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"

Authors: Coleman, Russell E., Burkett, Douglas A., Putnam, John L., Sherwood, Van, Caci, Jennifer B., et al.

Source: Journal of Medical Entomology, 43(4) : 647-662

Published By: Entomological Society of America

URL: https://doi.org/10.1603/0022-2585(2006)43[647:IOPSFO]2.0.CO;2

Forum

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"

RUSSELL E. COLEMAN,[1] DOUGLAS A. BURKETT,[2] JOHN L. PUTNAM,[2] VAN SHERWOOD,[3] JENNIFER B. CACI,[3] BARTON T. JENNINGS, LISA P. HOCHBERG,[4] SHARON L. SPRADLING,[2] EDGAR D. ROWTON,[4] KEITH BLOUNT,[2] JOHN PLOCH,[5] GRADY HOPKINS,[5] JO-LYNNE W. RAYMOND, MONICA L. O'GUINN,[6] JOHN S. LEE,[6] AND PETER J. WEINA

520th Theater Army Medical Laboratory, United States Army, Tallil Air Base, Iraq

J. Med. Entomol. 43(4): 647–662 (2006)

ABSTRACT One of the most significant modern day efforts to prevent and control an arthropod-borne disease during a military deployment occurred when a team of U.S. military entomologists led efforts to characterize, prevent, and control leishmaniasis at Tallil Air Base (TAB), Iraq, during Operation Iraqi Freedom. Soon after arriving at TAB on 22 March 2003, military entomologists determined that 1) high numbers of sand flies were present at TAB, 2) individual soldiers were receiving many sand fly bites in a single night, and 3) *Leishmania* parasites were present in 1.5% of the female sand flies as determined using a real-time (fluorogenic) *Leishmania*-generic polymerase chain reaction assay. The rapid determination that leishmaniasis was a specific threat in this area allowed for the establishment of a comprehensive Leishmaniasis Control Program (LCP) over 5 mo before the first case of leishmaniasis was confirmed in a U.S. soldier deployed to Iraq. The LCP had four components: 1) risk assessment, 2) enhancement of use of personal protective measures by all personnel at TAB, 3) vector and reservoir control, and 4) education of military personnel about sand flies and leishmaniasis. The establishment of the LCP at TAB before the onset of any human disease conclusively demonstrated that entomologists can play a critical role during military deployments.

KEY WORDS sand flies, leishmaniasis, Iraq, surveillance, control

In this article, we provide an overview of the general situation that the U.S. Military encountered at Tallil Air Base (TAB), Iraq, in March 2003 and discuss the factors that led to the establishment of a Leishmaniasis Control Program (LCP) at TAB. We also describe each of the four main components of the LCP (vector surveillance, personal protective measures [PPM], sand fly and reservoir control, and soldier education). Although brief summaries of this program have been published separately (Coleman et al. 2004; 2005), we believe that it is important to provide a comprehensive overview of one of the most significant modern day efforts to prevent and control an arthropod-borne disease during a military deployment. Although much of this article relies on anecdote, we believe that this information has both scientific as well as historical value. In the series of articles to follow, we will provide detailed results about the specific components of the program, including: 1) the general biology of phlebotomine sand flies at TAB, 2) our evaluation of a variety of surveillance devices for the collection of sand flies, 3) the impact of environmental conditions on sand fly activity, 4) the efficacy of a variety of area spray measures on sand fly abundance, 5) the efficacy of a variety of residual spray measures on sand fly abundance, 6) testing of sand fly populations for the presence of *Leishmania* parasites and the genetics of *Leishmania* parasites isolated from sand flies, 7) our evaluation of PPM as a means of protecting soldiers from sand fly bites, and 8) our overall evaluation of the risk of leishmaniasis at TAB and the efficacy of the LCP.

Material has been reviewed by the Walter Reed Army Institute of Research and the U.S. Army Medical Research and Material Command. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting true views of the Department of the Army or the Department of Defense.

[1] Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910 (e-mail: russell.coleman@us.army.mil).

[2] 407th Air Expeditionary Group, United States Air Force, Tallil Air Base, Iraq.

[3] 787th Medical Detachment, United States Army, Tallil Air Base, Iraq.

[4] Walter Reed Army Institute of Research, Silver Spring, MD.

[5] Kellog, Brown and Root Corporation, Tallil Air Base, Iraq.

[6] U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

Historically, leishmaniasis has been a major cause of infectious disease morbidity among military personnel deployed to the Middle East (Kinnamon et al. 1979, Cross et al. 1996). During World War II, 1,000–1,500 cases of cutaneous leishmaniasis (CL) and 50–75 cases of visceral leishmaniasis (VL) occurred in allied forces in the Middle East (Most 1968). During the "Six-Day War" in 1967, up to 50% of Israeli forces operating in parts of the Jordan Valley were infected with leishmaniasis (Naggan et al. 1970), and 20% (12/60) of at-risk personnel assigned to the Multinational Force and Observers (MFO) in the Sinai desert of Egypt in 1990 developed CL (Fryauff et al. 1993). During Operation Desert Storm (ODS), 20 cases of CL and 12 cases of viscerotropic leishmaniasis were diagnosed in the 697,000 allied soldiers deployed to the Arabian Peninsula in 1990 and 1991 (Gasser et al. 1991, Magill et al. 1993, Martin et al. 1998). Most recently, an outbreak of CL caused by *Leishmania major* Yakimoff and Schokhor occurred in U.S. Military personnel deployed to Iraq during Operation Iraqi Freedom (CDC 2003, 2004; Pehoushek et al. 2004; Weina et al. 2004; Berman 2005; Willard et al. 2005). As of November 2004, 1,178 cases of CL had been identified in U.S. Military personnel (Lay 2004); however, this number is probably an underestimate, and the actual number of cases is estimated to be between 1,500 and 2,000 cases as of 1 January 2006 (P.J.W., personal communication).

Leishmaniasis is a parasitic infection caused by various species of *Leishmania. Leishmania* parasites are transmitted by the bite of an infected female sand fly. Although primarily a zoonotic disease found in rodents and canids, some species of *Leishmania* may survive for decades in asymptomatic infected people (Magill 1995). In Iraq, the primary forms of leishmaniasis are CL and VL. CL, caused by *L. major, Leishmania tropica* (Wright), or both, in Iraq, is manifested as skin lesions or nodules (Weina et al. 2004). Although CL is normally self-healing, it can create serious disability and permanent scars. Individuals who have recovered from CL usually exhibit immunity to reinfection by the species of *Leishmania* that caused the disease (Magill 1995). Humans are the sole proven reservoir of *L. tropica,* with transmission from person to person by the vector (WHO 2003). In rural areas, yet to be determined animals are thought to be the reservoirs of *L. tropica;* however, the full transmission cycle of *L. tropica* is still under investigation in various foci (Jacobson 2003). For *L. major* in the Middle East, gerbils, *Meriones crassus* Sundevall and *Meriones lybicus* Lichenstein, and the fat sand rat, *Psammomys obesus* Cretzschmar, are the primary animal reservoirs, and *Phlebotomus papatasi* Scopoli is the primary vector (Yaghoobi-Ershadi and Javadian 1996, Yaghoobi-Ershadi et al. 2005). Between 1989 and 2001, the reported incidence of CL in Iraq ranged from a low of 2.3 per 100,000 (625 total cases) in 2001 to a high of 45.5 per 100,000 (8,779 total cases) in 1992 (WHO 2003).

VL, primarily caused by *Leishmania donovani* Laveran and Mensel and *Leishmania infantum* Nicolle, is characterized by irregular fever, weight loss, swelling of the liver and spleen, and anemia. It is the most severe form of leishmaniasis and is usually fatal if left untreated (Magill 1995). The incubation period for VL ranges from months to years. The intensity of infection is dependent on partial immunity resulting from previous exposure, concomitant illness, malnutrition, and other factors (WHO 2003). The vectors of VL in Iraq have not been fully elucidated; however, *Phlebotomus alexandri* Sinton has been incriminated in the transmission of *L. infantum* (Sukkar 1974, Sukkar et al. 1985). Reservoirs of *L. infantum* are believed to be domestic dogs, jackals, foxes, and potentially rats, whereas humans are the reservoirs of *L. donovani* (Armed Forces Pest Management Board 1999). An infected human host may serve as a source of infection to sand flies as long as the parasite persists in the circulating blood or skin, even after clinical recovery. Historically, the most important endemic area for VL in Iraq was in the center of the country and in the Greater Baghdad area. Since 1991, the disease has extended to new areas rarely affected before, such as Missan, Thi-Qar, and Basrah governates in southeastern Iraq (WHO 2003). Between 1989 and 2001, the reported incidence of VL in Iraq ranged from a low of 2.6 per 100,000 (491 total cases) in 1989 to a high of 20.0 per 100,000 (3,866 total cases) in 1992 (WHO 2003). During ODS, 12 cases in total of VL due to *L. tropica* were reported in U.S. soldiers deployed to Saudi Arabia (Hyams et al. 1995). None of these individuals had cutaneous manifestations normally associated with this parasite.

Currently, there are no prophylactic drugs or vaccines that can be used to prevent leishmaniasis (Magill 1995). Therefore, the prevention of leishmaniasis relies upon preventive measures taken to minimize exposure to biting sand flies (Martin et al. 1998). Transmission zones can be extremely focal because of the presumed limited flight range of vector species (Magill 1995). Infection in military personnel occurs when activities such as desert operations expose service members to foci where infected sand flies are found (Martin et al. 1998). Measures used to minimize sand flies bites include application of residual insecticides on tents and buildings, ultralow volume (ULV) space spraying of insecticides by using truck-mounted or aerial spray equipment, and use of PPM such as application of insect repellent on exposed skin, donning of permethrin-treated clothing, and use of insecticide-treated bed-nets (Alexander and Maroli 2003). Because the breeding sites of sand flies are generally unknown, control efforts that focus on immature stages are currently not feasible.

Terrain and Environmental Conditions at TAB

TAB (30° 56′ N, 46° 06′ E) is located 160 miles southeast of Baghdad and 140 miles northwest of Kuwait City (Fig. 1). The largest city in the immediate vicinity of TAB is An Nasiriyah, located ≈17 km to the northeast. TAB is a major tactical airfield in southern Iraq. It encompasses an area of ≈30 km^2 and has a fenced perimeter of ≈22 km. It is located in a region

Fig. 1. Map of Iraq and Kuwait showing the location of TAB in relation to Baghdad and Kuwait City.

of semiarid desert (Fig. 2). The terrain is flat with <1-m natural variation in elevation over the 30 km^2 of the base. There are no natural sources of water (e.g., streams or ponds); however, there is a network of abandoned irrigation canals that hold water during the infrequent rains. Conditions at TAB were best described by an airman assigned to the 407th Air Expeditionary Force: "Everything that does not move is covered in a grayish brown, powdery dust. The heat is oppressive—>120 degrees in the shade, and the open fields and roads bear craters large enough to swallow small trucks. In March 2003, the area around TAB looked more like the surface of the moon than the bustling tent city and flight-line area standing today. After the base fell to coalition

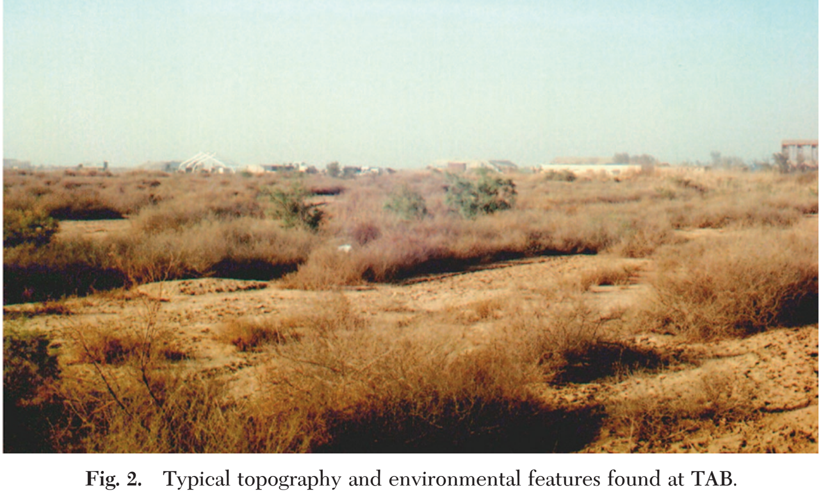

Fig. 2. Typical topography and environmental features found at TAB.

Fig. 3. Aerial view of the U.S. Air Force facility established at TAB in May 2003, including (A) hospital, (B) central dining facility, (C) air-conditioned living quarters, and (D) shower tents and latrine tents.

forces during Operation Iraqi Freedom, the landscape was desolate, save a few abandoned buildings, many of which still had extensive damage remaining from the first Gulf War" (http://www.globalsecurity. org/military/world/iraq/tallil.htm).

Environmental conditions at TAB are harsh. Annual precipitation ranges between 10 and 20 cm, with most rainfall occurring between November and April. From 23 April 2003 to 31 October 2004, there was a total of 18.9 cm of precipitation. Measurable precipitation occurred on 26 of 558 d during this period. Maximum daily temperatures in the summer (May–September) average above 45°C and exceed 50°C on occasion, with nighttime temperatures averaging 25°C in the summer and 0–10°C in the winter. During 2003 and 2004, the maximum daily temperature exceeded 37.8°C (100°F) on 160 and 162 d, respectively. During the summer, late afternoon storms with high winds and blowing

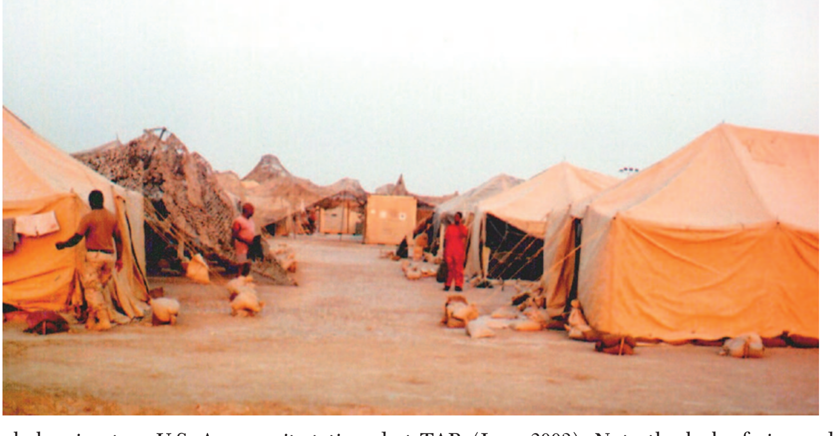

Fig. 4. Tents belonging to a U.S. Army unit stationed at TAB (June 2003). Note the lack of air conditioners on the tents.

Fig. 5. Typical sand fly bites acquired during a single night on U.S. military personnel stationed at TAB in 2003.

dust occur frequently and adversely affect all activity (human and sand fly). The impact of environmental factors (temperature, wind speed and direction, relative humidity, cloud cover and moon phase) on sand fly activity will be evaluated in a subsequent article.

Military Forces and Living Conditions at TAB

The U.S. military population at TAB ranged from a low of ≈500 individuals on 23 March 2003 to a high of almost 30,000 individuals (15,000 soldiers, airmen, and marines living at TAB with an additional 15,000 marines in the vicinity of An Nasiriyah) in June and July 2003. Living conditions at TAB were austere during the initial occupation (March–May 2003). During this period, most military personnel lived either inside abandoned buildings or hangers found on the base or in tents without air conditioners. By the end of May, U.S. Air Force personnel were living in air-conditioned tents (Fig. 3), whereas the majority of Army personnel continued to live in tents that had no air conditioning (Fig. 4) through summer 2003. By April

Fig. 6. Schematic map showing the layout of TAB and the locations of the standard traps used in sand fly surveillance.

2004, almost all personnel at TAB lived in air-conditioned tents or buildings.

Preventive Medicine Capabilities at TAB

One of the first medical units entering Iraq during OIF was the 520th Theater Army Medical Laboratory (TAML), which crossed into Iraq on 20 March 2003 and arrived at TAB during the night of 22 March 2003. Although the primary mission of the TAML was a confirmatory laboratory responsible for the detection of chemical and biological warfare agents in environmental and human samples, the TAML deployed with a wide range of medical diagnostic capabilities to include assays for endemic disease surveillance. TAML personnel supporting the endemic disease surveillance effort included an infectious disease physician, a medical entomologist, a veterinary pathologist, an environmental science officer, and several preventive medicine technicians.

Between late March and late April 2003, two additional medical units with preventive medicine capabilities deployed to TAB. The U.S. Army's 787th Medical Detachment (787th MED DET) deployed a four-person team led by a medical entomologist. The 787th MED DET was equipped with insect surveillance (light traps) and control (hand-held and truck-mounted sprayers) equipment along with a variety of insecticides. The U.S. Air Force's 407th Air Expeditionary Group (407th AEG) deployed a field hospital that included a public health officer, a medical entomologist, and several public health technicians. The 407th AEG also included a team of pest managers equipped with a variety of insecticide spray equipment and pesticides.

Establishment of a Leishmaniasis Control Program at TAB

Background

In early April 2003, several U.S. military units at TAB reported that soldiers were being bitten by insects. Early on the evening of 12 April, three unbaited CDC light traps were placed in a building where soldiers had received insect bites, and on the morning of 13 April four phlebotomine sand flies were collected from one of the light traps. During the next 2 wk, several hundred additional sand flies were collected at various locations throughout TAB. Because of the high numbers of sand flies collected, the TAML prepared a series of reports between 18 and 28 April that stated leishmaniasis should be considered a potential threat at TAB and recommended a prevention and control program be established. An outline of the proposed LCP was provided in one of these reports. The goal of the LCP was to protect all coalition forces in the vicinity of TAB from leishmaniasis. The LCP had four main objectives: 1) establish a vigorous surveillance program (collection of sand flies and testing for *Leishmania* parasites using a real-time polymerase chain reaction [PCR] assay) to assess risk, 2) ensure all U.S. military personnel had access to and used PPM (deet-containing repellents, permethrin-treated uniforms, and permethrin-treated bed-nets), 3) establish a sand fly control program, and 4) ensure all military personnel at TAB were informed about the risk of leishmaniasis and preventive measures required to protect themselves from the disease. The LCP was initiated in April 2003 and continued through October 2003, when surveillance indicated sand flies were no longer active due to cooling temperatures. The LCP was reinitiated in April 2004 and continued through October 2004.

The establishment of the LCP at TAB provided a unique opportunity for U.S. Army, Air Force, and Navy entomologists and pest control personnel to work together along with medical personnel from Britain, Italy, Korea, and The Netherlands. The goal of the LCP was to protect all military forces assigned to TAB from leishmaniasis. To accomplish this goal, we established a working group consisting of representatives from each military service. This working group met weekly at the TAML to review progress on the implementation of the LCP and to identify problems/issues and develop solutions to these problems.

Soldier Exposure to Sand Fly Bites

During late April 2003, it became apparent that increasing numbers of soldiers were receiving significant numbers of sand fly bites. To determine the impact of sand fly bites on military personnel, we reviewed sick call visits to military medical facilities at TAB. Between 1 April and 15 May 2003, 377 individuals in total sought medical treatment at the 1st Battalion (293rd Infantry Regiment) Aid Station—insect bites were the most common complaint and constituted 42% (172) of all sick call visits. The majority of these sick call visits were for either multiple sand fly bites (in some cases, >1,000 bites were received in a single night), for severe allergic reactions to the bites or for treatment of secondary infections of the bites that resulted when soldiers rubbed or scratched the bites raw (Fig. 5). A subsequent review of admission records of the 86th Combat Support Hospital revealed that 7.5% (142/1,893) of hospital visits between 23 March and 14 May 2003 were for insect bites—the majority of patients seen at the 86th Combat Support Hospital were referred there by lower level medical organizations (such as Battalion Aid Stations) and presumably represent the more severe insect bites that were occurring. An informal survey of selected units at TAB subsequently found that up to 75% of soldiers had received sand fly bites in April and May 2003. Although the presence of leishmaniasis at TAB had not been confirmed at this point, this cumulative data strongly suggest sand fly bites alone were a significant nuisance problem.

Sand Fly Surveillance Program

Establishment of the Sand Fly Surveillance Program. The initial focus of our surveillance effort was to determine what arthropods were biting U.S. military personnel. Once we had determined that phlebotomine sand flies were the primary cause of the bites, we established a program to monitor sand fly populations throughout TAB. Weather permitting, 14 CDC-style light traps in total (model 512, John W. Hock Company, Gainesville, FL) were set up two or more times per week beginning on 17 April 2003 and continuing through 25 October 2004. Traps were not set during winter (November 2003–March 2004). Traps were started at 1800 hours local time and collected by 0800 hours the next day. Ten of the traps were placed in areas where vector control activities (area spraying and application of residual insecticides) occurred (Trtd-1 thru Trtd-10), whereas two traps each were placed in two locations (Con-1 and Con-2) where vector control activities did not occur (Fig. 6). The primary goals of this program were to 1) monitor sand fly and mosquito populations, 2) determine sand fly species and changes in species composition over time, 3) evaluate the effectiveness of the control and spray program, and 4) test the sand flies for *Leishmania* parasites to aid in the identification of disease foci.

Upon return to the field laboratory at the TAML (and later at the Air Force field laboratory), collection bags containing sand flies were placed in a −70°C freezer. Flies were sorted and the numbers of male, unengorged female and engorged female sand flies were determined. Approximately 10–15% of female and 95% of male sand flies were shipped to the Walter Reed Army Institute of Research (Silver Spring, MD) where representative specimens were identified to species. Approximately 85–90% of female and 5% of male sand flies (separated by sex) were placed in pools of 1–20 for subsequent testing for *Leishmania* parasites by using a fluorogenic PCR assay. These samples were stored at −70°C until tested.

Testing of Sand Flies for *Leishmania*. The fluorogenic PCR reaction used in this study was modified from that of Wortmann et al. (2001). The assay detects all species of *Leishmania* parasites. DNA was extracted from sand flies using the QIAamp tissue kit (QIAGEN, Valencia, CA). Complete details on this *Leishmania*-generic assay as well as *L. major*-, *L. tropica*-, and *L. donovani*–*L. infantum*-specific assays will be provided in subsequent articles in this series

Summary of Results from the Sand Fly Surveillance Program. During 2003, 60,533 sand flies (mean = 45.9 per trap; SD = 99.5; range = 0–1,160) and 5,294 mosquitoes (mean = 3.9 per trap; SD = 38.9; range = 0–1,000) in total were collected at TAB. The first collection was made on 12 April and the last on 31 October. Collections (1,318 in total; trap nights) were made on 108 nights during this period. Approximately 92% (1,208/1,318) of the traps contained sand flies. A summary of the sand fly collection by month is presented in Table 1. Approximately 37% (22,296/60,533)

Table 1. Results of sand fly (2003 and 2004) and mosquito (2003) collections at TAB, Iraq

Mo	No. of trap nights	Total no. of sand flies (range)	Mean no. of sand flies/trap (SD)	Total no. of mosquitoes (range)	Mean no. of mosquitoes/trap (SD)
			2003 (17 April–31 Oct.)		
April	60	564 (0–50)	9.4 (11.2)	110 (0–18)	1.8 (3.8)
May	328	12,647 (0–700)	38.6 (70.2)	3,274 (0–1,000)	9.7 (70.7)
June	306	16,212 (0–800)	53.0 (90.0)	1,490 (0–400)	4.8 (31.0)
July	156	11,783 (0–955)	75.5 (134.2)	368 (0–155)	2.3 (13.9)
Aug.	214	13,418 (0–1,160)	62.7 (152.2)	46 (0–4)	0.2 (0.6)
Sept.	134	5,173 (0–466)	38.6 (72.4)	6 (0–2)	0.04 (0.3)
Oct.	120	736 (0–45)	6.1 (8.8)	0	N/A
Total	1,318	60,533 (0–1,160)	45.9 (99.5)	5,294 (0–1,000)	3.9 (38.9)
			2004 (20 April–25 Oct.)		
April	54	499 (0–128)	9.2 (23.7)		
May	156	4,115 (0–346)	26.4 (44.0)		
June	102	4,654 (0–974)	45.6 (104.5)		
July	194	14,981 (0–916)	77.2 (146.5)	Not recorded	
Aug.	134	5,019 (0–622)	37.5 (79.2)		
Sept.	51	1,697 (0–183)	33.1 (53.7)		
Oct.	87	1,145 (0–131)	13.2 (25.8)		
Total	778	32,110 (0–974)	41.3 (95.1)		

N/A, not applicable.

of the sand flies were collected in the standard traps and 63% (38,237/60,533) in a series of studies intended to evaluate the efficacy of surveillance devices and the various control measures that were implemented.

In 2004, 32,110 sand flies (mean = 41.3 per trap, SD = 95.1, range = 0–974) in total were collected during 82 nights of trapping (778 total trap nights). The first collection was made on 20 April and the last on 25 October. Approximately 87% (674/778) of the traps contained sand flies. Approximately 64% (20,665/32,110) of the sand flies were collected in the standard traps. A summary of the sand fly collection by month is presented in Table 1.

Phlebotomine sand flies collected at TAB included *P. papatasi*, *Phlebotomus sergenti* (Parrot), *P. alexandri*, and unidentified *Sergentomyia* sp. To date, a total of 6,979 sand flies have been identified, to include 3,569 males and females collected in 2003 and 3,410 males collected in 2004. The 3,569 sand flies identified from 2003 collections consisted of 655 *P. papatasi* (18.4% of the total 2003 collection), 63 *P. sergenti* (1.8%), 1,114 *P. alexandri* (31.2%), and 1,737 *Sergentomyia* sp. (48.7%). The 3,410 male sand flies identified from 2004 collections included 1,107 *P. papatasi* (32.5% of the total 2004 collection), 1,002 *P. alexandri* (29.4%), and 1,301 *Sergentomyia* sp. (38.2%). Additional informa-

Table 2. *Leishmania* infection rates in sand flies collected at TAB, Iraq, during 2003 and 2004

Mo collected	No. of pools of sand flies tested	Total no. of sand flies tested	No. of pools of sand flies positive	% pools of sand flies positive	Minimum field infection rate
			2003 (female sand flies)		
April	39	465	6	15.4	1.29
May	572	4,964	58	10.1	1.17
June	471	6,601	96	20.4	1.45
July	350	4,944	81	23.1	1.63
Aug.	266	3,942	69	25.9	1.75
Sept.	10	130	1	10.0	0.76
Oct.	0	0	N/A	N/A	N/A
Total	1,708	21,046	311	18.2	1.48
			2003 (male sand flies)		
All	26	397	0	0.0	0.0
			2004 (female sand flies)		
April	18	243	4	22.22	1.65
May	105	1,437	20	19.05	1.39
June	134	1,956	42	31.34	2.15
July	215	3,314	68	31.63	2.05
Aug.	100	1,500	29	29.00	1.93
Sept.	61	915	24	39.34	2.62
Oct.	50	750	15	30.00	2.00
Total	683	10,115	202	29.58	2.00

N/A, not applicable.

tion on the biology and ecology of phlebotomine sand flies at TAB will be provided in subsequent articles.

In 2003, 1,708 pools in total containing 21,046 female sand flies were tested for *Leishmania* parasites, along with 26 pools containing 397 male sand flies (male sand flies served as a negative control). An average of 12 sand flies (SD = 4.4, range = 1–25) were included in each pool. Three hundred and eleven pools of female sand flies (18.2%) tested positive by using the *Leishmania*-generic assay (Table 2). None of the pools of male sand flies was positive. The minimum field infection rate for the female sand flies was 1.48% (we assume that only one sand fly is infected in each pool). In 2004, 683 pools in total containing 10,115 female sand flies were tested. Two hundred and two (29.6%) of pools were positive, with a minimum field infection rate of 2% (Table 2).

We subsequently tested each of the *Leishmania*-positive pools from 2003 by using real-time PCR assays specific for *L. major, L. tropica,* and visceral complex (*L. donovani–L. infantum–L. chagasi*) parasites. Approximately 15% of the pools that had initially tested positive with the *Leishmania*-generic assay subsequently tested positive with one of the species-specific assays, conclusively demonstrating that pathogens capable of causing human disease were present at TAB. However, ≈85% of the pools that tested positive with the *Leishmania*-generic assay did not test positive with any of the species-specific assays. There are several possible explanations for these results. The species-specific *Leishmania* assays are ≈10-fold less sensitive than the *Leishmania*-generic assays, suggesting that some samples that initially tested positive using the *Leishmania*-generic assay may have been below the detection limit of the species-specific assays. However, it is also possible that some of the samples that tested positive with the *Leishmania*-generic assay may have resulted from pathogens that do not infect humans (*Leishmania tarentolae* Wenyon, *Leishmania turanica* Strelkova, Peters and Evans or *Leishmania gerbilli* Wang, Qu and Guan). We have conducted an initial study to determine the species of *Leishmania* parasites that were present in sand flies at TAB by assessing homology of the glucose-6-phosphate isomerase gene (Nyame et al. 1994). To date, 15 isolates in total have been characterized. Eleven (73%) of the isolates were *L. tarentolae,* three (20%) were *L. infantum,* and one (7%) was related to both *L. major* and *L. tropica. L. tarentolae* is a parasite of reptiles that is transmitted by sand flies of the genus *Sergentomyia* and is of no medical importance (Maroli et al. 1988). More detailed information on the testing of sand flies, to include our genetic analyses of the parasites, will be provided in subsequent articles.

Conclusions Drawn from the Sand Fly Surveillance Program. Although data from the sand fly surveillance program clearly revealed the presence of *Leishmania* parasites capable of causing human disease at TAB, it is critical these data be interpreted cautiously. The detection of *Leishmania* parasites in the sand flies by using PCR does not prove the sand flies were infectious (capable of transmitting the parasite) nor do they indicate whether the infected sand flies were feeding on humans. Approximately 4.7% (2,370/50,222) of the sand flies that were checked for blood contained a bloodmeal. We did not analyze any of these bloodmeals to determine the host. However, it was clear that almost all of the insect bites received by military personnel at TAB were due to sand flies, suggesting that infected sand flies were indeed feeding on these individuals. To fully evaluate the threat posed by the infected sand flies, additional bloodmeal and vector competence studies are required. Nevertheless, data from the sand fly surveillance program provided the first solid evidence that leishmaniasis posed a significant threat to U.S. military forces deployed to Iraq. Data from this surveillance program allowed us to implement a proactive prevention, control, and education program at TAB over 5 mo before the first case of leishmaniasis was confirmed in U.S. soldiers deployed to Iraq and allowed us to inform military medical policy makers about the threat that sand flies and leishmaniasis posed months before the first human case was detected. This early warning resulted in the preparation of plans to deal with leishmaniasis in U.S. military forces deployed to Iraq.

Use of PPM as a Means of Preventing Sand Fly Bites

The goal of this portion of the LCP was to ensure that all military personnel at TAB were using appropriate PPM to protect themselves from sand flies. PPM mandated by the Department of Defense (DoD) include use of deet-containing insect repellent on exposed skin, proper wearing of permethrin-treated uniforms, and sleeping under a permethrin-treated insect bed-net (Armed Forces Pest Management Board 2002). To determine the availability of these items at TAB as well as to evaluate compliance, we conducted a variety of different surveys and visited a number of units to evaluate their use of PPM.

An initial survey conducted in April 2003 found that only 5% of Army units at TAB had deployed with insect repellent, permethrin-treated uniforms, or insect bed-nets. Random visits to a number of different Army units confirmed these initial findings. A survey of Air Force units conducted in June 2003 found only 10–15% of deployed personnel arrived at TAB with these PPM items. This low compliance occurred in spite of Army and Air Force mandates requiring units to deploy with bed-nets, permethrin, and deet-based repellents.

Even when available, use of PPM was poor. Insect bed-nets were available to Air Force personnel at TAB by late May 2003 (insect bed-nets are part of the standard deployment package tent cities). A survey of Air Force personnel (excluding flying and security forces) conducted in June 2003 found that only 31% of males (67/214) and 12% (8/65) of females used a bed-net. In contrast to this low compliance, virtually all personnel in the flying and security forces units used bed-nets in their sleeping quarters. This high compliance presumably resulted from the fact that these units had assigned medical personnel who emphasized that bed-nets provided substantial protection from leishmaniasis. A subsequent survey of Air Force personnel conducted in August 2003 found that the compliance rate for insect bed-net use had increased to ≈75% for both male and females on beds or cots ($n = 320$). This increase in compliance between June and August presumably resulted from command emphasis along with increased education regarding the threat of leishmaniasis at TAB.

Because few of the Army, Navy, or coalition units assigned to TAB in early 2003 had insect repellent, permethrin-treated uniforms, or insect bed-nets and because none of these items were available at TAB, an emergency requisition of PPM supplies intended to support the 10,000 soldiers stationed at TAB was submitted in late April 2003. This requisition included 1,670 boxes of deet insect repellent (each box contains 12 2-oz tubes), 2,500 boxes of permethrin (each box contains 12 Individual Dynamic Absorption kits for the treatment of uniforms, insect bed-nets, or both), and 10,000 insect bed-nets. Unfortunately, most of these items were not delivered to TAB until July 2003 (a partial delivery of PPM supplies occurred in early June when a 787th MED DET entomologist drove from TAB to Kuwait and searched through the Theater Distribution Center to locate the missing items). A variety of problems contributed to this delay, including 1) incorrect entry of data into the military ordering system by untrained personnel; 2) cancellation of orders for a variety of reasons without notifying the individual who had placed the order; 3) unavailability of the required items within the logistics system, resulting in a back-order; and 4) delivery of supplies to the wrong location, contributing to further delay. During the interval between the requisition of the PPM supplies in April and delivery in July, soldiers continued to receive large numbers of sand fly bites.

Even after required PPM supplies had arrived at TAB, many soldiers complained that the provided products were not effective. Repeated discussions with soldiers in a variety of units led us to the following general conclusions regarding PPM:

- Due to the intense heat, off-duty personnel normally wore shorts and T-shirts, slept in nothing but shorts, and did not use insect bed-nets (even when these were available).
- Most soldiers failed to treat their insect nets and uniforms with permethrin (even when the appropriate products were available).
- Thousands of insect nets that were shipped to TAB provided no protection against sand flies because they were untreated and had a large mesh size that allowed sand flies to readily pass through them.
- Many soldiers did not know how to properly use the insect bed-nets. We frequently observed large gaps between the nets and the cots on which the majority of soldiers slept. These gaps were large enough to allow sand flies easy access to the soldier.
- Soldiers frequently slept with their body in contact with the insect bed-nets, allowing the sand flies to bite the soldiers through the net.
- Even when available, soldiers routinely did not use the DoD's Extended Duration Topical Insect and Arthropod Repellent (a polymer formulation containing 33% deet). Common reasons given for not using this product included 1) a belief that it was unsafe, 2) it has an unpleasant odor, 3) it is uncomfortable when applied to the skin, and 4) a belief that it was ineffective.
- Many soldiers complained that the Extended Duration Topical Insect and Arthropod Repellent did not protect them from sand flies. Soldiers routinely stated they applied insect repellent when they went to bed and in the morning they awoke with many bites. Observation of many of these soldiers led us to conclude that 1) in some instances, they had failed to completely cover exposed skin with repellent; and 2) that even when applied properly, they were sweating the insect repellent off during the night and were then receiving bites before they woke up. The general conclusion was that the DoD repellent did not provide 12 h of protection against sand flies under the harsh climatic conditions found at TAB.

Our observations, made primarily in summer 2003 when air conditioning was generally not available, convinced us PPM were not protecting soldiers from sand flies at TAB. We attributed the failure of PPM to unavailability, noncompliance, improper use, and ineffectiveness of some of the products. More specifically,

- most units did not bring PPM supplies with them when they deployed;
- the logistics system was unable to deliver PPM supplies to TAB in a timely manner;
- even when available, soldiers failed to use PPM for a variety of reasons (e.g., extreme temperatures, blowing sand, unfamiliarity with the products); and
- even when used, PPM did not protect soldiers from sand fly bites for a variety of reasons (e.g., improper use of the items, failure of the items under harsh conditions).

White et al. (2005) recently reported results from a survey of repellent use by U.S. military personnel arriving in Kuwait in 2004 for deployment to Iraq. Although the threat of leishmaniasis to U.S. military personnel deployed to Iraq had been well documented by early 2004 (CDC 2003, 2004), White et al. (2005) reported that only 36 and 47% of respondents had received any deet or permethrin, respectively. Of particular concern was the fact only 22 and 17% of U.S. Army Reserve soldiers had received deet or permethrin, respectively. Somewhat surprisingly, White et al. (2005) found that >80% of respondents had received a briefing on repellents. Although their analysis did not explain why a large proportion of respondents had received a briefing on repellent use but only a small proportion actually received the repellents themselves, White at al. (2005) suggested

Table 3. Summary of the various pesticides used to control sand flies in 2003 at TAB, Iraq

Pesticide applied (EPA registration no.)	Active ingredient (AI) (% AI)	Total vol		Amount AI applied (oz)	Dates applied	Total no of days applied
		Dilution (%)	Applied (oz)			
Area spray						
Atrapa ULV (1812-407)	Malathion (96.5)	Undiluted	4,256	4,107.3	4 May–2 June	26
Scourge (432-716)	Resmethrin (16.5)	Undiluted	24,808	4,108.2	3 June–2 Sept.	59
Pyronyl Oil (655-501)	Pyrethrins (3)	Undiluted	223	42.4	4 May–24 July	9
Residual spray						
Demand Pestabs (10182-387)	Lambdacyhalothrin (10)	0.03–0.06	94,592	42.1	26 April–24 Sept.	65
Tempo WP (3125-377)	Cyfluthrin (20)	0.05–0.1	135,296	79.8	29 May–16 Sept.	24
Talstar F (279-3162)	Bifenthrin (7.9)	0.02–0.06	11,456	6.5	25 Aug–16 Sept.	9
Dursban (464-571)	Chlorpyrifos (41.5)	0.5	5,440	23.5	13 Aprol–25 Aug.	7
Demon WP (10182-71)	Cypermethrin (40)	0.1	1,120	1.2	25 July–20 Aug.	3
Dust						
Sevin Dust (10107-43)	Carbaryl (5)	Undiluted	3,712	185.6	28 June–7 July	5

that a potential explanation was that health threat briefings were mandated by the Army, whereas units were responsible for ordering their own supplies.

Although a variety of factors affected the perceived failure of PPM as a means of preventing sand fly bites at TAB, all of the authors agree that in the absence of a vaccine or effective prophylactic drug against leishmaniasis, PPM continue to offer the best potential protection from sand flies. Most of the authors diligently used PPM while at TAB and successfully avoided sand fly bites. It is critical, therefore, that efforts be made to educate military personnel on these products so that they will deploy with them and will know how to most effectively use them when required. However, we also recognize there are a variety of inherent problems with some of the current PPM products. For example, many soldiers do not like the current military-issue insect repellent because it is a thick lotion that is unpleasant when applied to the skin (e.g., oily, sand sticks to it). Alternative formulations (e.g., a pump spray) need to be evaluated to determine whether they will be more acceptable. Similarly, soldiers and airmen were reluctant to use the products that contained the DoD labels. When given a choice of two identical products, one with a DoD and the other with a commercial label, personnel would invariably choose the commercial product. Many soldiers expressed dissatisfaction with the standard military insect bed-net, because the net is not pretreated with permethrin (soldiers must treat it themselves) and is difficult to properly set up and tuck in. Although a new self-supporting, permethrin-treated insect bed-net meeting many of the military's requirements is now available within the military logistics system, many units do not purchase this new bed-net due to the higher cost ($76 compared with $30 for the standard military insect bed-net). The new self-supporting bed-net is not necessarily a perfect product for all military personnel or for use in all situations, because it is relatively small and has limited space. Although ideal for rapid deployments and short exercises, a number of soldiers expressed an interest in a larger bed-net with space for a cot and personal belongings. A variety of new insect bed-net designs need to be evaluated to identify products that are both effective and acceptable to soldiers.

Establishment of a Vector Control Program at TAB

The goal of this portion of the LCP was to reduce the population of sand flies at TAB by using a variety of classic vector control techniques available to military forces operating in Iraq. After the collection of the first sand flies on 13 April 2003, the 787th MED DET and the Pest Control Section of 407th AEG assumed responsibility for all pest control operations at TAB. Doctrinally, the 787th MED DET and the 407th AEG were responsible for support to U.S. Army and U.S. Air Force units, respectively; however, out of necessity both units worked together to provide support to all U.S. and coalition forces stationed at TAB without regard to military service or country of origin. The 787th MED DET conducted pest control operations from 13 April to 1 October 2003, whereas the Pest Managers of the 407th AEG operated from 1 May 2003 to 1 October 2004. In May 2003, the Army contracted Kellog, Brown and Root (the engineering and construction subsidiary of Halliburton Corporation) to provide pest control at TAB. Kellog, Brown and Root contractors joined the LCP in May 2003. After the departure of the 787th MED DET in October 2003, Kellog, Brown and Root assumed responsibility for all pest control for Army units stationed at TAB.

The sand fly/leishmaniasis control program established at TAB had four main components: 1) application of space sprays via truck-mounted and hand-held sprayers, 2) application of residual insecticides on tents and buildings, 3) habitat destruction, and 4) reservoir control. The goal of these efforts was to kill adult sand flies. Complete data on pesticide applications at TAB were not available for 2004 as this article was being written and are not discussed here. During 2003, pesticides for sand fly control were applied on 129 of 165 total days between 13 April and 24 September. A summary of insecticides applied in 2003 is provided in Table 3. The following are details on the area and residual spray programs carried out in 2003.

Area Spraying. Area spraying commenced on 4 May 2003 and continued through 2 September 2003. During this period, area spraying was conducted on 62 nights by using Atrapa ULV insecticide (96.5% malathion), Scourge insecticide (16.5% resmethrin) or pyronyl oil insecticide (3% permethrins). Spraying was conducted on every night that weather conditions permitted (wind speeds of <5 miles/h). Atrapa and Scourge insecticides were applied using a truck-mounted Beecomist Pro Mist sprayer according to label directions of the respective pesticide. Pyronyl oil insecticide was applied using a Colt hand-fogger. Collectively, 29,287 oz of insecticide containing 8,258 oz of active ingredient was applied during 2003 (Table 3). Our area spray program will be thoroughly described and results evaluated in a subsequent article; however, evaluation of the number of sand flies collected in the 12–14 standard light traps indicated our area spray program was ineffective and did not substantially reduce sand fly populations at TAB.

Residual Spraying. Residual spraying commenced on 13 April 2003 and continued thru 24 September 2003. During this period, residual insecticides were applied on 94 of 165 d. Active ingredients applied to tents and buildings included lambda-cyhalothrin, cyfluthrin, bifenthrin, chlorpyrifos, and cypermethrin (Table 3). Residual insecticides were originally applied using hand-held or back-pack sprayers; however, Kellog, Brown and Root contractors began using a 300-gal truck-mounted sprayer for this purpose in July 2003. In addition to the application of residual insecticides to tents and buildings, Sevin (carbaryl) dust insecticide was used to control sand flies in rodent burrows. Our residual spray program will be thoroughly described and results evaluated in a subsequent article; however, our sand fly surveillance program indicated residual pesticides applied primarily to the exterior surfaces of tents had a limited impact on sand fly populations. We believe that harsh climatic conditions (intense UV light, high temperatures, and blowing dust and sand) had a detrimental effect on the effectiveness of the residual insecticides (Wilamowski and Pener 2003).

Conclusions drawn from our Vector Control Program. We established a comprehensive vector control program in April 2003 and continued this effort through September 2003. During this period, we applied a variety of pesticides by using several different methods. Although a more thorough review of this effort will be provided in a subsequent article (to include results from a number of studies intended to fully evaluate the impact of insecticide application on sand fly populations), all of our data suggested that our control program had a minimal impact on sand fly abundance. Certainly, we never saw a dramatic reduction in sand fly abundance after a pesticide application as we have experienced with mosquito control efforts. Nonetheless, data from our control (untreated) light traps suggest that we were able to gradually reduce sand fly populations over the course of the year. A significant issue was that our control methods were only targeting adult sand flies. Because sand fly larval habitats are not well known, control of immature stages is generally not feasible (Alexander and Maroli 2003).

Establishment of a Reservoir Control Program at TAB

Documented animal reservoirs of *Leishmania* parasites in the Middle East include dogs and other canids, hedgehogs, and a variety of rodents (Peters et al. 1985, Yaghoobi-Ershadi and Javadian 1996, Baneth et al. 1998). The extermination of infected reservoirs can be an effective method of preventing and controlling leishmaniasis outbreaks in human populations (Alexander and Maroli 2003). Potential animal reservoirs at TAB included feral dogs, jackals and foxes, and a variety of small rodents. In April 2003, we established a program to eliminate potential animal reservoirs of leishmaniasis at TAB.

Canid Control. The TAML veterinary pathologist worked closely with other veterinarians, pest control technicians, and security personnel to establish a program to reduce feral dog populations at TAB. Feral dogs were shot by security personnel or captured in traps by veterinarians and pest control personnel and brought to the TAML where they were humanely euthanized. Blood and tissue samples were collected for subsequent testing for *Leishmania* parasites. Although >200 feral dogs were killed in 2003, we were never able to eliminate dogs at TAB. Dogs from outside the base were attracted to TAB by the waste produced by coalition forces and continuously replaced the dogs that were exterminated. In addition to the feral dogs, six golden jackals, *Canis aureas* L., were collected in live traps. *Leishmania* parasites were not evident in histologic sections or immunohistochemical stains of liver, spleen, and lymph node nor were parasites detected using the real-time *Leishmania* PCR assay in any of the dogs or jackals examined.

Rodent Control. In June 2003, the Army contracted with Kellog, Brown and Root to provide pest control at TAB. Kellog, Brown and Root pest control supervisors worked with TAML, 407th AEG and 787th MED DET personnel to establish an aggressive rodent control program at TAB. The most common rodent at TAB was an unidentified species of mouse (most likely a species of *Mus*) that was commonly found in tents. Mouse populations were initially low but exploded over the course of the summer as the military population grew at TAB. The mice typically found harborage under the wooden pallets used as floors in the military tents. Although mice are not normally considered an important reservoir of *Leishmania* parasites, they are used in the laboratory to infect sand flies (Belkaid et al. 1998, Sadlova et al. 1999). We were concerned that the large number of potentially infected mice living in proximity with military personnel could result in increased transmission rates to our soldiers. Mice were trapped using snap traps, Sherman traps, or both, or they were killed using Maki parrafinized rodent pellets [active ingredient *3-[3-(4′-bromo[1,1′-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2*H*-1-benzopyran-2-one; EPA registration no. 7173-187]. In 2003, we estimated that several thousand mice were exterminated at TAB. Blood and tissue samples from a number of mice were tested using the *Leishmania*-generic real-time PCR assay. One of 34 mice (2.9%) tested positive for *Leishmania* parasites. *Leishmania* parasites were not detected in histological sections of the liver, spleen, and lymph nodes. Based on this evidence, we were unable to determine whether mice at TAB were a source of infection to sand flies.

In addition to the mice, a variety of potential mammalian reservoirs of *Leishmania* parasites were collected in Sherman traps, including 12 long-eared hedgehogs, *Hemiechinus auritus* Gmelin; 11 jirds (*Meriones* spp.); gerbils (*Rhombomys* spp.); and 11 fat sand rats. None of these animals was collected inside of tents; however, they were frequently found within 10–15 m of tents. None of these animals tested positive for *Leishmania* parasites by the real-time PCR assay, nor were *Leishmania* parasites detected in histological sections of the liver, spleen, and lymph node.

Establishment of a Program to Educate Soldiers on Leishmaniasis

Almost all U.S. military personnel (to include medical personnel) stationed at TAB were unfamiliar with leishmaniasis. Very few knew the cause of the disease, preventive measures, signs and symptoms, or diagnosis and treatment procedures. Together, a number of significant issues suggested we needed a leishmaniasis education program at TAB. Specific issues that led to this decision included the following:

- Leishmaniasis has a long incubation period (ranging from weeks to months), suggesting military personnel could become infected at TAB but might not develop symptoms until a much later date.
- Many National Guard and Army Reserve units were stationed at TAB. Because of the long incubation period of leishmaniasis, these soldiers could be infected at TAB but not develop symptoms or seek treatment until after their return to civilian life in the United States. We were concerned these individuals would receive initial medical care from civilian physicians unfamiliar with leishmaniasis, potentially delaying diagnosis and treatment.
- Because vaccines and prophylactic drugs are unavailable for leishmaniasis, PPM remained the best method of preventing the disease. We felt all military personnel at TAB needed training on the proper use of PPM.
- U.S. Army and Marine units were highly mobile (in contrast to U.S. Air Force units) and there was concern units could travel to areas where little or no preventive medicine support was available, making it critical they receive as much information as possible on leishmaniasis prevention while at TAB.
- Because vector control efforts and PPM seemed relatively ineffective, we believed a significant number of military personnel stationed at TAB would contract leishmaniasis, and we therefore needed to teach them the signs and symptoms of both CL and VL and to provide guidance on the proper diagnosis and treatment of the disease.
- Because the majority of U.S. physicians (particularly civilian physicians) are unfamiliar with leishmaniasis, we believed it was critical that soldiers be provided with contact information for military experts on the disease.

The education program at TAB was targeted at three key groups: medical providers, key leaders (commanders and their senior staff), and individual units. Although the depth and breadth of the education program differed according to the target audience, the overall objectives were similar for each group. Each group was provided critical information on leishmaniasis, including 1) etiology, 2) prevention, 3) symptoms and diagnosis, and 4) treatment. Because Air Force personnel arrived and departed TAB as a single group (in contrast to army units, which were constantly arriving and departing), the method of briefing Air Force personnel was different from that used for Army and Marine personnel. Air Force personnel would normally receive a "new-comers" briefing that included information on leishmaniasis when they arrived at TAB. In contrast, we found it necessary to identify each new Army unit arriving at TAB and make arrangements to brief them. Because of this basic difference between the Army and Air Force, the following information pertains primarily to the Army.

The method of presenting information on leishmaniasis varied according to which type of group (medical personnel, key leaders, or individual units) we were briefing. Groups of 20–30 medical personnel would normally receive a comprehensive briefing (to include a Powerpoint presentation as well as hands-on training on the collection of samples for the diagnosis of CL) from a physician. Key leader briefings were normally given to senior (Colonel and Lieutenant Colonel) commanders and their staffs. Briefings were normally given during the commanders' weekly staff meetings when the commander and his staff would be informed of the threat and asked to assist in the implementation of the LCP. A key objective of these briefings was to inform the commanders we would be working on the implementation of the LCP with the subordinate units within their respective commands.

Unit level briefings were intended for the average soldier stationed at TAB. Briefings were given at the unit, with all personnel assigned to each unit encouraged to attend. A key objective was to ensure all soldiers were aware of leishmaniasis and understood how to protect themselves. More than 100 U level briefings were given to Army units stationed at TAB. These briefings lasted anywhere from 30 min to over an hour, with ≈30 min required for the briefing and as much time as was necessary to answer questions. A typical pattern emerged wherein newly arrived units not yet exposed to sand flies had few questions, whereas units with soldiers who had received many sand fly bites or had contracted leishmaniasis asked many questions.

A variety of products were used to disseminate information on leishmaniasis to military personnel assigned at TAB. Color posters providing basic information on the disease as well as contact information for diagnosis or treatment were posted throughout TAB (e.g., in latrines, dining halls, shower tents). This poster was translated into several different languages and provided to coalition forces stationed at TAB. The TAML worked with the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) at Aberdeen Proving Ground, Maryland, to develop a laminated, wallet-sized "Leishmaniasis Information Card." USACHPPM produced >150,000 of these cards in 2003 and sent them to the TAML. These cards were provided to all military personnel stationed at TAB and were subsequently distributed to U.S. military forces throughout Iraq. A modified version of this card is currently available from USACHPPM (http://chppm-www. apgea.army.mil/news/ Leishmaniasis_files/LeishmaniasisCard.pdf). The Tallil Times, a newspaper initiated by the Army Public Affairs Office and later continued by the Air Force Public Affairs Office at TAB, published a number of articles on leishmaniasis in 2003 and 2004. The first of these articles was published in May 2003, months before the first case of CL occurred in a U.S. soldier stationed in Iraq. Finally, a Standard Form 600 (Chronological Record of Medical Care) was prepared that documented the risk of leishmaniasis at TAB and the fact that soldiers stationed at TAB were at risk of being infected. The completed form contained information on the prevention, control, and treatment of the disease as well as contact information for military physicians familiar with the disease. The Standard Form 600 was provided to each Army unit at TAB. Each unit was then responsible for placing this form in each individual's medical record. This form was initially used only by U.S. Army and Marine units at TAB; however, it was subsequently adopted by the U.S. Air Force.

Summary

In this article, we described the evolution of a program intended to prevent and control a potential outbreak of leishmaniasis in U.S. military forces stationed at TAB, Iraq. We believe that our identification of *Leishmania* parasites in sand flies clearly demonstrates the value of medical entomology to the U.S. military. The early identification of *Leishmania* parasites in sand flies allowed us to implement our prevention and control program several months before the first documented case of leishmaniasis was reported in a U.S. soldier deployed to Iraq. Although it is impossible to determine how many cases of leishmaniasis were actually prevented by our efforts, we believe the comprehensive program established should serve as a model for the control of vector-borne diseases during military deployments. Subsequent to the establishment of the LCP at TAB, the U.S. DoD has implemented measures to decrease the risk of leishmaniasis throughout Iraq. These measures are similar to those we had established at TAB and include 1) improving living conditions (providing air conditioners for tents) for deployed personnel, 2) heightening awareness that leishmaniasis is a threat (through publicity about cases of leishmaniasis in military personnel and pre- and postdeployment briefings about leishmaniasis), 3) emphasizing the importance of using PPM, and 4) enhancing vector control activities (CDC 2004).

The assessment of sand fly abundance and the testing of sand flies for *Leishmania* parasites by using a real-time PCR assay in the field were invaluable for assessing risk. Information on sand fly infection rates provided a powerful tool for convincing skeptics that we faced a potentially serious situation. The detection and identification of the threat months before the first human case was diagnosed provided us with a "window of opportunity" of which we took full advantage by implementing our comprehensive LCP. Although PCR assays are a powerful tool for vector-borne diseases surveillance, care must be used in interpreting results. PCR provides information on the presence of a pathogen and not on the ability of a vector to transmit the pathogen. It is possible the sand flies collected at TAB were not competent vectors of the parasites detected by PCR. Although in many instances we were unable to determine the species of *Leishmania* parasite present in the sand flies, in many instances we conclusively demonstrated that pathogens capable of causing human disease (*L. major*, *L. tropica*, or *L. infantum*) were found. The primary species of sand flies found at TAB included *P. papatasi, P. alexandri,* and *P. sergenti.* Each of these species is a documented vector of disease-causing *Leishmania.* It is therefore highly likely that active transmission of medically important *Leishmania* parasites was occurring at TAB.

A significant limitation of the *Leishmania*-generic PCR assay was identified in this study. This assay was originally designed to detect parasites in clinical (human) samples (Wortmann et al. 2001). Intuitively, the detection of nonpathogenic species of *Leishmania* in clinical samples is of minimal concern. If the assay detects *Leishmania* in a sample taken from a soldier with symptoms indicative of leishmaniasis, it is likely that it is a pathogenic parasite. However, the detection of *Leishmania* in sand flies must be interpreted more cautiously. Our genetic analyses of *Leishmania* DNA isolated from sand flies indicated that >85% of the positive samples resulted from nonpathogenic parasites, whereas only 15% of the parasites were a threat to our deployed soldiers. Clearly, it is critical that the species of parasite be determined to truly identify foci in which our soldiers will be at risk for leishmaniasis.

Conversely, data from our sand fly studies indicated that soldiers at TAB may have been exposed to *L. infantum.* Visceral-complex parasites (e.g., *L. infantum* or *L. donovani*) were detected in a number of pools of sand flies collected at TAB. Analysis of the glucose-6-phosphate isomerase gene of these parasites demonstrated the samples were identical to *L. infantum,* a potentially lethal pathogen known to occur in southern Iraq (WHO 2003). To date, of the >1,100 confirmed cases of leishmaniasis in U.S. military personnel deployed to Iraq (Lay 2004), only two cases of visceral disease have been detected (P.J.W., unpublished data). Our sand fly data suggest a significant number of soldiers may have been exposed to *L. infantum* at TAB. *L. infantum* normally causes symptomatic disease only in young children or in immunocompromised adults (Davies and Mazloumi Gavgani 1999); however, the parasite can persist for years as an asymptomatic infection (Magill 1995). The long-term implication of asymptomatic *L. infantum* infections in exposed military personnel is unknown.

Although we implemented a comprehensive prevention and control program specifically designed to reduce the risk of leishmaniasis at TAB, data from a variety of sources indicated our program may have had limited success. Our vector and reservoir control efforts did not seem to significantly reduce sand fly or canid populations, and PPM did little to reduce sand fly bites. In spite of these apparent limitations, all of the individuals participating in the LCP felt our efforts had an overall positive impact. The challenges inherent in establishing a disease control program in a war-time environment made it difficult to assess the true impact of our effort. For example, our sand fly surveillance efforts focused on the collection of sand flies in light traps placed outside of buildings and tents, whereas the majority of sand fly bites were occurring inside of tents and buildings. It is possible that although our control efforts did not reduce the numbers of sand flies collected in the light traps they could have reduced the numbers of sand flies inside tents and building where soldiers were sleeping. Data to support this possibility will be presented in subsequent articles on vector control. Although light trap data did not indicate a significant reduction in sand fly populations, anecdotal evidence from several studies indicates that soldiers perceived a reduction in sand fly bites after application of residual insecticides on tent walls. Alternatively, even though light trap data did not show a significant decrease in sand fly abundance, it is possible control efforts (the almost daily application of insecticides) reduced the longevity of sand flies at TAB. Because sand fly control efforts targeted adult flies only, it is likely that adult populations were being constantly replenished as larval stages matured. Despite the constant replenishment, the daily application of insecticides may have reduced sand fly longevity such that transmission of *Leishmania* parasites from sand flies to humans was not possible.

In retrospect, it seems only 20–50 cases of leishmaniasis were acquired at TAB. These numbers demand explanation for two reasons. First, they are lower than predicted. In April 2003, preventive medicine experts at the TAML estimated up to 400 soldiers stationed at TAB would develop leishmaniasis. This estimate was based on sand fly abundance, infection rates, and biting rates. Second, the infection rates at TAB are lower than expected relative to other locations in Iraq, because surveillance indicated sand flies were much more abundant at TAB and the infection rates in TAB's sand flies was higher than most other locations. Unfortunately, no sand flies were collected from two areas in Iraq from which the highest number of human cases occurred. There are several explanations for the low numbers of cases from TAB. First, our data suggest *L. infantum* was the most common species of disease-causing *Leishmania* present in sand flies at TAB. This parasite normally does not cause symptoms in healthy, immunocompetent adults (Magill 1995). Although no cases of visceral disease caused by *L. infantum* have occurred in soldiers stationed at TAB, it is likely that a significant number of soldiers have been exposed to the parasite. Second, it is possible control efforts were more effective than light trap data alone suggested and that our control measures in fact did significantly reduce the number of sand fly bites received by soldiers at TAB. Third, it is also possible the extremely high biting rates early in 2003 (presumably by sand flies that were not yet infectious) could have sensitized the soldiers and thus protected them from subsequent infectious bites, as suggested by Kamhawi et al. (2000). Finally, our original calculations assumed that all sand flies were infected with parasites capable of causing human disease. Because only 7% of *Leishmania*-positive sand flies were infected with *L. major*, only 28 cases of CL in total (400 originally estimated $\times$ 0.07) would have been expected.

Acknowledgments

A number of military medical entomologists provided invaluable support to our effort to control leishmaniasis at TAB. These individuals include Colonel (COL) Steve Berte, COL James Swaby, COL Steve Bennett, COL Deborah Niemeyer, Lieutenant Colonel (LTC) Mustapha Debboun, COL Leon Robert, LTC Eric Milstrey, COL Gene Cannon, and LTC Steve Horosko.

References Cited

Alexander, B., and M. Maroli. 2003. Control of phlebotomine sand flies. Med. Vet. Entomol. 17: 1–18.

Armed Forces Pest Management Board. 1999. Disease vector ecology profile: the Middle East. Defense Pest Management Information Analysis Center, Forest Glen Section, Walter Reed Army Medical Center, Washington, DC.

Armed Forces Pest Management Board. 2002. Personal protective measures against insect and other arthropods of military significance. Defense Pest Management Information Analysis Center, Forest Glen Section, Walter Reed Army Medical Center, Washington, DC.

Baneth, G., G. Dank, E. Keren-Kornblatt, E. Sekeles, I. Adini, C. L. Eisenberger, L. F. Schnur, R. King, and C. L. Jaffe. 1998. Emergence of visceral leishmaniasis in central Israel. Am. J. Trop. Med. Hyg. 59: 722–725.

Belkaid, Y., S. Kamhawi, G. Modi, J. Valenzuela, N. Noben-Trauth, E. Rowton, J. Ribeiro, and D. L. Sacks. 1998. Development of a natural model of cutaneous leishmaniasis: powerful effects of vector saliva and saliva preexposure on the long-term outcome of *Leishmania major* infection in the mouse ear dermis. J. Exp. Med. 188: 1941–1953.

Berman, J. 2005. Recent developments in leishmaniasis: epidemiology, diagnosis, and treatment. Curr. Infect. Dis. Rep. 7: 33–38.

[CDC] Centers for Disease Control and Prevention. 2003. Cutaneous leishmaniasis in U.S. military personnel–

Southwest/Central Asia, 2002–2003. Morb. Mortal. Wkly. Rep. 52: 1009–1012.

[CDC] **Centers for Disease Control and Prevention. 2004.** Update: cutaneous leishmaniasis in U.S. military personnel–Southwest/Central Asia, 2002–2004. Morb. Mortal. Wkly. Rep. 53: 264–265.

Coleman, R. E., D. A. Burkett, V. Sherwood, J. Caci, L. Hochberg, and P. A. Weina. 2004. Prevention and control of leishmaniasis during Operation Iraqi Freedom. Wing Beats 15: 10–16.

Coleman, R. E., D. A. Burkett, J. Caci, V. Sherwood, B. Jennings, L. Hochberg, P. A. Weina, and J. Putnam. 2005. Assessment of the leishmaniasis threat at Tallil Air Base, Iraq and development of a prevention and control program. Archives De L'Institut Pasteur de Tunis: Proceedings of the Fifth International Symposium of phlebotmine sand flies. 82: 65.

Cross, E. R., W. W. Newcomb, and C. J. Tucker. 1996. Use of weather data and remote sensing to predict the geographic and seasonal distribution of *Phlebotomus papatasi* in southwest Asia. Am. J. Trop. Med. Hyg. 54: 530–536.

Davies, C. R., and A. S. Mazloumi Gavgani. 1999. Age, acquired immunity and the risk of visceral leishmaniasis: a prospective study in Iran. Parasitology 119: 247–257.

Fryauff, D. J., G. B. Modi, N. S. Mansour, R. D. Kreutzer, S. Soliman, and F. G. Youssef. 1993. Epidemiology of cutaneous leishmaniasis at a focus monitored by the multinational force and observers in the northeastern Sinai Desert of Egypt. Am. J. Trop. Med. Hyg. 49: 598–607.

Gasser, R. A., Jr., A. J. Magill, C. N. Oster, and E. C. Tramont. 1991. The threat of infectious disease in Americans returning from Operation Desert Storm. N. Engl. J. Med. 324: 859–864.

Hyams, K. C., K. Hanson, F. S. Wignall, J. Escamilla, and E. C. Oldfield, 3rd. 1995. The impact of infectious diseases on the health of U.S. troops deployed to the Persian Gulf during operations Desert Shield and Desert Storm. Clin. Infect. Dis. 20: 1497–1504.

Jacobson, R. L. 2003. Leishmania tropica (Kinetoplastida: Trypanosomatidae) – a perplexing parasite. Folia Parasitol. (Praha) 50: 241–250.

Kamhawi, S., Y. Belkaid, G. Modi, E. Rowton, and D. Sacks. 2000. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. Science (Wash., DC) 290: 1351–1354.

Kinnamon, K. E., E. A. Steck, P. S. Loizeaux, L. D. Hendricks, V. B. Waits, W. L. Chapman, Jr., and W. L. Hanson. 1979. Leishmaniasis: military significance and new hope for treatment. Mil. Med. 144: 660–664.

Lay, J. C. 2004. Leishmaniasis among U.S. Armed Forces, January 2003–November 2004. Medical Surveillance Monthly Report, U.S. Army Center for Health Promotion and Preventive Medicine 10: 2–5.

Magill, A. J., M. Grogl, R. A. Gasser, Jr., W. Sun, and C. N. Oster. 1993. Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. N. Engl. J. Med. 328: 1383–1387.

Magill, A. J. 1995. Epidemiology of the leishmaniases. Dermatol. Clin. 13: 505–523.

Maroli, M., M. Gramiccia, L. Gradoni, P. D. Ready, D. F. Smith, and C. Aquino. 1988. Natural infections of phlebotomine sandflies with Trypanosomatidae in central and south Italy. Trans. R. Soc. Trop. Med. Hyg. 82: 227–228.

Martin, S., J. Gambel, J. Jackson, N. Aronson, R. Gupta, E. Rowton, M. Perich, P. McEvoy, J. Berman, A. Magill, and C. Hoke. 1998. Leishmaniasis in the United States military. Mil. Med. 163: 801–807.

Most, H. 1968. Leishmaniasis. *In* Infectious Diseases and General Medicine, pp 1–28. Office of the Surgeon General, U.S. Government Printing Office, Washington, DC.

Naggan, L., A. E. Gunders, R. Dizian, Y. Dannon, S. Shibolet, A. Ronen, R. Schneeweiss, and D. Michaeli. 1970. Ecology and attempted control of cutaneous leishmaniasis around Jericho, in the Jordan Valley. J. Infect. Dis. 121: 427–432.

Nyame, K., C. D. Do-Thi, F. R. Opperdoes, and P.A.M. Michels. 1994. Subcellular distribution and characterization of glucose phosphate isomerase in *Leishmania mexicana mexicana.* Mol. Biochem. Parasitol. 67: 269–279.

Pehoushek, J. F., D. M. Quinn, and W. P. Crum. 2004. Cutaneous leishmaniasis in soldiers returning from deployment to Iraq. J. Am. Acad. Dermatol. 51: S197–200.

Peters, W., S. Elbihari, C. Liu, S. M. Le Blancq, D. A. Evans, R. Killick-Kendrick, V. Smith, and C. I. Baldwin. 1985. *Leishmania* infecting man and wild animals in Saudi Arabia. 1. General survey. Trans. R. Soc. Trop. Med. Hyg. 79: 831–839.

Sadlova, J., M. Svobodova, and P. Volf. 1999. Leishmania major: effect of repeated passages through sand fly vectors or murine hosts. Ann. Trop. Med. Parasitol. 93: 599–611.

Sukkar, F. 1974. Study on sandflies as vectors of kala azar in Iraq. Bull. Endemic Dis. (Baghdad) 15: 85–104.

Sukkar, F., F. Yawanis, N. A. Al-Doori, and S. K. Al-Mahdawi. 1985. The possible vector of infantile leishmaniasis in Iraq. Bull. Endemic Dis. (Baghdad) 26: 27–36.

Weina, P. J., R. C. Neafie, G. Wortmann, M. Polhemus, and N. E. Aronson. 2004. Old world leishmaniasis: an emerging infection among deployed US military and civilian workers. Clin. Infect. Dis. 39: 1674–1680.

White, D. J., P. Davis, and M. H. Walter. 2005. Survey of repellent use by service members arriving in Kuwait for Operation Iraqi Freedom 2. Mil. Med. 170: 496–500.

Wilamowski, A., and H. Pener. 2003. Efficacy of microencapsulated insecticides against the sandfly, *Phlebotomus papatasi* Scopoli. J. Vector Ecol. 28: 229–233.

Willard, R. J., A. M. Jeffcoat, P. M. Benson, and D. S. Walsh. 2005. Cutaneous leishmaniasis in soldiers from Fort Campbell, Kentucky returning from Operation Iraqi Freedom. J. Am. Acad. Dermatol. 52: 977–987.

[WHO] **World Health Organization. 2003.** Communicable disease profile – Iraq. Communicable Disease Working Group on Emergencies, HQ Division of Communicable Disease Control, EMRO, WHO Office, Baghdad, Iraq.

Wortmann, G., C. Sweeney, H.-S. Houng, N. Aronson, J. Stiteler, J. Jackson, and C. Ockenhouse. 2001. Rapid diagnosis of leishmaniasis by fluorogenic Polymerase Chain Reaction. Am. J. Trop. Med. Hyg. 65: 583–587.

Yaghoobi-Ershadi, M. R., and E. Javadian. 1996. Epidemiological study of reservoir hosts in an endemic area of zoonotic cutaneous leishmaniasis in Iran. Bull. World Health Organ. 74: 587–590.

Yaghoobi-Ershadi, M. R., A. A. Akhavan, A. R. Zahraei-Ramazani, A. R. Jalali-Zand, and N. Piazak. 2005. Bionomics of *Phlebotomus papatasi* (Diptera: Psychodidae) in an endemic focus of zoonotic cutaneous leishmaniasis in central Iran. J. Vector Ecol. 30: 115–118.

Received 30 August 2005; accepted 5 April 2006.