#301

William Beaumont Army Medical Center

A Stealth Parasite: Prevalence and Characteristics of Risks for Latent Visceral Leishmaniasis in a Cohort of US Soldiers deployed to Operation Iraqi Freedom (OIF)

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Abstract

Background: Leishmaniasis is a zoonotic parasitic disease transmitted by sand fly bites. Visceral leishmaniasis (VL) is a chronic intracellular infection which, when symptomatic, can be fatal without therapy. Subclinical or latent VL may occur in a majority of those infected with lifelong risk of activation when immunosuppressed. Symptomatic VL has been described in Soldiers deployed to Operation Iraqi Freedom (OIF). We report the prevalence and risk characteristics of latent VL infection in OIF Soldiers.

Methods: Healthy Soldiers deployed during summer months (2002-2011) to VL endemic areas of Iraq were recruited from Fort Bliss, Texas. Responses to a risk factor survey and blood samples were obtained. *Leishmania* research diagnostics were performed on serum and/or white blood cells to include ELISA, rk39 immunochromatography, qPCR, and interferon gamma release (IGRA) assays. Analyses included descriptive percentages and other summary statistics. Fisher's Exact test and logistic regression were used for group comparisons.

Results: Out of 88 subjects enrolled, 76/88 (86%) were male with median age 39 years and deployment duration of 365 days. The prevalence of latent VL was 10.2% (CI 4.8%-18.5%) with 7 IGRA positive and 2 ELISA positive. Travel to Ninewa governate correlated with VL, p<0.05. No significant differences were noted in occupation, personal protective measures, deployment timeframe, or sleeping conditions between VL positive and negative individuals. In persons with latent VL, 4/9 (44.4%) and 6/9 (66.7%) deployed to Ninewa and Baghdad respectively, 7/9 (77.8%) were outdoors most nights, 5/9 (55.6%) slept on the ground during deployment, 5/9 (55.6%) were medical personnel, 7/9 (77.8%) slept in less than full uniform, and 8/9 (88.9%) never or rarely used insect repellent.

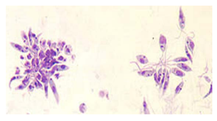
Conclusions: Latent VL was identified in asymptomatic OIF Soldiers (10.2%). Travel to Ninewa governate correlated with VL latent infection. In the latent VL group, many were healthcare workers, slept on the ground or in less than full uniform, and rarely used insect repellent. Further studies are needed to inform risk of reactivation disease in latently infected US Soldiers and to target measures for broader surveillance and safety, such as the screening of military blood donors.

Background

- Visceral Leishmaniasis (VL): zoonotic and parasitic disease endemic in Southwest Asia
  - Transmitted by the sand fly vector (Genus *Phlebotomus* and *Lutzomyia*) with the primary infecting species *Leishmania infantum* in Iraq
  - Can manifest in a spectrum as asymptomatic (Latent Visceral Leishmaniasis or LVL) infection to symptomatic overt disease (estimated ratio of 1:100)
  - Disease manifestations: chronic fever, weight loss, hepatosplenomegaly, and pancytopenia
- Epidemiology
  - Iraq: In Basrah up to north of Baghdad (Figure 1B), with VL rates ranging from 1-22%[1]
  - OIF is a protracted armed conflict that started in 2003, in which over 373,000 Army Soldiers have deployed [2]
  - In U.S. Armed Forces: 25 cases of visceral leishmaniasis, 1186 cases of cutaneous leishmaniasis reported from 2003-2011[3]
- Why is this a concern?
  - Uncertain prevalence of latent disease in deployed military personnel
  - Potential concern for activation with biologic disease-modifying drug usage, organ transplant, chemotherapy agents, and AIDS
  - Rates of progression to symptomatic VL vary from 7.9-35.6% in asymptomatic seropositive individuals, while symptomatic VL relapses in transplant patients were as high as 25.7%[4]
- **Study Aims:**
  - **Measure LVL prevalence in OIF deployers in Fort Bliss, TX.**
  - **Identify VL infected individuals using research blood assays**
  - **Query subjects for risk factors for VL infection**

A

B



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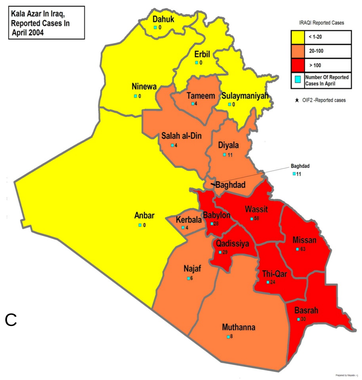


Figure 1. (A) *Phlebotomus* fly taking a blood meal and (B) *Leishmania* promastigotes in culture (CDC). (C) Reported cases of visceral leishmaniasis in Iraq, Jan-Apr 2004 (Iraqi Ministry of Health)

Materials and Methods

•**Study Design and Selection:** A surveillance study with A) blood sample and B) risk factor survey assessing for sand fly exposure risks. The study was conducted from October 2016 to January 2017 at Fort Bliss, El Paso, TX. This is part of a multi-site cohort study that also included the U.S. Pentagon and the Walter Reed National Medical Center in the National Capitol Region (Washington DC/Bethesda, MD). Volunteers enrolled were screened for higher risks of vector exposures based on timeframe and location of deployments utilizing data on VL distribution provided by the Iraqi Ministry of Health (in Figure 3). 93 OIF deployers screened, 88 OIF deployers enrolled

•**Inclusion/Exclusion Criteria**

- **Inclusion**: Department of Defense beneficiary, deployed *to* leishmaniasis endemic areas of Iraq for at least one month between May to September in the years 2002-2011, experienced outdoor evening exposure regularly
- **Exclusion**: 3rd trimester of pregnancy, history of HIV, cancer or chemotherapy in past two years, solid organ transplant recipient, receiving immune modifying medications, received blood transfusion after return from Iraq

• **Lab Analysis:** LVL infection was determined using standard of care rK39 immunochromatographic testing (Kalazar Detect™,InBios), ELISA[5], quantitative PCR[6], and Interferon Gamma Release Assay (IGRA)[6]. Initial lab processing was conducted at WBAMC, with diagnostic studies performed at USUHS. Chagas serology on ELISA positive samples was done to rule out cross reactivity with *Trypanosoma cruzi* infection.

• **Statistical Analysis:** LVL infection was defined as a positive result for any of the above four lab results. Descriptive data analysis was applied involving proportions (percentages), and means. Fisher's Exact test was used to compare answers to survey questionnaire between respondents who were VL positive and negative. Logistical regression was used to determine correlations between deployment frequency to locations in Iraq.

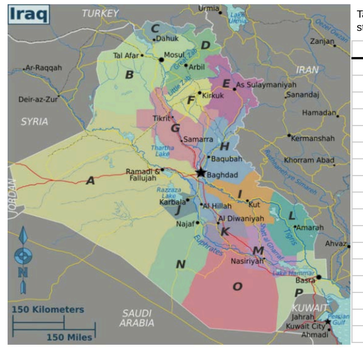


Table 2. Deployment geographic distribution (% deployed in province/governate) (n=88). *p<0.05, statistically significant by Logistical regression

Map Symbol	Governate	Leish Positive	Leish Negatives	P Value
★	Baghdad	66.7	54.4	0.6634
Anbar	A	33.3	12.7	0.7777
Ninewa	B	44.4	16.5	0.0473*
Dohuk	C	11.1	0	not calculated
Arbil	D	11.1	2.5	0.0871
Al-Sulaimaniyah	E	33.3	37.9	0.1066
Kirkuk	F	33.3	8.9	0.1208
Saladdhin	G	33.3	37.9	0.2914
Diyala/Babylon	H	22.2	46.8	0.3714
Wasit	I	33.3	18.9	0.9764
Karbala	J	11.1	6.3	0.6862
Qadislyah	K	11.1	21.5	0.5787
Misan	L	22.2	7.5	0.0808
ThiQat	M	11.1	16.5	0.7246
Al-Najaf	N	0	7.5	0.8384
Al-Muthanna	O	11.1	10.1	0.8839
Basrah	P	11.1	16.5	0.6106

Results

Figure 2. Demographic characteristics of the subject population presented by relative percentage. [Numbers denote percentage per category]

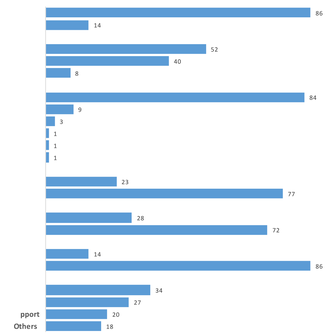
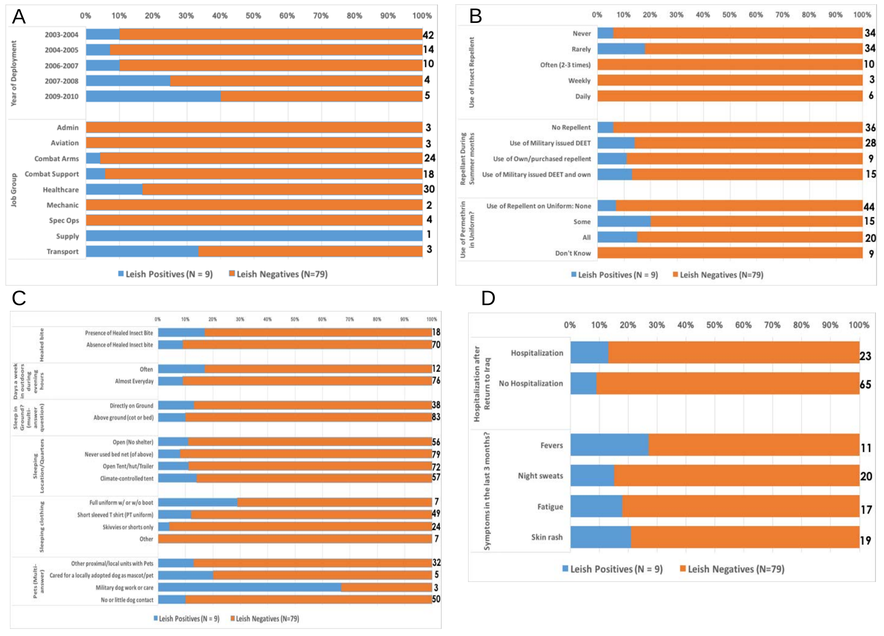


Table 1. Laboratory testing results by type of test using sera obtained at the time of survey. No cross reactivity with *T. cruzi* on further testing.

Tests	N (%)
Number of Total Subjects	88
Negative	79 (89.8%)
rk39	0
Leishmania ELISA+	2 (2.3%)
Leishmania IGRA+	7 (7.9%)
Leishmania qPCR+	0
Total Positive	9 (10.2%)

Figure 3. Prevalence of risk factors of leishmaniasis in positive and negative deployers. (A) Year of deployment and job description. (B) Use of insect repellent and permethrin impregnated uniforms. (C) Sleeping attire and locations, presence of pets/animals. (D) Post deployment symptoms and hospitalizations. Numbers on right indicate the number of respondents in each of the queried categories. All potential associations were p = Not Significant (NS), (Fisher's exact).



Discussion and Implications

- **Approximately 10.2% of tested subjects were positive for LVL.** Ft. Bliss cohort is part of a larger cohort in a multi-site study in the U.S. Armed Forces.
- Previous rate at 1.2% of overt VL in U.S. Armed Forces from 2001-2016[3]
  - Potentially large number of undiagnosed deployed military members
- No evidence for association of LVL infection with:
  - Standard demographic or occupational characteristics
  - Year of deployment
  - Use of preventative countermeasures
  - Animal encounters
  - Current reported symptoms or evidence of illness
- Deployment to **Ninewa governate**, (includes Mosul) conferred increased risk of *Leishmania* infection
  - Possible reasons: geographic factors, differences in Soldier and/or vector activity, differences in methodology/underreporting [8]
  - LVL geographic risk may have been differently distributed compared to initial reports from Iraq Ministry of Health
- Study Limitations
  - Low sample size
  - Experimental assays with performance parameters being evaluated
  - Potential recall bias
  - Multiple deployments and potential travel to *Leishmania*-endemic areas not related to deployments
- Higher risk of VL reactivation in infected if ever immunosuppressed
  - Possible need for testing prior to start of therapy and/or vigilant monitoring?
- Additional prospective studies planned:
  - Evaluate burden of LVL in currently deployed Soldiers
  - Longitudinal assessment of risks and clinical consequences of VL reactivation

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