

Asymptomatic Visceral Leishmaniasis in Iraq: Prevalence and Determinants

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Visceral Leishmaniasis is one of the world's neglected tropical diseases

Endemic

>60 countries

Global Incidence

500 000 cases/year

Global Deaths

>50 000

Population at risk

350 million

The Public Health Impact of the disease worldwide is grossly underestimated

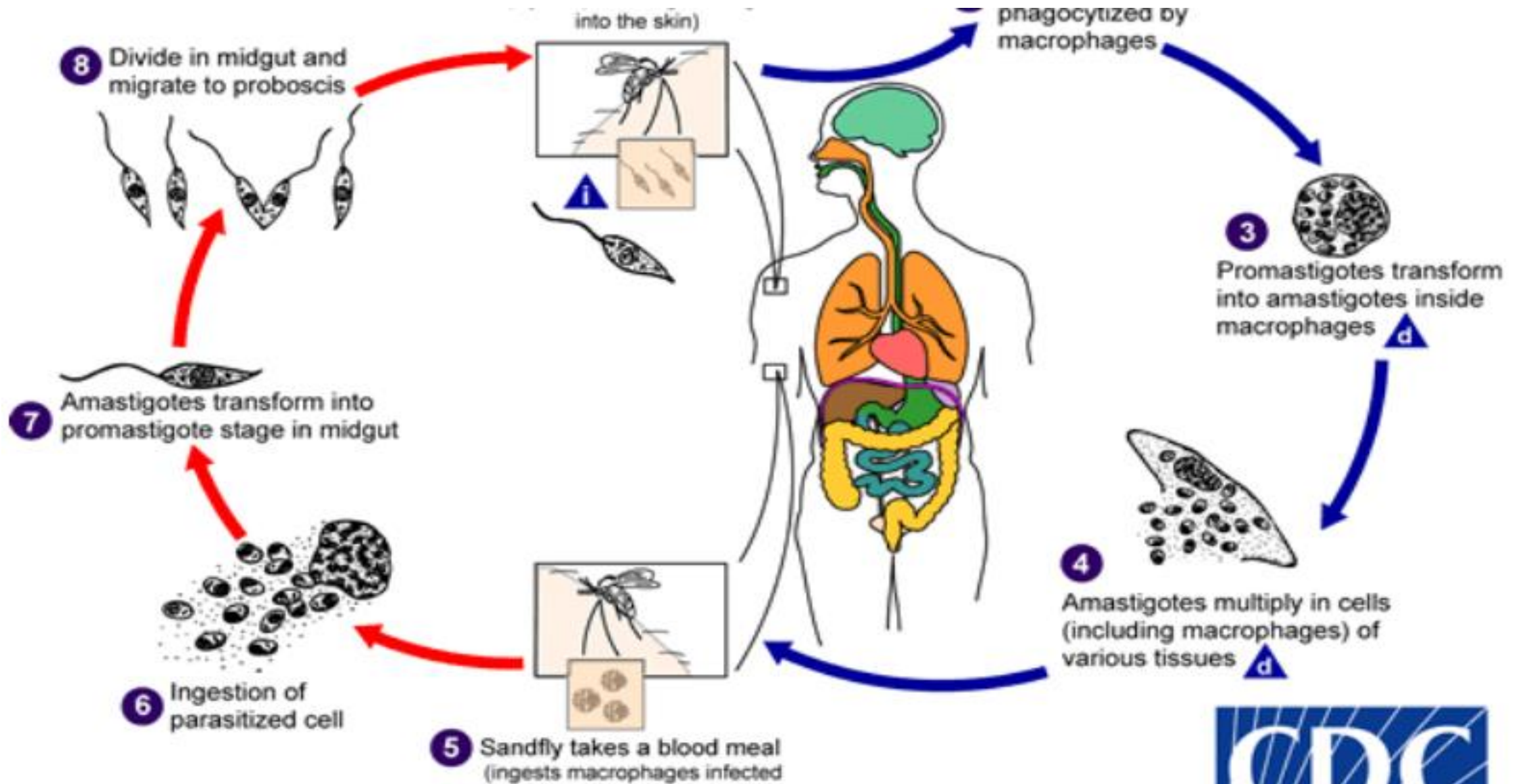
- **Notification is compulsory in only few countries**
- **# of unrecorded cases and prevalence must be substantial**

Causative Parasite of Visceral Leishmaniasis

- Various strains of *Leishmania donovani* complex
- *Leishmania donovani*, and *L. infantum*, in the old world
- *L. chagasi* in the new world
- In Iraq, *L. infantum* the main causative agent



Leishmania parasite life cycle



Mode of Transmission

- About 30 species of phlebotomine sand flies
- In Iraq, *Phlebotomus papatasi* is predominant, but *Phlebotomus sergenti* is responsible for Visceral Leishmaniasis transmission.
- From the host, sand fly takes infective blood meal with macrophages containing amastigotes
- In the fly's midgut, amastigotes transform into promastigotes
- Female phlebotomine sand flies inoculate promastigote into human skin.



Reservoirs

- Dogs, human, and other mammals
- In Iraq, Dogs are the main reservoirs



Risk Factors of Visceral Leishmaniasis

- **Living in rural area**
- **Low socioeconomic status**
- **Malnutrition**
- **Immuno deficiencies**
- **Environmental changes**
- **Living in proximity to ponds**
- **Large family size**
- **Mud-type housing**
- **Owning domestic dogs**

Spectrum of Clinical Course

- Asymptomatic infection**
- Acute self-limited febrile illness**
- Prolonged nonspecific systemic illness**
- Overt Visceral Leishmaniasis**

Clinical Manifestations

- Incubation period : 2-6 months
- Symptoms and Signs:
 - Prolonged fever
 - Weight loss
 - Hepatomegaly
 - Splenomegaly
 - Pancytopenia
 - Hypergammaglobulinemia
- Usually fatal if not adequately treated .

Differential Diagnosis of Visceral Leishmaniasis

- **Diagnosis is complex**
- **Clinical features shared by other commonly occurring diseases:**
 - **Malaria**
 - **Typhoid Fever**
 - **Tuberculosis**
 - **Brucellosis**
- **Many of these diseases can be present along with Visceral Leishmaniasis**

Laboratory diagnosis of Visceral Leishmaniasis

- **Non-leishmanial tests:**

Pancytopenia, Polyclonal hypergamaglobulinemia (formal gel test)

- **Microscopic examination of aspirates from lymph nodes, bone marrow or spleen**
- **Molecular techniques as PCR**

Antibody detection:

- **Gel diffusion**
- **Complement fixation test**
- **Indirect hemagglutination test**
- **Indirect florescent agglutination test (IFAT)**
- **The direct agglutination test (DAT)**
- **Enzyme-linked immunosorbent assay (ELISA)**
- **Immunochromatographic test (ICT)**

Treatment: Currently 4 drugs available for Visceral Leishmaniasis, but only one used in Iraq

- **Pentavalent antimonials: first-line treatment for over 70 years; the only drug used in Iraq**
- **Miltefosine: Oral drug**
- **Amphotericin B: used in two formulations: “Conventional” Amphotericin B and “Liposomal” Amphotericin B (Ambisome)**
- **Paromomycin (PMM)**

Epidemiology of Visceral Leishmaniasis in Iraq

Wide extension of affected area over the last 25 years

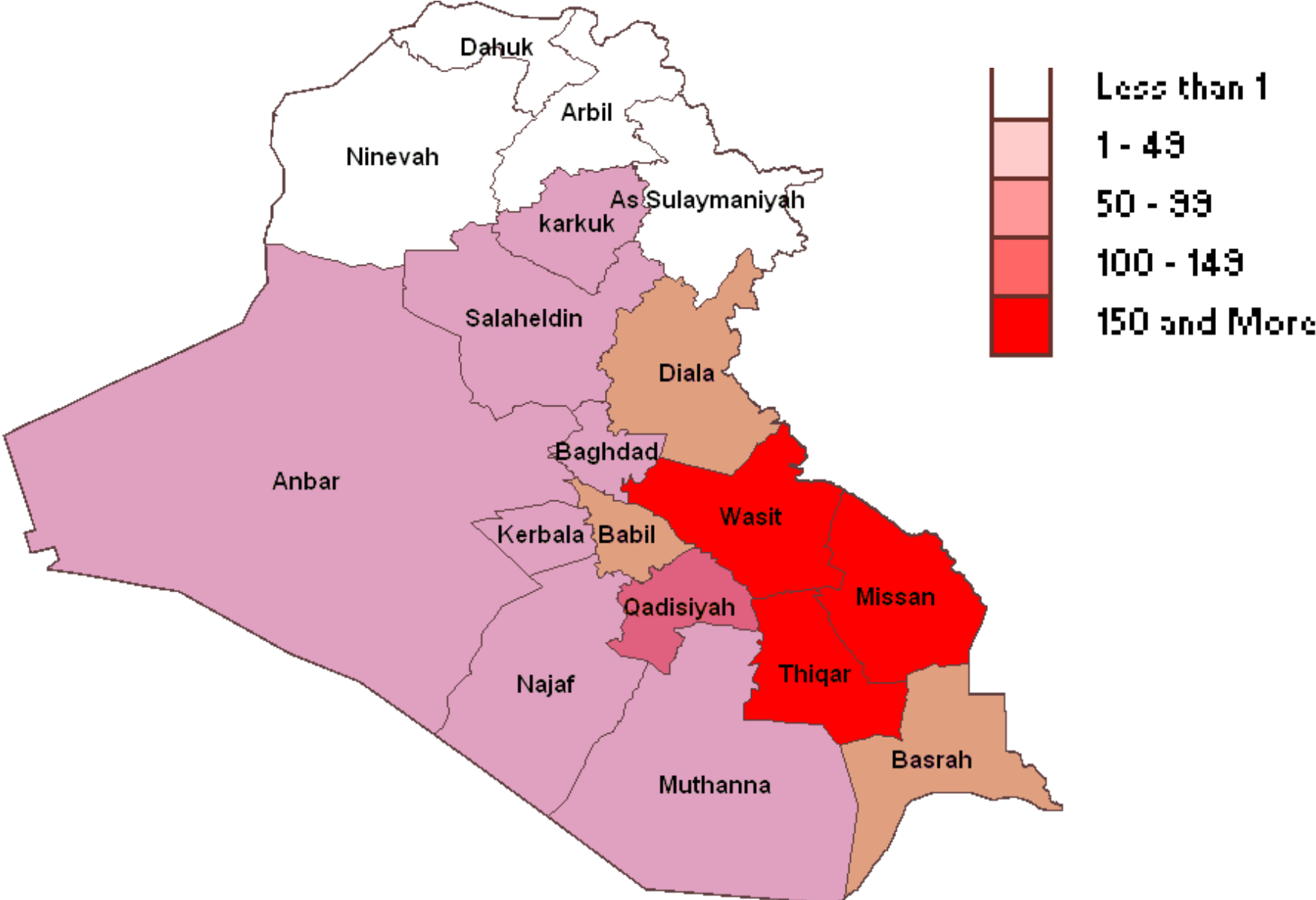
- Historically, the areas surrounding the Capital Baghdad within 30-40 Km radius were the most affected zone**
- Since 1991, Visceral Leishmaniasis widely extended to new zones as the southern provinces of Iraq (Missan, Thi qar, Wasit and Basrah)**

The reported incidence of Visceral Leishmaniasis in Iraq per 100,000 under 5 year old children:

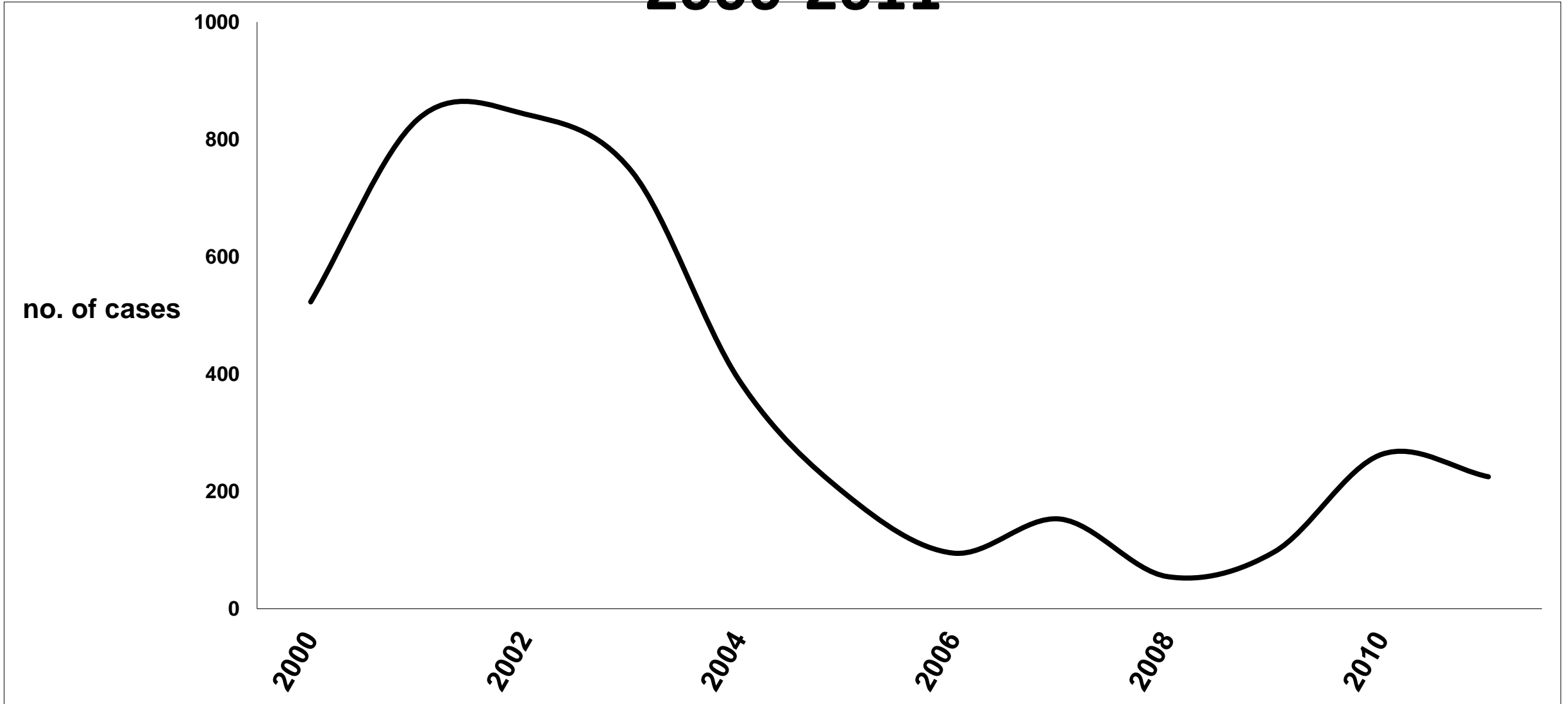
Year	Incidence
1989	2.6
1992	20
2001	109
Thiqar Province, 2002	360

18,665 Visceral Leishmaniasis cases reported in Iraq in the last 10 year; 16% (3,017) in Thiqar province

Visceral Leishmaniasis rate per 100,000 by province, Iraq, 2002-2011.



Visceral Leishmaniasis trend, Thi qar Province, 2000-2011



In Iraq, Visceral Leishmaniasis is a disease of childhood

<5 years	90%
<1 year	40%
Case Fatality Rate	3%

Asymptomatic Visceral Leishmaniasis

- **In Visceral Leishmaniasis endemic areas only 20% of infected subjects will have symptomatic Visceral Leishmaniasis**
- **Majority of infected individuals will have a sub-clinical infection that may remain completely asymptomatic**
- **In Iraq, No Data about asymptomatic Visceral Leishmaniasis**

Objectives

Determine the: existence, prevalence and risk factors of asymptomatic Visceral Leishmaniasis among contacts of clinical Under 5 years Visceral Leishmaniasis cases, Thiqr Province, Iraq, 2012

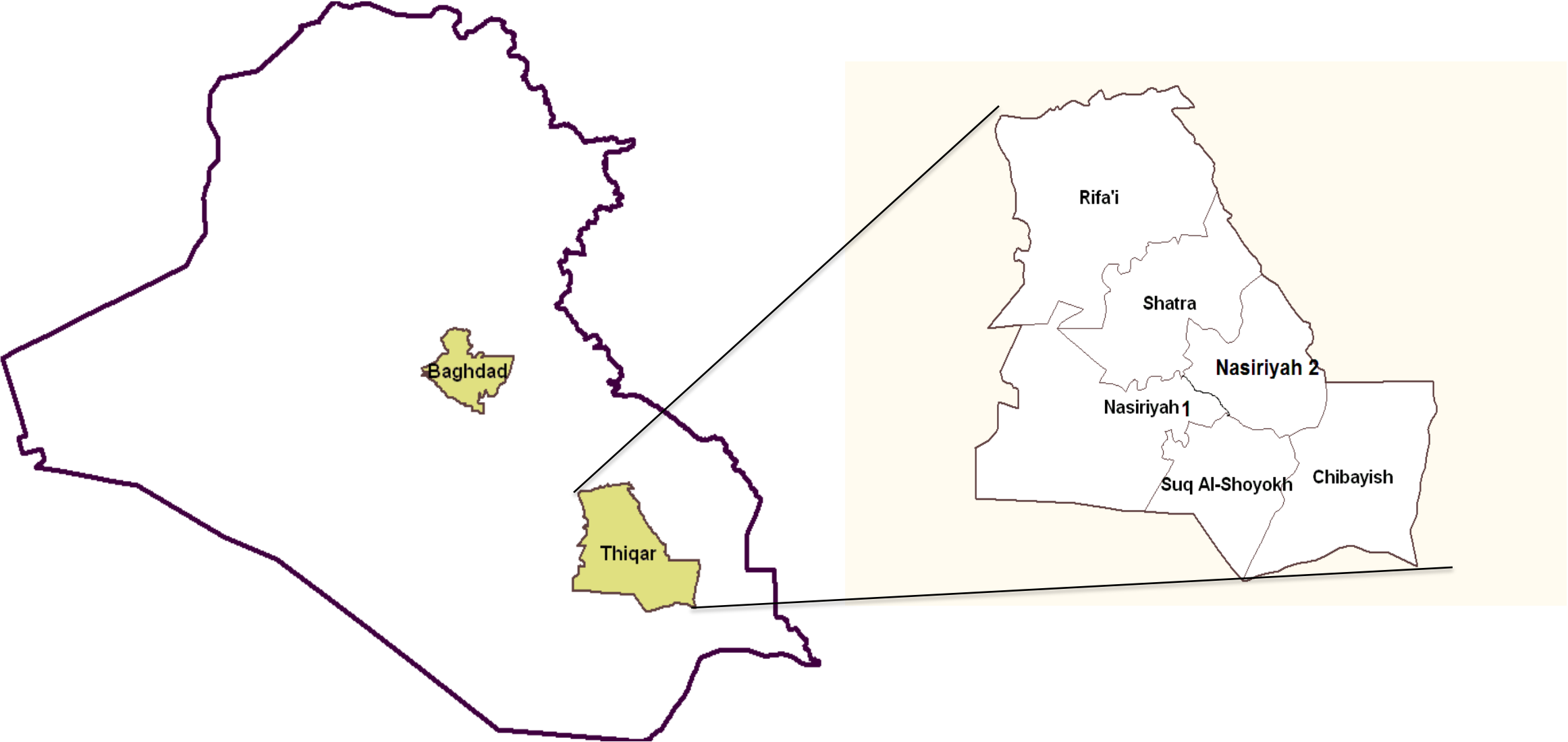
Subjects and Methods

Study design	A screening cross sectional study
Setting	Five out of the six health districts of Thiqar province (about 1,800,000 inhabitants)
Data Collection Time	Feb. 15th to Mar. 31st , 2012, and a follow-up for clinical findings done every two months for six months .

Study Population and Sample Size:

- The last reported 50 confirmed Visceral Leishmaniasis cases that were admitted to the only Pediatrics Hospital inThiqar province**
- For each case, five healthy contacts living in the same house or in the nearest neighborhood were included in the study.**
- The total was 250 contacts**

Iraq map show Thiqar province location and districts



Inclusion Criteria

- **No sign or symptoms of Visceral Leishmaniasis on enrollment into the study**
- **No history of Visceral Leishmaniasis**
- **less than five years old**
- **Live in the same house or in the closest neighborhood**

Methods

- **Contacts screened for Visceral Leishmaniasis using the dipstick rk39 rapid leishmaniasis test (sensitivity > 95%. and specificity about 100%)**
- **Positive samples confirmed by indirect immunofluorescent agglutination test (IFAT); sensitivity >95% and specificity about 100%**
- **All positive subjects were followed every two months for the maximum incubation period of Visceral Leishmaniasis (six months) for possible appearance of Visceral Leishmaniasis sign and symptoms.**

Operational Definitions

- **Asymptomatic case**: under five years contact who test positive for rk39 and IFAT, and did not develop signs and symptoms of Visceral Leishmaniasis during the six months follow up period
- **Latent case**: Asymptomatic child with positive rk39, and IFAT who develops symptoms during the six months follow up period.
- **Confirmed case**: A case that is clinically and laboratory confirmed (using IFAT)

Data Collection Tool

- **A questionnaire developed and filled by the investigator through direct interview.**
- **Blood samples obtained by finger prick and immediately examined by rk39 dipstick .**
- **Positive asymptomatic contacts recall for another serological confirmatory test which is IFAT.**

Analysis Plan

- Prevalence of latent and asymptomatic cases was estimated
- Association with basic and housing variables was studied
- Epi-info used for data entry and analysis
- Chi-square and Fisher's exact test used
- P value < 0.05 considered significant.

Ethical Approval

The study conducted after granting approval of:

- Ministry of Health/Thiqar Health Directorate (written)**
- Children's family (verbal)**
- Blood sample taken under completely aseptic conditions**

Demographic characters of the 50 selected VL cases, Thiagar, 2012

Characters	Frequency	%
<u>Gender</u>		
•male	30	60%
• Female	20	40%
<u>Age group</u>		
•0-12 months	28	56%
•13-59 months	22	44%
<u>Residency</u>		
•Rural	47	94%
•Urban	3	6%
<u>House type</u>		
• cement- block	12	29%
• Mixed	11	21%
•Mud	27	50%

Demographic characters of the 50 selected VL cases, Thiqar, 2012

Characters	Frequency	%
<u>District</u>		
•Chebaish	11	22%
•Nasiriyah	2	4%
•Refai	19	38%
•Shatra	17	34%
•Suq-alsheuikh	1	2%
<u>Presence of domestic animal</u>		
•Yes	48	96%
•No	2	4%

Comparison between VL cases and the contact

Characters	Cases (N=50)	Contact (N=250)
Age range	3 – 36 months	6- 48 months
Male to Female ratio	1.5: 1	1.2: 1

Main Outcomes

Characteristics	No	%
Children show positive result for rk39 dipstick test	86	34.0
Children positive for rk39 and positive for IgG antileishmanial antibody by IFAT	86	100.0
Latent Visceral Leishmaniasis	0	0.0
Children lost to follow-up	0	0.0

Distribution of study group by lab. results and demographic character

Characters	Frequency N (%)	Seropositive N (%)	Prevalence Ratio	P value
<u>Gender</u>				
• Male	134 (46)	54(39)	1.69	0.8
• Female	112(54)	32(29)		
<u>Age groups</u>				
0—12 months	83(33)	29(35)	1.03	0.90
13—59 months	167(67)	57(34)		

Distribution of study group by lab. results and residence and house type

Characters	Frequency N (%)	Seropositive N (%)	Prevalence Ratio	P value
<u>Residency</u>				
Rural	235(94)	85(36)	85	0.02
Urban	15(6)	1(7)		
<u>House type</u>				
Cement-Block	60(29)	11(18)	5.2	0.003
Mixed	55(21)	17(31)	3.4	
Mud	135(50)	58(43)		

Distribution of study group by lab. results and type of contact and district

Characters	Frequency N (%)	Seropositive N (%)	Prevalence Ratio	P value
<u>Type of contacts</u>				
Household	166(69.5)	65(39)	3.1	0.026
neighborhood	84(30.5)	21(25)		
<u>Districts</u>				
Chebaish	55(22)	17(31)		
Nasiriyah	10(4)	3(30)		0.82
Refai	95(38)	37(39)		
Shatra	85 (34)	28(33)		
Suq alsheukh	5(2)	1(20)		

Distribution of study group by lab. results and presence of sand flies and domestic animals

Characters	Frequency N (%)	Seropositive N (%)	Prevalence Ratio	P value
<u>Presence of sandfly</u>				
Yes	240(96)	85(35.4)	85	0.087
No	10(4)	1(10.0)		
<u>Presence of domestic animals</u>				
Yes	100(40)	33(33)	0.93	0.70
No	150(60)	53(35.5)		

Asymptomatic Visceral Leishmaniasis is existent in Iraq as in other endemic countries

- Around one third of close contacts had asymptomatic Visceral Leishmaniasis infection**
- Thiqr province is a high-transmission area, as the seroprevalence in a healthy under 5 population >30%**

The results of current study are consistent with other studies in different countries

- In India Topno et al, 2010 found that 37% of the asymptomatic contacts is seropositive by using rk39 test**
- In Brazil endemic areas Silva et al, 2011, the prevalence was 13%.**

Household versus Neighborhood Contacts

- In agreement with other studies, the prevalence of asymptomatic Visceral Leishmaniasis was higher among household contacts than neighborhood contacts
- Singh et al, 2002, Bihar state, India used recombinant K39 (rK39) antigen, found the prevalence among family contacts: 44% and among neighbors contacts: 33%
- Genetic factors or exposure factors??

Mud Made Houses

- **A higher prevalence in children live in mud-made houses**
- **Consistent with other studies. (Schenkel et al, 2006 and Ranjan et al, 2005)**
- **Cracks and crevices appear in mud walls may function as breeding sites for the female sandflies**

Do we need to treat asymptomatic cases?

- **No**
- **No drug is currently safe enough to treat asymptomatic infections**

Limitations

- **Inability to do invasive lab tests as confirmatory test and lack of PCR for VL at national level**
- **The study done in one province**
- **Incomplete environmental assessment for density of sand flies**

Conclusions

- 1) Asymptomatic Visceral Leishmaniasis is common among contacts of clinical Visceral Leishmaniasis cases**
- 2) Household contacts and living in mud made house increase the risk**
- 3) No latent Visceral Leishmaniasis infection**

Public Health Impacts

- **Screening for asymptomatic infection is part of the epidemiological investigation.**
- **Unlike previous years, the control measures now depend on vector density rather than appearance of symptomatic cases**

Recommendation

- 1) Further studies involving older age groups, and non contacts of Visceral Leishmaniasis cases**
- 2) Screening and follow-up of under five contacts of Visceral Leishmaniasis cases**
- 3) Screening survey for asymptomatic Visceral Leishmaniasis infections, in other endemic and non-endemic areas**

Acknowledgement

- **Zoonotic disease section, Iraq CDCC**
- **PHC section, Zoonotic diseases unit, and PH Lab in Thiqr Province**
- **Annasiriyah Pediatrics Hospital, Thiqr Province**
- **R. Fountain and T. Nagachinta, US CDC**



Thank you