VL sign and symptoms.

Operational definition

Confirmed VL case: A case that is clinically and laboratory confirmed (using IFAT) in Al Nasiriya pediatric hospital.

Contact: Under five years child lives in the same house or the next-door neighbor house.

Latent case: Asymptomatic child with positive rk39, and IFAT with a titer of 1:10, who develops symptoms during the six months follow up period.

Asymptomatic (sub-clinical) case: under five child with no VL history or sign and symptoms and test positive for rk39 and IFAT with a titer of 1:10, and did not develop signs and symptoms of VL during the six months follow up period.

Data collection tool

A questionnaire was developed and filled through direct interview with the parents or other care givers of the contacts pertaining basic demographic and epidemiological data. Blood samples were obtained by simple finger prick which was immediately examined by rk39 dipstick. Results appeared within 5 to 10 minutes. Positive asymptomatic contacts were recalled for another serological confirmatory test (indirect immunoflurecent test, IFAT) done by having another blood drop on a filter paper and examined in Thiqar Public Health Laboratory. A cut off of 1:10 was considered according to manufacturer’s instructions. Positive cases were followed up through bi-monthly assessment of the contacts (with co-operation with epidemiological investigation team of Thi-qar health directorate) for any sign or symptom of VL for six months (the maximum incubation period for appearance of VL signs and symptoms)

Statistical analysis

The prevalence of latent and asymptomatic cases was determined and their distribution by sociodemographic variables was demonstrated. Epi-info software was used for data entry and analysis. Chi-square and fisher exact test were used and P value < 0.05 was considered significant.

Ethical Issues

The study conducted after documented approval from ministry of health/Thiqar Health Directorate. Children’s family verbal consents were approved. All blood sample obtained under completely aseptic conditions.

Results

The demographic characteristics of the 250 children aged less than five years who were close contacts of confirmed VL cases are shown in table 1. One hundred sixty six (66.4%) lived in the same cases’ houses, and the remaining lived in neighbor houses. Male: female ratio was 1.2:1. The mean age was 24.6 months (± 13.5). The screened children were from five districts; 72% were from Refai and Shatra. Around 94% lived in rural areas. Regarding type of houses, 135 (54%) live in mud-made houses, 60(24%) lived in cement-block-made houses and remaining live in mixed houses. Domestic animals were present in 240 (96%) of houses of screened children. Animal shelter were present in 100 (40%) of the screened children’s houses, and sandflies were present in all 250 (100%) of the screened children’s houses.

Eighty sex children showed positive result for rk39 dipstick test; all were positive for IgG anti-leishmanial antibody by IFAT, making the prevalence of asymptomatic VL 34.4%, with 95% confidence interval of 28.4% - 40.4%.

Among males, 39% were positive compared to 29% among females; no statistically significant association with sex was found (P=0.8).

The mean age of the positive group was 25.1 months (± 13.3 months), compared to 24.4 (± 13.7 months) for the negative group, with no statistically significant difference (p > 0.05). Eighty five (36.2%) rural residents were positive compared to only one (6.7%) among urban residents; a significant association was found (P=0.02). Sixty five (39%) of household contacts were positive compared to 21 (25%) among neighborhood contacts; a statistically significant association was found (P=0.026). The prevalence of asymptomatic VL ranged between 10% in Suq-alsheiukh district to 39% in Al Refai district; no statistically significant association was found (P= 0.77). By house type, the prevalence of asymptomatic VL children was 43% among children living in mud made houses, compared to 18% among those living increment blocks made houses and 31% among those living in mixed houses, a significant association was found (P=0.003). The prevalence of asymptomatic VL was 35% among children living in houses with domestic animals compared to 10% among those without domestic animals; no statistically significant association was found (P=0.087).

The prevalence was 33% among children living in houses with animal shelter, compared to 35.3% in houses without animal shelter; no statistically significant association was found (P= 0.70).
The follow-up revealed that none of the 86 (100%) positive children became a clinical "latent" case. None of the cases were lost during the follow-up period.

**Discussion**

In Iraq, no studies were conducted before to determine the existence and the prevalence of asymptomatic VL cases, inspite of the importance of such data to complete the epidemiological picture and to understand the natural history of the disease.

The current study had confirmed the existence of asymptomatic VL with a prevalence of 34.4% (95% CI: 28.4% - 40.4%) among household and neighborhood under five years contacts of VL cases. This result is consistent with other international studies. Topno et al, 2010 in Bihar province, India showed that 36.7% of asymptomatic contacts are seropositive by using rk39 test [16]. In Brazil, Silva et al, 2011, used five serological tests: ELISA using promastigote antigen, ELISA using recombinant K39, ELISA using rk26 antigens, an indirect immunofluorescence test using promastigote antigen, and an immunochromatographic test using rk39 antigen, the prevalence was 44.7%, 42.7%, 77.6%, 12.6%, and 12.6%, respectively [22].

The seroprevalence shown in the current study, indicated that Thiqar province is a high-transmission area, as the seroprevalence in healthy populations varies from <10% in low to moderate endemic areas, to >30% in high-transmission foci [23-25].

The prevalence of asymptomatic infection among household contacts was significantly higher than among the neighborhood contacts. This is in agreement with studies conducted in India by Singh et al, 2002, in Bihar state, and Sharma et al, 2000 [26,27]. The presence of a case of visceral leishmaniasis in the family or vicinity, suggesting exposure to the same risk factors, including risk of infection between family members whether this is due to genetic or exposure factors to Leishmania parasites, remains to be elucidated. [28, 29].

The current study showed higher prevalence among children lived in mud-made houses, as compared to cemented block-made or mixed houses, as mud-made houses are a well-known risk factor for...
Leishmania infection [30,31]. Schenkel et al, 2006, in southeastern Nepal identify mud-made houses as a risk factor for VL infection (OR=3, 95% CI =1.1-7.6) [32], Ranjan et al, 2005, in India also showed that mud-plastered house walls act as a risk factor for VL [33]. Cracks and crevices appear in mud walls may function as breeding sites for the female sandflies [32,34,35].

The prevalence of asymptomatic VL showed no significant gender difference. This is consistent with studies conducted in Brazil by Moreno et al, 2006 and de Oliveira et al, 2008. [17]. Similarly, no significant association with age, as the study included only below five years children. Sakru et al, 2007 in Turkey found that about 83% of asymptomatic infection was in adult persons. Romero, et al, 2009, and Topno et al, 2010 found a positive correlation between age and prevalence of asymptomatic VL. This is mainly due to a longer period of exposure in the endemic area would increase the chance of contracting of this infection [18].

The prevalence of asymptomatic VL was varied between different districts, although not statistically significant. Adini et al, 2003 in northern Palestine also showed such variations. Geographical variations were presumed to reflect differences in parasite virulence and host population characteristics, such as nutritional status and immunogenetic factors [36, 37].

The higher prevalence of asymptomatic VL in those living in houses with domestic animals (mainly dog), can be based on epidemiological and experimental evidences, that infected dogs with signs and symptoms of leishmaniasis are the main reservoir hosts of L. infantum in Mediterranean and Middle East regions for human and susceptible animals [38-40]. Dogs can remain infected by L. infantum without displaying apparent clinical signs of VL for years even for their entire life [41]. The presence of animal shelters (particularly cattle shelters) as a risk factor is a matter of controversy. Bern et al, 2000 in Nepal, and Bern et al, 2005 in Bangladesh, found a decrease in VL risk with increased cattle density [42-44]. While a village-based study in eastern Sudan, by Bucheton et al, 2002 showed increased risk associated with cattle ownership, and presumed that animal shelters are good site for sandfly breeding [45]. Kolaczinski et al, 2008, in Uganda indicated that treating livestock with topical insecticide against ectoparasites increased kala-azar risk, suggesting a displacement of sand fly feeding to humans [46]. The protective effect of presence of animal shelters explained by Mukhopadhyay et al, 1987 when they conducted Sandfly blood-mal analysis in India, and found that; Phlebotomus feed predominantly on bovines, with humans as their second choice [47].

None of the seropositive children show overt clinical VL after six months follow-up. This is consistent with Oliveira et al, 2008 study in Brazil; they found none of the asymptomatic person progressed to symptomatic case after six months of follow up [48]. Also Silva et al, 2011 mentions that; about 3 years after the first evaluation, none of the subjects had progressed to clinical VL. [22]. But Topno et al, 2010 found that, about 18.4% of asymptomatic person progress to clinical case within 12 months [16]. Sharma et al, 2000 found that 25% of seropositive house contacts became symptomatic within three months [27].

The diagnosis of asymptomatic VL would serve as a marker of possible progression to the disease particularly in immunosuppression states [19,49,50]. As they harbor Leishmania parasites in peripheral blood, they can act as reservoirs of parasites in the transmission of disease by bites of sand flies [51].

The main limitations of the current study are inclusion of only VL endemic areas, restricted included age group, unavailability of Leishmania direct agglutination test (DAT) in Iran and not testing those with negative rk39 for IFAT test to determine proportion of false negative rk39 dipstick test.

Conclusions

Asymptomatic VL infections are frequent, among less than five years old household and neighborhood contacts of clinical VL cases in Thiqar province VL endemic areas. The prevalence shown in this study indicates that Thiqar province is considered a high- transmission foci and the transmission cycle is more than what was expected. Children living in the same cases house are more likely contracts infection, than those children living in neighbor houses. The prevalence of asymptomatic VL was higher among those live in mud-made houses, than those living in cement- block made houses. No latent VL infection was identified over a six months follow up period.

References


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