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Asymptomatic Visceral *Leishmania infantum* Infection in US Soldiers Deployed to Iraq

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Background. Visceral leishmaniasis (VL), due to *Leishmania infantum,* is a persistent intracellular parasitic infection transmitted by the bite of infected sand flies. Symptomatic VL has been reported in U.S. soldiers with Iraq deployment. Untreated symptomatic VL can be fatal; asymptomatic VL (AVL) may establish a lifelong risk of reactivation. We report prevalence and AVL risk factors in Operation Iraqi Freedom (OIF) deployers during 2002–11.

Methods. Healthy soldiers exposed to VL endemic areas in Iraq and 50 controls who never traveled to endemic regions were recruited through military healthcare facilities (2015–17). Responses to a risk factor survey and blood samples were obtained. *Leishmania* research diagnostics utilized included enzyme-linked immunosorbent assay (ELISA), rk39 test strips, quantitative polymerase chain reaction (PCR), and interferon gamma release (IGRA) assays. Statistical analyses included Fisher exact test, Pearson χ^2 test, Mann-Whitney *U* test, and logistic regression.

Results. 200 deployed subjects were enrolled, mostly males (84.0%), of white ethnicity (79.0%), and median age 41 (range 24–61) years. 64% were seropositive for *Phlebotomus alexandri* saliva antibodies. Prevalence of AVL (any positive test result) was 39/200 (19.5%, 95% confidence interval 14.4%–25.8%). Two (1.0%) PCR, 10 (5%) ELISA, and 28 (14%) IGRA samples were positive. Travel to Ninewa governorate increased risk for AVL ($P = .01$).

Conclusion. AVL was identified in 19.5% of OIF deployers; travel to northwest Iraq correlated with infection. Further studies are needed to inform risk for reactivation VL in US veterans and to target additional blood safety and surveillance measures.

Keywords. visceral leishmaniasis; asymptomatic; US soldiers; deployed; Iraq.

In Iraq, a bite from an infected sand fly (*Phlebotomus alexandri*) can transmit *Leishmania infantum*, the parasite that causes visceral leishmaniasis (VL) [1, 2]. No prophylactic medicine or vaccine exists. North Americans typically have no protective immunity; the deployment of over 1 million US service members to Iraq potentially exposed many to *Leishmania* [3]. This parasite can persist inside human macrophages lifelong. Activated VL manifests as a chronic illness with fever, weight loss, cytopenias, and hepatosplenomegaly. Host immune responses (similar to tuberculosis) hold latent VL in check. Reported risks for activation in European/ Brazilian adults include biologic response modifying agents, human immunodeficiency virus (HIV), organ transplant, poorly controlled diabetes, and alcohol abuse [4–8]. Untreated active VL has been associated with >90% mortality but can be successfully treated with liposomal amphotericin [9, 10].

In the past decade, asymptomatic visceral leishmaniasis (AVL) has been recognized as common in endemic areas with infection to disease ratios ranging from 50–100:1 in Spain [11], 8.9:1 in India, Nepal [12, 13], 13:1 in Iran [14] and 6.5:1 to 89:1 in Brazil [15–17]. In Iraq, among asymptomatic close contacts of children with active VL, 86/250 (34%) tested rK39 and *Leishmania* seropositive [18]. The risk of asymptomatic infection in healthy travelers from non-endemic areas, such as deployed military, is poorly understood [19]. Although cases of cutaneous and active visceral leishmaniasis have been described in US soldiers deployed to Iraq, chronic asymptomatic visceral infection with *L. infantum* has not been previously recognized in US forces [20, 21]. Due to risk of AVL reactivation among soldiers with service in Iraq, we investigated 200 higher risk military persons approximately 11.3 years after Iraq deployment studying blood samples with multiple assays to include rK39 test strips, *Leishmania* enzyme-linked immunosorbent assay (ELISA), *Leishmania*

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interferon gamma release assay (IGRA), and quantitative polymerase chain reaction (qPCR) measuring blood parasite load.

METHODS

Ethical Considerations

This research protocol was approved by the Uniformed Services University (USU), Walter Reed National Military Medical Center (WRNMMC), William Beaumont Army Medical Center (WBAMC), and the Food and Drug Administration (FDA) Institutional Review Boards (IRB). All participants provided written informed consent.

Patient Population

Subjects completed a risk factor survey and donated blood samples for VL research assays. Enrollment occurred at WRNMMC/USU in Bethesda MD, DiLorenzo Tricare Health Clinic-Pentagon, Washington, DC, and WBAMC in El Paso TX. Subjects were US military personnel in good health, age 18–60 years, who traveled to Iraq between 2002–11. Higher likelihood of sand fly exposure was selected by requiring at least one summer month spent in Iraq, at least three nights per week spent outdoors, and deployment to governorates where the Iraq Ministry of Health reported VL (Figure 1A courtesy of Eric Milstrey). Exclusion criteria included receiving blood transfusion since leaving Iraq and underlying immunosuppressive conditions. Controls were healthy individuals aged 18–60 years who had no life-long travel to areas endemic for leishmaniasis.

Risk Factor Survey

Participants completed a 20-item questionnaire detailing demographic and military information, dates and details of first deployment to endemic areas of Iraq, including risk factors for exposure to sand flies. Participants were queried about other deployments to VL endemic areas, postdeployment hospital admissions, and systemic symptoms occurring in the 3 months preceding enrollment.

Serum and Risk Factor Data From the Immediate Iraq Deployment Period

Concurrent data about personal protective measures (PPM) and medications collected in the immediate postdeployment period were obtained from the Defense Medical Surveillance System

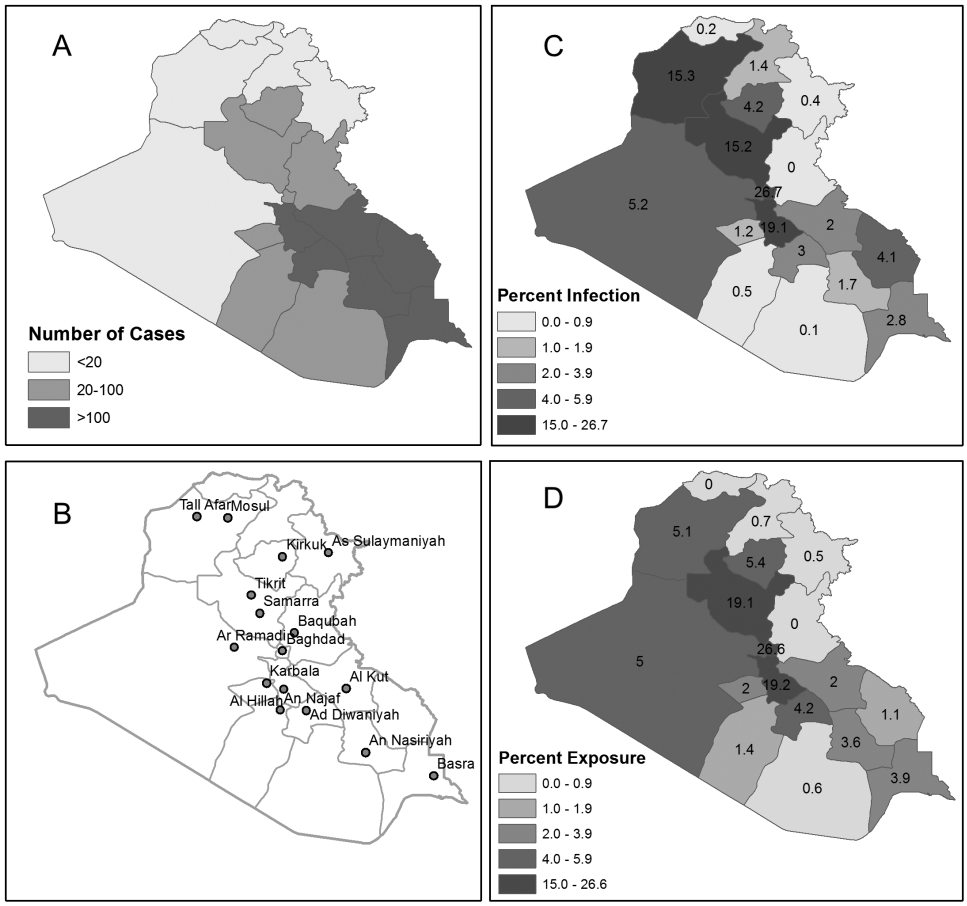

Figure 1. Figure 1 shows distribution of reported Iraqi Visceral leishmaniasis cases in 2004 by goverante (*A*); major cities in Iraq (*B*); distribution of deployed locations (as % time spent) for US infected (n = 39) (*C*); and US not infected (n = 161) (*D*). * $P = .01$, Mann-Whitney *U* test comparing person time fractions spent in Ninewa governate (15.3 vs. 5.1).

(DMSS) [22]. Matched, banked serum specimens provided within 6 months of the end of the first Iraq deployment were retrieved from the Department of Defense Serum Repository (DoDSR), The Armed Forces Health Surveillance Branch, US Department of Defense, Silver Spring, MD; release dates 2015–17.

Parasite Culture and Antigen Preparation

L. infantum parasites (MHOM/BR/00/1669, courtesy Dr. M. Wilson, University of Iowa) were grown at 26° C in hemoflagellate modified minimal essential medium with 10% fetal bovine serum until reaching stationary phase [23, 24]. Soluble *Leishmania* antigen (SLA) was prepared as described [25]. SLA was tested for endotoxin using Limulus lysate assay (Lonza) with measured level of 0.38 EU/ug.

Interferon Gamma Release Assay

One mL of whole blood or 1 × 10^6 peripheral blood mononuclear cells (PBMC) were stimulated with either 20 ug/mL SLA or 10% phytohemagglutinin (PHA, Life Technologies) for 72 hours at 37° C, 5% CO2. Supernatants were collected and tested in duplicate using human interferon gamma (IFN-γ) ELISA kits (Ready-SET-Go, eBioscience). Assay cutoff was determined by calculating mean of IFN-γ in control subjects plus 2 standard deviations (SD).

Soluble *Leishmania* Antigen ELISA

SLA ELISA was performed as previously described [26] with modification. Coating was done with 0.5 ug/well of SLA, serum samples were diluted 1:400, and secondary antibody was 1:10 000 goat anti-human IgG-HRP (Southern Biotech). Reaction was developed in the presence of 3,3′,5,5′tetramethylbenzidine substrate for 30 minutes, then stopped (KPL, Inc.). ELISA cutoff was defined as the average optical density (OD) of control subjects plus 3 SD.

rK39

Reactivity against rK39 was tested using the immunochromatographic Kalazar Detect™ Rapid test (InBios).

L. infantum Real-time Quantitative PCR

DNA was extracted from 5 × 10^6 PBMC using the DNAeasy Blood & Tissue Kit (Qiagen) with modification. Samples were incubated overnight at 56°C in the presence of proteinase K and AL buffer, and DNA was eluted in 50 uL H_20. The target REPL repeat was amplified from 5 uL DNA as previously described [27].

Phlebotomus (Paraphlebotomus) alexandri Collection and Salivary Gland Homogenates ELISA

Sand fly collections were conducted using CDC light traps (John W. Hock Company) at Waqqa, in the north Jordan Valley, Jordan, in August 2016. Salivary glands from *Ph. alexandri* females were dissected at Jordan University of Science and Technology, frozen at −20 °C, and shipped to USU [28, 29]. Salivary gland homogenate (SGH) ELISA was performed as previously described with modification; coating was done with 0.1 ug SGH/well, sera 1:200 dilution, and secondary antibody at a 1:5000 dilution [30]. ELISA cutoff was defined as the average OD of control subjects plus 3 SD.

Statistical Analysis

A sample size of 200 deployers was chosen to determine deployed population VL prevalence within 5% bounds of a 15% point estimate. Categorical data were analyzed using Fisher exact and χ^2 tests [31], whereas continuous data were analyzed using independent samples *t*-test or Mann-Whitney *U* test. The level of statistical significance was set at $P < .05$. Univariate logistic regression models (using SPSS 24) were constructed to screen for potential confounders among risk factors associated with VL infection at *P*-value threshold <.25.

RESULTS

Population

In sum, 250 volunteers were enrolled: 200 OIF deployers and 50 controls. The majority of deployers were males (84.0%) of white race or ethnicity (79.0%) with median age 41 (range 24–61) years. Most served in the Army (88.5%) as enlisted soldiers (64.5%). Average time since first deployment to Iraq was 11.3 years (range 5–14). Controls were mainly white (72.0%) males (74.0%) with median age 32 years (range 19–58). Four control subjects were excluded from the final analysis: 1 was unable to obtain blood, 1 did not reveal full travel history initially, and 2 had elevated not stimulated IFNγ levels and did not reach threshold for adequate stimulation.

Laboratory Assays

In this study, AVL was defined as a positive result on any test. Out of 200 OIF deployers, 39 (19.5%; 95% confidence interval [CI]: 14.4%, 25.8%) had positive test results using *L. infantum* assays conducted with blood samples obtained at enrollment (remote from OIF deployment). None had positive rK39 immunochromatographic test results. Two (1.0%) deployers were qPCR positive (quantified as 5 and 15 parasites/mL), 9 (4.5%) tested SLA ELISA positive, 27 (13.5%) tested *Leishmania* IGRA positive; 1 person (0.5%) was both ELISA and IGRA positive. IFN-γ levels in deployers and controls are shown in PBMC versus whole blood assays (Figure 2A and 2B). Figure 3 shows antibody levels measured by SLA ELISA comparing deployers versus controls. We cultured buffy coat samples from PCR positive individuals but were not able to isolate *Leishmania.* Four OIF deployers had a history of cutaneous leishmaniasis with IGRA tests positive with higher IFN-γ levels after *L. infantum* SLA compared to *L major* SLA stimulation (data not shown). Three controls were IGRA positive. One control had positive SLA ELISA; all sera with positive SLA ELISA showed no cross reactivity with ELISA for *T. cruzi* (Quest Diagnostics).

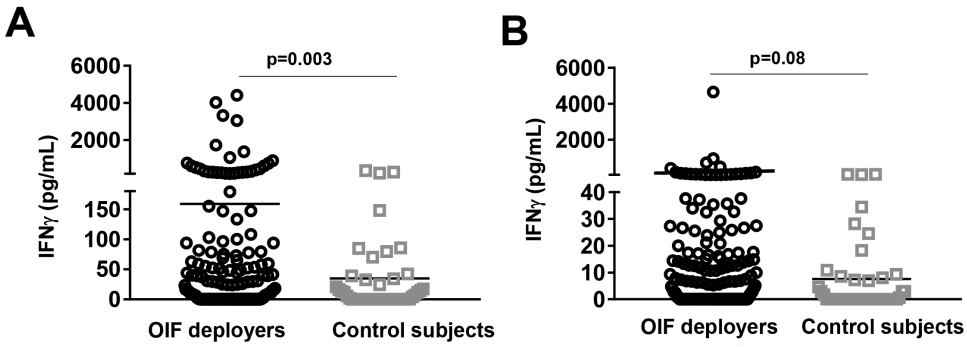

Figure 2. IFN-γ levels in SLA proliferated PBMC and whole blood. IFN-γ (pg/mL) production by (A) PBMC or (B) blood stimulated with *L. infantum* SLA in Iraq deployers (n = 200) and not-exposed control subjects (n = 46). The assay cutoff is defined as the mean OD + 2 SDs for the values obtained from control samples (180 pg/mL for PBMC and 40 pg/mL for blood). The solid lines represent the mean. The significance of differences between groups was evaluated by the Mann-Whitney *U* test. *P* values <.05 were considered significant. Abbreviations: IFN- γ, interferon γ; PBMC, peripheral blood mononuclear cell; OD, optical density; OIF, Operation Iraqi Freedom; SD, standard deviation; SLA, soluble *Leishmania* antigen.

Epidemiologic Risks for Infection

Risk characteristics of exposed OIF deployers were assessed (Table 1). No statistical association was found comparing infected versus uninfected subjects in military rank and job type, early (before 2006 with limited support infrastructure) versus late deployment, duration of deployment, night activities, attire, sleep accommodations, illness post-deployment, exposure to dogs, or use of PPM. Presence of antibodies to *Ph. alexandri* saliva was assessed; 77% of the AVL infected were positive for SGH antibodies compared to 61% of the noninfected, odds ratio 2.14, (95% CI: 0.95, 4.81). Evaluating geographic exposure within Iraq, deployment to Ninewa governorate was associated with infection, *P* = .01 (Figure 1A–1D). Using logistic regression, factors potentially associated with VL infection examined were deployment to Ninewa for ≥30 days (*P* = .01), occupation (combat arms, combat support, health care) (*P* = .10), and presence of SGH antibody (*P* = .06). Deployment to Ninewa remained significantly associated with infection after adjusting for job code and sand fly ELISA (OR for infection = 3.09, 95% CI 1.18–8.09). SGH antibodies and job code were not found to be associated with infection in the adjusted model.

Data from DD Form 2796 (post deployment health assessment) were retrieved. In sum, 177 volunteers completed the forms; no significant differences were found in recall of PPM or symptoms immediately postdeployment compared to current responses.

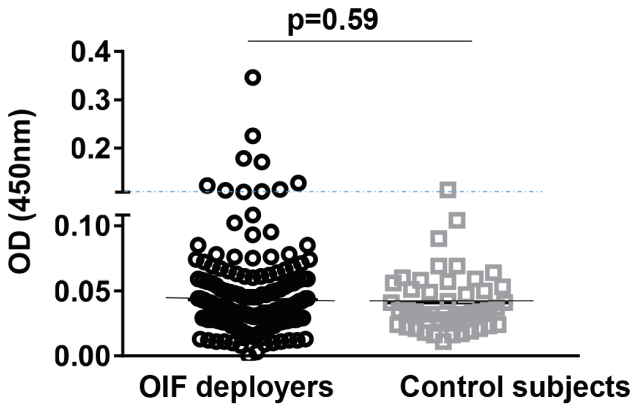

Figure 3. Serum IgG measured using *L. infantum* SLA ELISA in Iraq deployers and control subjects. The dotted line shows the cutoff value, which is defined as the mean OD + 3 SDs for the values obtained with sera from nonexposed controls (0.109). Solid lines indicate mean OD values. The significance of differences between groups was evaluated by the Mann-Whitney *U* test. *P* values <.05 were considered significant. Abbreviations: ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; OD, optical density; OIF, Operation Iraqi Freedom; SD, standard deviation; SLA, soluble *Leishmania* antigen.

DISCUSSION

Leishmaniasis has emerged as a parasitic disease of relevance to US Armed Forces in recent conflicts with reports detailing cutaneous, viscerotropic, and visceral leishmaniasis [20, 32–35]. Approximately 2040 diagnoses of leishmaniasis were reported from 2001–16 in US Armed Forces with over 1000 cutaneous and 25 visceral leishmaniasis cases reported in DMSS [20].

This study is the first to our knowledge to look at AVL in a cohort of US forces deployed during OIF; approximately 20% were found to be *Leishmania* infected by blood assays performed at least a decade after deployment to Iraq. Most tested positive by IGRA (14%), fewer by ELISA (5%), rarely PCR (1%), and none by rK39. Vector exposure was high with 64% testing positive for *Ph. alexandri* saliva antibody. Our results are similar to another deployed population: 1048 United Nations volunteers in nonendemic Austria where 4.5% persons tested serologically positive and 0.4% were PCR positive for leishmaniasis [19].

There are few well-defined diagnostic tests to detect AVL. Invasive tissue diagnostics are too aggressive, less sensitive in asymptomatic individuals, and unsuitable for surveillance [27]. Rapid tests such as ELISA and PCR are useful, but sensitivity/specificity depends on the antigen [36] or DNA target used

Table 1. Comparison of Characteristics and Potential Risk Factors of Exposed Infected versus Noninfected OIF Veterans (n = 200)

	Infected n (%)	Not Infected n (%)	*P*
	39 (100)	161 (100)	
Mean current age (range)	40.5 years (28–58)	41.2 years (24–61)	NS[d]
Sex			NS[e]
Male	35 (89.7)	133 (82.6)	
Female	4 (10.3)	28 (17.4)	
Race			NS[e]
White	32 (82.1)	126 (78.3)	
Black/African American	5 (12.8)	20 (12.4)	
Asian	0	6 (3.7)	
Pacific Islander	1 (2.6)	2 (1.2)	
Other	1 (2.6)	7 (4.3)	
Service			NS[e]
Army	34 (87.2)	143 (88.8)	
Marine Corps	2 (5.1)	9 (5.6)	
Navy	3 (7.7)	6 (3.7)	
Air Force	0	3 (1.9)	
Rank			
Officer	15 (38.5)	56 (34.8)	NS[e]
Enlisted	24 (61.5)	105 (65.2)	
Military occupation in Iraq			NS[e]
Combat arms	4 (10.3)	37 (23.0)	
Aviation	3 (7.7)	8 (5.0)	
Special operations[a]	0	11 (6.8)	
Combat support	7 (18.0)	31 (19.3)	
Healthcare	16 (41.0)	46 (38.6)	
Administration	4 (10.3)	16 (9.9)	
Supply	4 (10.3)	4 (2.5)	
Mechanic	0	4 (2.5)	
Transportation	1 (2.6)	4 (2.5)	
Median days deployed[b] in Iraq (range)	361(93–517)	321(30–790)	NS[f]
Total deployments to Iraq or Afghanistan since 2001			NS[e] (for trend)
1	12 (30.8)	56 (34.8)	
2–4	22 (56.4)	95 (59.0)	
5 or more	5 (12.8)	10 (6.2)	
Summer evening outdoor activities[c]			NS[e]
2–4 days/week	7 (18.5)	19 (11.8)	
Five or more days/week	31 (81.6)	142 (88.2)	
Typical summer sleepwear			NS[e]
Full uniform ± boots	7 (18)	16 (9.9)	
Shorts and t-shirt (physical fitness uniform)	23 (59.0)	87 (54.0)	
"Skivvies" only	7 (18)	46 (28.6)	
Other	2 (5.1)	11 (6.8)	
Summer sleep conditions			
Total no. of nights (mean)	7228 (185)	27681 (188)	
Percentage of all nights on ground (vs. bed/cot)	19.5%	13.5%	NS[e]
Exposure to dogs			NS[e]
Had a local pet	4 (10.3)	14 (8.7)	
Others in unit had a pet	14 (35.9)	62 (38.5)	
Worked with military working dogs	3 (7.7)	12 (7.5)	
Little or no direct contact	23 (59)	89 (55.3)	
Use of DEET insect repellent			NS[e]
None	16 (41)	64 (39.8)	
Brought own brand	4 (10.3)	15 (9.3)	
Used military issued repellent	10 (25.6)	54 (33.5)	
Used both	9 (23.1)	28 (17.4)	

Table 1. Continued

	Infected n (%)	Not Infected n (%)	*P*
Frequency of repellent use			NS[e]
Never	17 (43.6)	60 (37.3)	
≤ 1/week	13 (33.3)	69 (42.9)	
>1/week	4 (10.3)	21 (13.0)	
Daily	5 (12.8)	11 (6.8)	
Permethrin–treated uniforms			NS[e]
None	15 (38.5)	64 (39.8)	
Unknown	2 (5.1)	20 (12.4)	
Some uniforms treated	12 (30.8)	34 (21.1)	
All uniforms treated	10 (25.6)	43 (26.7)	
[†]Sand fly saliva antibodies detected	30 (76.9)	98 (60.9)	*P* = .06[e]
Constitutional symptoms (in previous 3 months)			NS[e]
Fever	5 (12.8)	15 (9.3)	
Night sweats	9 (23.1)	36 (22.4)	
Weight loss	0	5 (3.1)	
Fatigue	12 (30.8)	44 (27.3)	
Skin rash	10 (25.6)	37 (23.0)	
Multiple symptoms reported			NS[e]
>3	4 (10.3)	1 (.06)	
3	1 (2.6)	2 (1.2)	
2	6 (15.4)	2 (1.2)	
Postdeployment hospitalization	11 (28.2)	43 (26.7)	NS[e]

Infection defined as positive by polymerase chain reaction, interferon-γ release assay, or serology. Individual comparison level of significance *P* = .05. No correction for multiple positive comparisons was performed.

Abbreviations: DEET, *N,N*-diethyl-*meta*-toluamide; NS, nonsignificant; OIF, Operation Iraqi Freedom.

[a]Salivary gland homogenate ELISA antibody.

[b]During first Iraq deployment.

[c]No response from one infected participant.

[d]Means compared with Student's *t*-test.

[e]Proportions compared with χ^2 or Fisher's exact test.

[f]Rank sums compared with Mann-Whitney test.

[37]. In our study, we adapted immunological (SLA ELISA and IGRA) and molecular (real-time PCR) tools that identified a prevalence rate of 20% among OIF deployers. Because of individual variability in host immune responses, combined assays testing Th1 and Th2 responses and parasite antigen presumably enhanced the capacity to detect asymptomatic infection [38].

Prevalence of asymptomatic infection in OIF deployers was within the ranges published from endemic regions. AVL may be seen in up to 30% or more in endemic areas and seropositivity has been reported in 7% of worldwide blood donors [39, 40]. Molecular testing in two subjects showed parasite loads (mean 10, range 5–15 parasites/mL) consistent with active parasitemia since parasite DNA degrades shortly after amastigote death [41]. Parasitemia in OIF deployers appears similar to parasite loads previously reported in AVL; *L. donovani* cases had a median of 7.7 parasites/mL [42] and 10–56 parasites/mL (*L. infantum*) were reported among healthy Brazilian children [43].

Progression of AVL is determined by parasite virulence and host factors [39, 44, 45]. AVL infected persons may be at risk for clinical progression with risk ranging from 1.5% to 23% up to 3 years after AVL diagnosis in India/ Nepal versus no risk of progression after seroconversion seen in Brazil over a decade [12, 45–49]. *Leishmania* infection of macrophages and the leishmaniacidal activity of these cells is moderated by cell mediated immunity. Cytokine release of IFN-γ and tumor necrosis factor results in eradication and/or containment, an appropriate long term durable response paralleling immune responses in latent tuberculosis [45, 50–52]. Host antibody responses (measured in ELISA, rK39) correlate with more recent infection with waning response over months to years [47, 53]. Molecular diagnostics have shown high sensitivity and specificity and have been utilized in the diagnosis of AVL and for monitoring therapy response [54, 55]

In our study, most soldiers with AVL had positive IGRA results indicative of ongoing immunity and/or containment of infection more than a decade after exposure. The SLA ELISA results in this group (anticipated to be short-lived) could be explained by differences in individual immune responses, boosting with potential re-exposure to leishmaniasis on other deployments, or nonspecific cross reactivity of antigens used. Supporting nonspecific cross reactivity, four control participants (4/50, 8%) with no apparent exposure to leishmaniasis

had positive results (3 IGRA, one ELISA). Although this proportion is excluded from the 95% CI for the prevalence in the exposed group, the 95% CIs for the proportions in the 2 groups overlap and the 95% CI for the difference in the 2 proportions includes zero.

The AVL prevalence noted in this cohort raises concerns regarding the potential for future reactivation of latent disease in the setting of acquired or iatrogenic immunosuppression. There are reports of leishmaniasis reactivation, including fatal VL, in populations given TNF antagonists and those with impaired cell mediated immunity due to HIV, transplant, lymph/hematopoietic neoplasms, and high dose corticosteroids [7, 56–59]. Extrapolating from these reports, those identified as positive for AVL would presumptively be at risk of reactivation disease if immunosuppressed.

Acquisition of cutaneous leishmaniasis in US Armed Forces in Iraq has been associated with exposure to sand fly bites in summer months, using less insect repellant, nightly activities, and inadequate PPM with a majority of cases reported in 2003–4 and subsequent decline after 2005 [20, 60]. Epidemiologic risks assessed in this study, however, showed no statistical differences between infected and noninfected in occupation, early versus later deployment (when improved infrastructure present), number of deployments, use of PPM, or animal reservoir exposure. Some differences in exposure risk compared to prior evaluations could be due to recall bias and the small sample size of this cohort. However, subject responses to similar questions on post-deployment assessments performed immediately after they returned home did not differ from their information obtained on our survey. Deployment to Ninewa governorate was positively associated with the risk for AVL. It is uncertain why deployment to Ninewa would be associated with greater risk as this Iraq region is not reported as VL endemic [2], although with increasing cases of VL in the neighboring Syrian Arab Republic [61] it is possible that differences in vector or vector activity, local reservoirs for infection, insufficient public health reporting, and soldier activity in this region contributed to this finding.

There are several limitations of this study. Issues with recall have been noted (ie, reporting exposure history more than a decade later). Despite focusing on initial deployments to Iraq, many soldiers had multiple deployments, which complicates ascertaining the exact timeframe or exposure location. Other than rK39, study assays were experimental and their performance characteristics have received limited investigation.

This study suggests the burden of VL in US military deployed to Iraq is greater than previously realized. 25 cases of overt VL were reported between 2001 and 2016 in US Armed Forces, but in this surveillance study approximately 20% of the cohort had AVL [20]. The natural course of AVL infection in healthy individuals, especially in nonendemic areas, is not well understood. Infected soldiers in our study were evaluated clinically and universally found to be asymptomatic. Unexplained splenomegaly was noted in one although no evidence of disease progression was seen. However, because of persistent intracellular infection, there is a potential threat for future VL reactivation in this population. No prospective studies are available to sufficiently gauge this risk [4]. Unrecognized AVL may also result in transmission risk when infected individuals donate blood. Our study suggests that the likelihood of an individual with AVL donating blood may be higher than anticipated [62].

Optimal management of AVL is unknown with practice guidelines advocating close monitoring of those who will be immunosuppressed and initiation of VL therapy if symptoms develop [63]. FDA-approved AVL diagnostic screening tests, a clinically available quantitative *L. infantum* PCR, and future prospective studies are needed to improve AVL detection and discern risks for disease progression in this population. Overall, due to the high mortality of undiagnosed/untreated overt VL, clinicians caring for US personnel who have remotely deployed to leishmaniasis endemic areas should be aware of possible AVL reactivation with immunosuppression and entertain VL as a diagnosis when their patients develop a consistent clinical syndrome.

Notes

Author contributions. All the authors contributed to data collection, analysis, interpretation, and drafting of this manuscript. N. E. A. and R. F. D. contributed to study design and had full access to the combined database set. R. M. M., J. E. S., and N. E. A. had responsibility for submission of the manuscript for publication.

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