

SHORT REPORT

Serological evidence of arboviral infection and self-reported febrile illness among U.S. troops deployed to Al Asad, Iraq

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SUMMARY

Understanding the epidemiology of current health threats to deployed U.S. troops is important for medical assessment and planning. As part of a 2004 study among U.S. military personnel deployed to Al Asad Air Base, in the western Anbar Province of Iraq, over 500 subjects were enrolled, provided a blood specimen, and completed a questionnaire regarding history of febrile illness during this deployment (average ~4 months in country). This mid-deployment serum was compared to pre-deployment samples (collected ~3 months prior to deployment) and evaluated for seroconversion to a select panel of regional arboviral pathogens. At least one episode of febrile illness was reported in 84/504 (17%) of the troops surveyed. Seroconversion was documented in nine (2%) of deployed forces tested, with no association to febrile illness. Self-reported febrile illness was uncommon although often debilitating, and the risk of illness due to arbovirus infections was relatively low.

Throughout history arthropod-borne viruses, or arboviruses, have been a significant threat to military forces deployed in the Middle East [1]. During the Second World War, U.S. and British forces deployed to Iran and Iraq suffered large numbers of casualties due to Sandfly fever [1, 2]. Based largely on these experiences, it was assumed U.S. forces deployed to this region during Operation Desert Storm/Desert Shield would also be at significant risk for arbovirus infection, but incidence was not as high as anticipated.

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Studies conducted during Operation Desert Storm/Desert Shield evaluated the risk of arbovirus infection to deployed U.S. forces in the region and found a lower than expected incidence [3, 4]. However, the current war in Iraq is much different than the previous operations in this region and may result in different risks to deployed troops in this region. A systematic cross-sectional survey performed at Marine Corps Air Station Al Asad, Anbar Province, Iraq from June to July 2004 was implemented to evaluate the epidemiology of self-reported febrile illness and to report the incidence of arbovirus infection among U.S. forces deployed to Iraq in support of Operation Iraqi Freedom.

Two-person teams from the Naval Medical Research Unit No. 3 (NAMRU-3) research group,

Cairo, Egypt, were randomly assigned to 12 sectors on base. A convenience sample of U.S. Department of Defense (US DoD) military personnel or civilian contractors encountered within those sectors were asked to participate in the study regardless of current health status. Most personnel domiciled in tents or buildings with central air-conditioning. To avoid interfering with military operational activities, personnel within high-security areas in assigned sectors were not approached to enrol in the study. After informed consent, participants completed a pre-tested epidemiological questionnaire which explored self-reported fever during deployment. Blood samples were collected and tested in parallel with available pre-deployment serum obtained from the US DoD serum repository to evaluate for the occurrence of seroconversion against a panel of regional arboviruses. Pre- and post-deployment serum was tested at NAMRU-3 by indirect ELISA for IgM and IgG antibodies or five viruses: family Flaviviridae, genus *Flavivirus*, species *West Nile* (WNV); family Bunyaviridae, genus *Phlebovirus*, species *Sandfly Fever Naples* (SFNV) and *Sandfly Fever Sicilian* (SFSV); family Togaviridae, genus *Alphavirus*, species *Sindbis* (SINV); and family Bunyaviridae, genus *Phlebovirus*, species *Rift Valley Fever* (RVFV) using in-house antigen assays [5]. Initially plates were coated with respective antigens and incubated overnight at 4 °C. Plates were then washed and incubated 37 °C for 1 h with a 1 : 100 dilution of test sera. This was followed by incubation with goat anti-human IgG horseradish peroxidase (HRP) conjugate at 1 : 5000 dilution for 1 h at 37 °C, followed by incubation with substrate (ABTS) for 30 min. Optical density (OD) of the reaction was determined using a spectrophotometer at 405 nm. Samples with OD values greater than 3 standard deviations above the mean of the negative control sera were considered positive. Positive samples were confirmed by a two-fold serial dilution. Seroconversion was defined as a fourfold rise in titre in mid-deployment serum positive as compared to pre-deployment value.

In addition to demographic information, subjects reported incidence of self-reported fever as well as any contemporaneous clinical features, medical treatment, and/or operational impact linked with the febrile illness. Based on the constellation of symptoms reported in association with the fever, febrile illness categories were categorized into differentiated febrile illness syndromes (febrile respiratory and febrile diarrhoea illness) and undifferentiated febrile illnesses.

Febrile diarrhoea was defined as an illness characterized by fever with diarrhoea and cramps without any respiratory symptoms (including rhinorrhoea, cough, and pharyngitis). Febrile respiratory illness was characterized by fever with no diarrhoea, and at least two of the following respiratory symptoms: pharyngitis, rhinorrhoea or cough. Undifferentiated fever was defined as an illness characterized without any diarrhoea or respiratory symptoms.

Questionnaire data were double-entered into Epi-Info 6 (CDC, Atlanta, GA, USA) and merged by unique identifier with laboratory results. For statistical testing of continuous variables, normality testing was conducted, followed by either parametric (Student's *t* test or ANOVA) or non-parametric (Kruskal–Wallis test) assessments. Categorical variables (proportions) were tested using either χ^2 or Fisher's exact tests. Point estimates and 95% confidence intervals for incidence of febrile illness and arbovirus seroconversion were calculated based on self-reported illness or seroconversion events and the associated person-time in theatre based on a Poisson distribution. Stata version 9 (Stata Corp., College Station, TX, USA) was used for all analyses, and statistical significance was set as $P \leq 0.05$. This study was approved by the Institutional Review Board of the U.S. NAMRU-3, Cairo, Egypt under GEIS Work Unit Number GN30019_06_N3.

In total, 549 volunteers, representing about 6% of the base population, completed a detailed questionnaire and provided a blood specimen. Of these, 504 had pre-deployment serum, which were collected on average 3 months prior to deployment, available from the US DoD Serum Repository for serological testing. The majority was male (98%), mostly Caucasian (59%), junior enlisted (E1–E3, 71%) and in the Marine Corps (88%) which is comparable to the active duty population currently deployed. Median age of military personnel was 23 years [interquartile range (IQR) 21–27 years] with a median time in theatre of 127 days (IQR 120–132 days) mostly being captured in the late winter and early spring. There were no differences in demographic or primary outcome variables between those for whom there was or was not pre-deployment serum available (data not shown).

Of the 504 volunteers which underwent serological testing, 496 completed the question on 'history of febrile illness during the current deployment'. Of these 496 respondents, 84 (17%) reported at least one febrile illness during their current deployment with

Table. *Sample population descriptive and clinical characteristics of self-reported febrile illnesses (n = 504)*

Characteristic	Parameter estimate
Age in years, median (IQR)	23 (21–27)
Male gender, <i>n</i> (%)	496 (98)
Service, <i>n</i> (%)	
Marine Corps	444 (88)
Navy	54 (11)
Army	6 (1)
Active duty status, <i>n</i> (%)	466 (92)
Rank, <i>n</i> (%) (<i>n</i> = 502)	
E1–E3	228 (45)
E4–E6	241 (48)
Senior enlisted/Officer	33 (7)
Race, <i>n</i> (%)	
Caucasian	298 (59)
African-American	39 (8)
Hispanic	108 (21)
Asian	18 (4)
Other, missing	43 (8)
Days in country, median (IQR)	127 (120–132)
Self-reported febrile illness, <i>n/N</i> (%)	84/496 (17)
Headache	75 (89)
Extreme fatigue	65 (79)
Diarrhoea	59 (70)
Chills	58 (69)
Night sweats	56 (67)
Nausea	56 (67)
Myalgia	51 (61)
Abdominal cramps	46 (55)
Rhinorrhoea	39 (46)
Cough	39 (46)
Pharyngitis	38 (45)
Swollen glands	33 (39)
Arthralgia	28 (33)
Vomiting	23 (27)
Ophthalmalgia	13 (15)
Scleral/conjunctival injection	13 (15)
Rash, unspecified	7 (8)
Skin ulcer	0

IQR, Interquartile range.

a median fever length of 2 days (range 1–12 days) resulting in a minimum self-reported incidence of 4.1 episodes/100 person-months (95% CI 3.2–5.0). Thirty-three (6.5%) reported more than one febrile illness. Associated symptoms with the febrile illnesses are described in the Table. Three-quarters (*n* = 63) of those with fever reported having respiratory symptoms, diarrhoea, or a combination of both in

association with their fever (differentiated), with the remaining 25% (*n* = 21) having other symptoms, including those often associated with arbovirus infection such as headaches, body aches and nausea (data not shown). Reported categories of medical utilization for illness were not mutually exclusive and included 68% self- or buddy-aide, 45% sought care at a medical treatment facility, 6% required an ER visit, and 5% were hospitalized. Mission impact due to febrile illness was reported with 71% reporting at least one day of decreased work productivity, and 44% missing one or more days of work. Only 10% reported that back-up personnel were required to be called in to cover a shift, however, 30% felt their illness impacted operational readiness of the unit. The only statistically significant difference between differentiated and undifferentiated febrile illnesses was that the undifferentiated febrile illness group were less likely to report that other members of their unit were sick during their time with fever (29% vs. 63%, *P* = 0.005). In general, undifferentiated fevers tended to be milder (as measured by operational impact indicators) and more frequently associated with self-treatment (86% vs. 62%, *P* = 0.06).

Overall, a seroconversion rate of 1.8% (*n* = 9) was calculated for the study group; eight SFSV and one SINV. Two subjects had baseline SFSV (IgG 1:100), one of which had a fourfold rise (seroconverter). The remaining seven SFSV seroconverters had negative baseline titres with three developing SFSV IgG titres of 1:800, and three developing titres of 1:400. The one seroconverter to SINV had a positive mid-deployment SINV IgG titre of 1:800, and a negative pre-deployment titre. Positive sera were also tested for IgM antibodies and were negative. Three enrollees had IgG positive for WNV on pre-deployment sera (IgG titre >1:1600), but no rise in titre at mid-deployment. Only one seroconverter reported febrile illness during deployment; an Army Reservist with one day of fever associated with muscle aches and fatigue who self-treated and did not seek medical care seroconverted to SFSV. He did not miss any days of work or experience decreased work productivity. There were no significant differences found in demographic variables between the group that seroconverted and those who did not seroconvert (data not shown).

We found that febrile illness was a common occurrence in deployed troops with a self-reported monthly attack rate of ~7%. A majority of subjects reported febrile illnesses which were characterized by diarrhoea

and/or respiratory symptoms. This is not surprising as the known incidence of invasive diarrhoeal disease and febrile respiratory infections during past deployments in Iraq have been described recently [6, 7]. This study found lower incidence of seroconversion in deployed forces with fever as did previous studies from Operation Desert Storm/Desert Shield which found 3.6% seroconversion rate to arboviruses in forces with a febrile illness [8]. Both of these studies show arbovirus infection and/or exposure to be far less of a threat than previously expected based on the experiences of previous military campaigns in the region [1].

Arboviruses are considered endemic in the region and have been responsible for significant febrile outbreaks in the past, but this study documents low risk at present. Antibodies to SFSV indicate, at least, exposure to sandflies. A recent report from Iraq after deployment of U.S. troops, has determined high numbers of sandflies in areas in Iraq and military personnel have reported many sandfly bites in a single night [9]. It is unclear if these lower rates were due to geographical differences or better vector control and personal protective measures employed, as other parts of the Middle East and Africa continue to experience high rates of Sandfly fever infection and decreased mission capability [10, 11]. Time of year may also play a role; historically, warmer temperatures are known to be associated with higher risk of disease. Our sample was taken among marines during the months of June and July who had been on deployment for a median of about 4 months. This would have placed their earliest exposure period in February or March. It is also a very strong possibility that aggressive vector control programmes and widespread use of personal protective measures against the insect vectors are protecting U.S. forces against higher rates of vector-borne disease. Though it appears that individual use of repellents is probably uncommon based on a recent published report among troops in Iraq and Afghanistan which showed that while 68.5% of the troops knew *N,N*-diethyl-m-toluamide (DEET) was readily available, only 14.6% of troops reported using DEET more than occasionally and 51.2% never used the repellent [6]. The use of permethrin-treated uniforms among the sample population was unknown.

The findings of our study must be interpreted with caution as there are several identified limitations. The definition of a febrile illness was based on self-report, therefore recall bias and misclassification may be

present and it is difficult to know if the estimates over- or under-estimate the actual risk of disease. Taylor *et al.* [12] describe a self-reported febrile illness rate of 2.9 episodes per 100 person-months (95% CI 2.0–3.9) among returning Gulf War I troops during a 7.5-month deployment period which is relatively low compared to the 4.1% we report in our mid-deployment study. This finding may be the result of truly different rates of febrile illnesses between the two combat deployments, or possibly the result of recall bias diminishing the estimate in the post-deployment survey of Taylor *et al.*, compared to the present study. Only one geographic region was sampled during this survey. Selection bias could have influenced the results as personnel within high-security areas in assigned sectors were excluded from participation. Vector-borne threat could have been higher in some of these areas most frequented by these soldiers (e.g. base perimeter where more suitable vector habitats may be present). Although occupational exposure which could have elucidated this issue was not recorded, the troops that were sampled were probably a heterogeneous group including soldiers who often went out on patrol in the surrounding areas, as well as those who did not leave the base during the deployment. Arbovirus transmission usually occurs in focalized environmental conditions [13]. Our survey was not designed to classify the range of ecological exposures within Anbar Province which stretches from the Tigris river to the Syrian border. Further studies evaluating febrile illness and arbovirus infection risk from other regions within Iraq would be of interest to evaluate the generalizability of our findings. In addition, our survey was designed to only ask exposure and risk factor questions to those with self-reported fever. In retrospect, it would have been useful to have a non-febrile illness comparison group to assess risk factors for undifferentiated febrile illness and arboviral seroconversion. Last, additional studies to evaluate the epidemiology of febrile respiratory, diarrhoeal and undifferentiated illness in deployed troops are warranted to determine aetiologies and possible counter-measures.

The self-reported symptom of fever was noted to affect approximately one of every five troops with attendant morbidity and health-care utilization, although a majority of these illnesses could be attributed to diarrhoea or respiratory syndromes. More clinic-based epidemiological studies to identify aetiology and operational impact of undifferentiated febrile illnesses are needed.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Oldfield EC, Wallace MR, Hyams KC.** Endemic infections of the Middle East. *Review of Infectious Diseases* 1991; **13**: S199–S217.
2. **Quin NE.** The impact of diseases on military operations in the Persian Gulf. *Military Medicine* 1982; **147**: 729–734.
3. **Richards AL, et al.** Arbovirus and rickettsial infections among combat troops during Operation Desert Shield/Desert Storm. *Journal of Infectious Diseases* 1993; **168**: 1080–1081.
4. **Hyams KC, et al.** The impact of infectious diseases on the health of U.S. troops deployed to the Persian Gulf during operations Desert Shield and Desert Storm. *Clinical Infectious Diseases* 1995; **20**: 1497–1504.
5. **Botros BA, et al.** Prevalence of hantavirus antibody in patients with chronic renal disease in Egypt. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2004; **98**: 331–336.
6. **Sanders JW, et al.** Impact of illness and non-combat injury during Operations Iraqi Freedom and Enduring Freedom (Afghanistan). *American Journal of Tropical Medicine and Hygiene* 2005; **73**: 713–719.
7. **Thornton SA, et al.** Gastroenteritis in US Marines during Operation Iraqi Freedom. *Clinical Infectious Diseases* 2005; **40**: 519–525.
8. **Richards AL, Hyams KC, Merrell MS.** Medical aspects of Operation Desert Storm [Letter]. *New England Journal of Medicine* 1991; **325**: 970.
9. **Coleman RE, et al.** Impact of phlebotomine sand flies on U.S. Military operations at Tallil Air Base, Iraq: 1. background, military situation, and development of a ‘Leishmaniasis Control Program’. *Journal of Medical Entomology* 2006; **43**: 647–662.
10. **Niklasson B, Meegan JM, Bengtsson E.** Antibodies to Rift Valley fever virus in Swedish U.N. soldiers in Egypt and the Sinai. *Scandinavian Journal of Infectious Diseases* 1979; **11**: 313–314.
11. **McCarthy MC, et al.** Evaluation of arthropod-borne viruses and other infectious disease pathogens as the causes of febrile illnesses in the Khartoum Providence of Sudan. *Journal of Medical Virology* 1996; **48**: 141–146.
12. **Taylor DN, et al.** *Helicobacter pylori* infection in Desert Storm troops. *Clinical Infectious Diseases* 1997; **25**: 979–982.
13. **Feliciangeli MD.** Natural breeding places of phlebotomine sandflies. *Medical and Veterinary Entomology* 2004; **18**: 71–80.