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Old World Leishmaniasis: An Emerging Infection among Deployed US Military and Civilian Workers

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Many veterans of Operation Iraqi Freedom are now returning to the United States after potential exposure to leishmaniasis. In the past year, large numbers of leishmaniasis cases of a magnitude not encountered in the United States since World War II have challenged clinicians in both the military and the civilian sectors. Many Reserve and National Guard troops were deployed to Iraq and are now back in their communities. Hundreds of leishmaniasis cases, which were managed by a few practitioners initially, permitted further appreciation of the epidemiology and diagnostic and treatment options for Old World leishmaniasis. We describe the current situation, with on-the-ground experience, complimented by a literature review, and we provide a practical list of options for the clinician likely to encounter this parasitic infection in the coming months and years.

In the past year, there were >600 cases of cutaneous leishmaniasis and 4 cases of visceral leishmaniasis diagnosed in American soldiers deployed to Iraq, Kuwait, and Afghanistan [1]. Leishmaniasis is a sandfly-borne parasitic disease caused by protozoa that live inside macrophages in mammals. Sandflies become infected after feeding on the reservoir animal (rodents, dogs, and other small mammals) or infected humans and can then transmit the parasite to other humans. Leishmaniasis is characterized by diverse clinical manifestations ranging from asymptomatic infections to self-limited cutaneous disease to life-threatening visceral disease. There are 3 major clinical syndromes: visceral disease (in which the parasite replicates throughout the reticuloendothelial system), cutaneous disease (in which it replicates in the dermis), and mucosal disease (in which it involves in the naso-oropharyngeal mucosa).

Old World cutaneous leishmaniasis (OWCL) is most often associated with the species *Leishmania major* and *Leishmania tropica*. In patients with cutaneous disease, ⩾1 skin ulcer or nodule forms in the absence of fever, anemia, spleen, and/or liver enlargement (figure 1). Cutaneous leishmaniasis may self-heal without drug treatment after a period of 7–12 months [2]. Cutaneous leishmaniasis may (infrequently) locally disseminate with subcutaneous nodules or regional lymphadenopathy in which amastigotes accumulate. In some unusual cases, *L. major* and *L. tropica* infection can develop into diffuse cutaneous leishmaniasis [3].

Old World visceral disease is associated with *Leishmania infantum* and *Leishmania donovani*. Visceral leishmaniasis usually begins in the absence of any recognizable skin lesion or scar. The symptoms are nonspecific and include some or all of the following: irregular high fever, cough, weight loss, anemia or pancytopenia, hepatosplenomegaly, lymphadenopathy, and fatigue. In untreated adults, especially in those who are protein malnourished or coinfected with other pathogens (e.g., HIV), and in young children, visceral leishmaniasis can be fatal.

In Operation Iraqi Freedom, US soldiers had intense vector exposures and often reported receiving hundreds of insect bites starting in late April 2003. Over 50,000 sandflies were collected from 14 sites in Iraq. Infection rates in sand flies, which were determined using batch PCR testing, ranged from 0.06% to 2.78% [4]. Of 310 patients with leishmaniasis who were interviewed at the Leishmaniasis Treatment Center of Walter Reed Army Medical Center (Washington, DC), 80% had used topical

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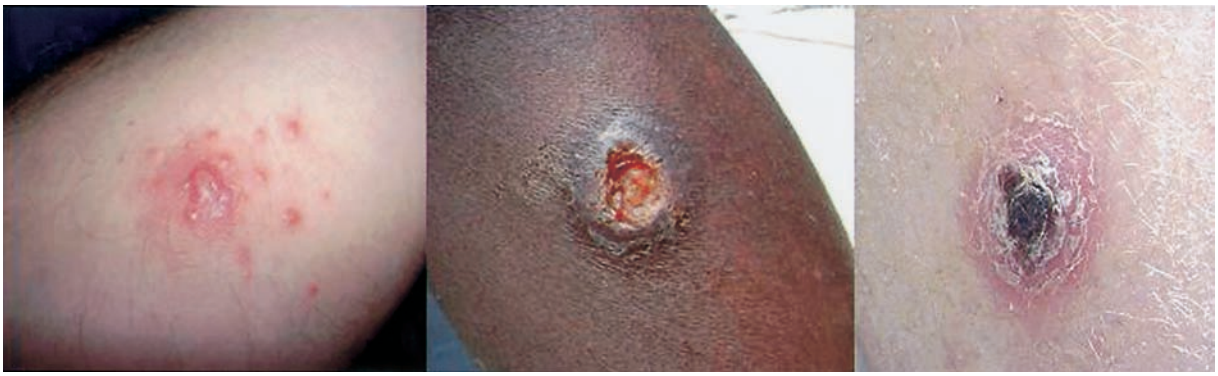

Figure 1. Leishmaniasis lesions in individuals involved in Operation Iraqi Freedom ranged from papular eruptions *(left)* to more classic erosive craters *(center)* and were sometimes surrounded by concentric desquamation *(right).*

repellents, but 26% noted that these were unavailable at some times during their deployment, 17% had ever treated their uniforms with permethrin, and 10% slept under a bednet.

Demographically, the majority of the cases of cutaneous leishmaniasis occurred among US Army troops. Distribution by sex and rank reflected the usual distribution of soldiers in the units most affected in the theater. The mean time to presentation (± SD) for medical attention was 9 ± 5 weeks. Most soldiers reported the onset of skin lesions between August and November 2004, with nearly one-half having onset during the months of September and October 2004 [1]. This parallels published information from Iraq [5]. The distribution of cases over time based on date of diagnosis is shown in figure 2.

DIAGNOSIS

Lesions of cutaneous leishmaniasis seen in Operation Iraqi Freedom ranged from small papular eruptions to large ulcerative erosions (figure 1). Most patients had multiple lesions (range, 1–47). Lesions presented most often on nocturnally exposed skin, especially that of the arms, followed by the lower extremities, more so than the trunk and back; and lesions presented least often on the face and neck. If a patient presented with a nonhealing lesion of >3 weeks' duration, had been in a known area of endemicity with sandfly exposure, and was not successfully treated with a course of broad-spectrum antibiotic therapy, the pretest probability for leishmaniasis was very high. Before use of a 7–10-day course of amoxicillin-clavulanate became standard for treatment of chronic skin lesions, there were ~5 bacterial infections for every case of leishmaniasis detected; after antibiotic pretreatment was instituted, this ratio reversed.

The first diagnostic decision is how to acquire a skin sample. A scraping of the lesion is the desired procedure in areas where lesions are cosmetically apparent, such as the face and hands, as well in areas where the skin is thin with underlying vital structures, whereas the punch biopsy is acceptable for areas where a 4–6-mm wide by 2–3-mm deep skin defect would have little consequence. Scrapings are much easier to interpret microscopically (a monolayer) and are less traumatic to the patient. Tissue sections are usually of uneven thickness and show an uneven distribution of amastigotes that may result in lengthy searching.

Scrapings are obtained by the following protocol. After the lesions are cleaned with alcohol, local anesthesia with 1% lidocaine (with epinephrine, unless contraindicated) is provided, and eschars are unroofed, samples are obtained by horizontally scraping (enough to elicit an exudate, but not vigorously enough to cause bleeding) the base of the underlying ulceration with a scalpel blade. The dermal tissue is then thinly applied in a circular fashion to a nickel-sized area on a slide. The center of the lesion is the easiest and highest-yield area in which to perform a lesion scraping.

Biopsy was the preferred technique to maximize the volume

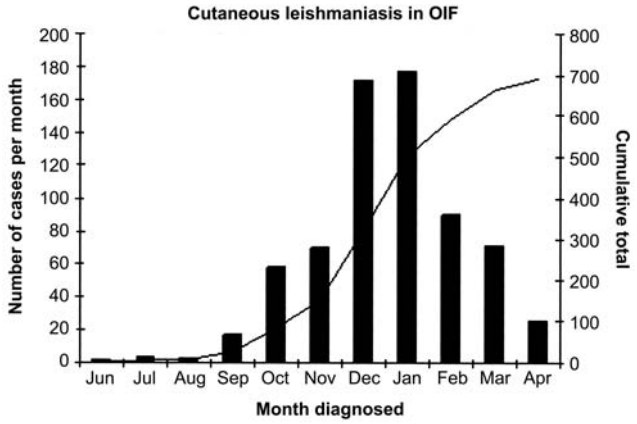

Figure 2. The "epidemic" curve of parasitologically confirmed cases of leishmaniasis during the months following the first confirmed leishmaniasis case from Iraq in US and coalition soldiers in Operation Iraqi Freedom (OIF), June 2003 through mid-April 2004.

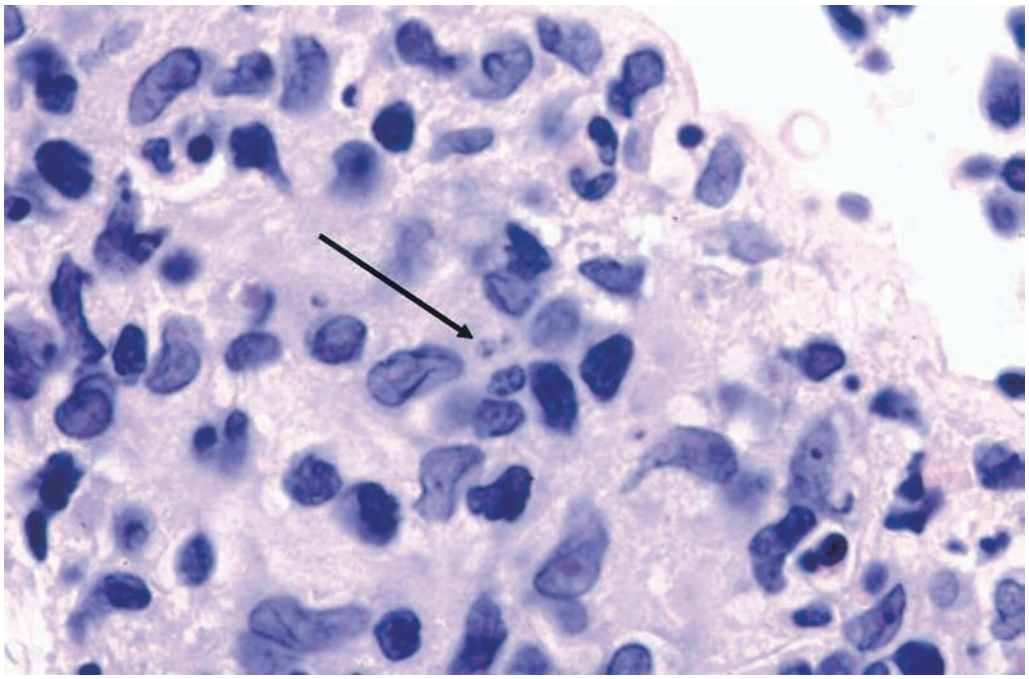

Figure 3. Tissue section cut at 4 microns illustrating an amastigote, with thin cell membrane, cytoplasm, nucleus, and kinetoplast *(arrow)* (hematoxylin and eosin stain; original magnification, ×1000).

of tissue obtained (in areas other than the face and hands), to identify conditions other than leishmaniasis, and for skin lesions that are not ulcerative. After cleansing and provision of anesthesia, a 4-mm, sterile, disposable punch or sterile scalpel should to be used to remove a piece of tissue ~1 mm deep from the edge of the lesion. The biopsy tissue specimen should then be briefly placed on sterile, clean, dry gauze to absorb excess blood that may interfere with the reading; the tissue is then touched or smeared onto the slide. These slides should be stained with Giemsa stain and are evaluated under oil immersion. The biopsy specimen should be taken from the edge of the ulcer to include both necrotic and viable tissue.

Tissue sections should be cut at 4 μm and stained with hematoxylin and eosin, which, in most instances, is adequate for demonstrating amastigotes (figure 3). The Brown-Hopps tissue Gram stain is sometimes helpful, because it accentuates the kinetoplast. All slides should be examined using the oil immersion objective. Most amastigotes are round to oval in shape and are 2–3 μm in greatest dimension. Amastigotes are comprised of a thin cell membrane, cytoplasm, nucleus, and a rod-shaped kinetoplast (figure 4). To identify an amastigote, all 4 of these structures must be together in a single cell.

Schneider's drosophila media supplemented with fetal bovine serum was used exclusively for parasite culture in the forward laboratory in southern Iraq. Even in that austere environment, parasites were recovered from >60% of the samples cultured. In the Leishmania Diagnostics Laboratory at Walter Reed Army Institute of Research (Silver Spring, MD), it was standard practice to use Schneider's, modified media, and Novy-MacNeal-Nicolle media. Recovered parasites were available for isoenzyme analysis to determine the species of leishmaniasis. Three hundred eight (60%) of the first 500 cases evaluated yielded a specimen on culture that could be expanded and maintained in the laboratory. Three hundred four (99%) of those 308 cultures were found, by cellulose acetate electrophoresis, to be positive for *L. major*. Some of these samples were further isotyped and found to be zymodeme MON-26, which is a common agent of zoonotic cutaneous leishmaniasis in the sub-Saharan region in the near and Middle East and has previously been reported from Iraq.

PCR was performed for diagnosis of leishmaniasis using a genus-specific primer and probe developed by Wortmann et al. [6]. This technique was used with both a LightCycler (Roche) and a SmartCycler (Cepheid) real-time PCR platform. These 2 platforms had excellent agreement. The advantages of this technique are the rapidity with which results can be obtained, the lack of reliance on expertise in microscopy, and the lack of expertise, expense, and specialized equipment needed to cultivate the parasite.

As of the middle of April 2004, there were >600 unique individual cases of leishmaniasis diagnosed either with culture or PCR at the Walter Reed Army Institute of Research (WRAIR) and/or by review of tissue smears and biopsies at the Armed Forces Institute of Pathology (Washington, DC). Diagnostic

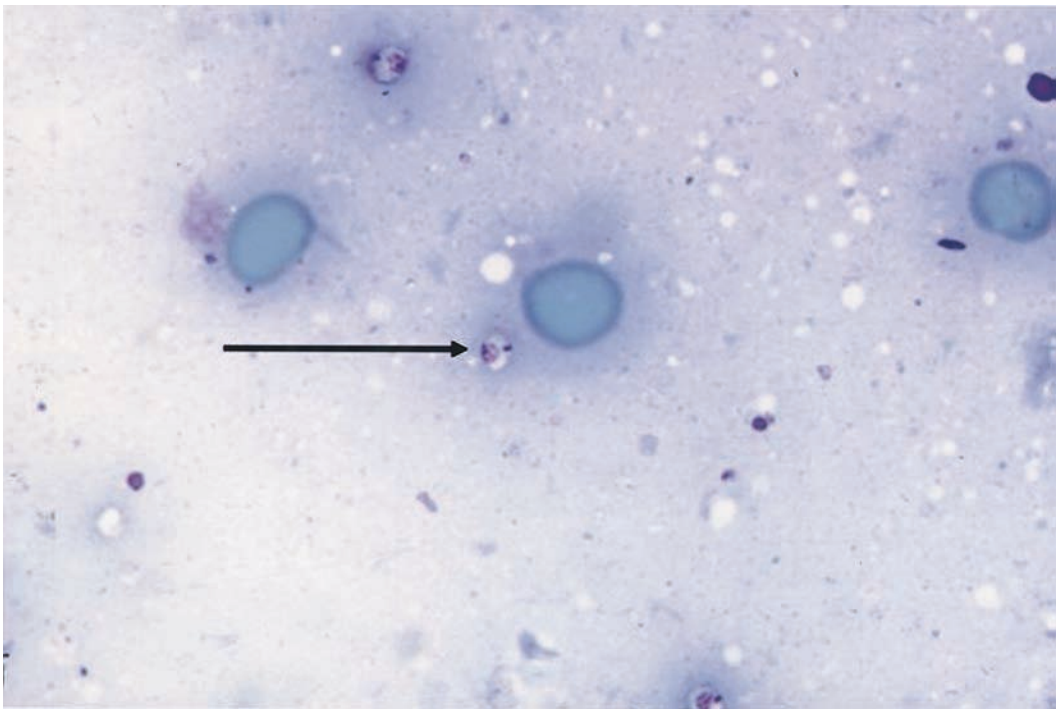

Figure 4. Tissue impression smear illustrating the characteristics of an amastigote, with thin cell membrane, cytoplasm, nucleus, and kinetoplast *(arrow)* (Giemsa stain; original magnification, ×1000).

techniques are best compared using the first 165 cases diagnosed at a US military field laboratory in Iraq. Extraordinary efforts were made to find amastigotes in multiple tissue impressions from every patient; PCR was performed on all specimens, and multiple parasite cultures were done whenever possible. Only 1 of the 165 patients had soley a positive PCR result, and of the remaining 164 persons, visualized amastigotes were found in samples obtained from 164 (100%), and parasites were cultured in samples obtained from 56 (84%) of 67 persons.

TREATMENT OF OWCL

The first question for the management of OWCL is whether to treat or not to treat. *L . major* infection is a generally self-limited cutaneous illness that heals in ⩽12 months [2]. *L. tropica* infection may have a more chronic course (up to years in duration) but is usually self-healing with the reported complications noted of leishmaniasis recidivans and viscerotropic leishmaniasis [7]. The primary consequence that may be affected by early administration of treatment is cosmetic (i.e., a lesser scar and no local dissemination). For small, self-healing lesions in areas of lesser cosmetic concern, it is reasonable not to treat, especially when the parasite is known to be *L. major.* Rahim and Tatar [5] noted that "a life long immunity to reinfection seems to be the rule after recovery from cutaneous leishmaniasis in Iraq. We have noted only two cases of reinfection…" (p. 45).

In our opinion, treatment of OWCL should be provided for the following circumstances: lesions are present on the face, ear, or other cosmetically evident areas; lesions have not healed for many months; lesions are present over areas with joints, such as fingers, the wrist, and the elbow, where scarring could impede future range of motion; lesions are present on the hands and feet, where secondary infection is of concern; lesions suggest evidence of local dissemination (e.g., there are satellite papules, regional lymphadenopathy, or sporotrichoid subcutaneous nodules); sores are occurring on immunocompromised hosts; or there are multiple lesions (more than 5–10) or large lesions (size, >4 cm). In these instances, systemic therapy should be considered.

The currently employed methods of treatment for OWCL include physical methods, topical ointments, oral agents, local injection, and systemic use of pentavalent antimony. Consensus on the best OWCL treatment awaits more well-controlled, definitive clinical trials. One of the earliest treatments was curettage, in which all inflamed surfaces are removed [8]. There is some concern that curettage could facilitate dissemination along lymphatic drainage [9]. Cryotherapy (using liquid nitrogen either topically with swab or via a cryomachine) has been used with variable results [10–12]. In one controlled trial [12], 57% of lesions were healed after 1–3 fortnightly treatments: if combined with use of an intralesional antimonial, then the rate of healing increased to 91%. Use of this treatment in dark-

skinned individuals has been associated with hypopigmentation. Local heat therapy has been of interest, because *Leishmania* species are susceptible to heat. In 2003, the US Food and Drug Administration (FDA) approved a device for the treatment of cutaneous leishmaniasis (ThermoMed; Thermosurgery Technologies) that generates heat in the skin by radio frequency. A placebo-controlled trial of a prototype device for treatment of New World cutaneous leishmaniasis (NWCL) with 3 treatments revealed a rate of healing (73%) that was similar to that of parenteral pentavalent antimony [13]. A recently completed trial of ThermoMed for cases of *L. tropica* infection in Afghanistan revealed an efficacy of 69% at 3 months for a single administration treatment [14].

Topical treatment has great appeal for *L. major* infection. Recently, SNAP cream (S-nitroso-N-acetylpenicillamine), which generates nitric oxide, was used for treatment of NWCL in 16 patients, all of whom improved (compared with none of the control subjects) [15]. Other nitric oxide–generating creams (e.g., ascorbic acid, salicylic acid, and nitrite) had no significant effect in 40 Syrian patients with *L. tropica* infection [16]. Topical paromomycin (aminosidine) has been studied in several randomized trials of patients with OWCL with variable results; the hypothesis is that the vehicle may be quite important. P-ointment (15% paromomycin and 12% or 5% methylbenzethonium chloride in soft white paraffin) used for treatment of *L. major* infection in Israeli was associated with a cure rate of 74% after 10–20 days [17]. In randomized clinical studies from Iran and Tunisia, no clear clinical benefit of 15% paraffin and 10% urea in paraffin ointment could be demonstrated [18–20].

Oral imidazoles have shown that species is important in treatment effect. For example, *L. tropica* infections showed poor clinical response to azoles [21], although in vitro data suggested that ergosterol biosynthesis disruption by itraconazole was effective [22]. *L. major* may be more responsive to azoles. In patients with *L. major* infection in Iranian, itraconazole (7 mg/kg q.d. for 3 weeks) cured 59% of subjects, compared with 44% of subjects in the placebo arm [23]. In another trial, which involved 96 patients randomized to receive ketoconazole (600 mg q.d. for 30 days) or intralesional meglumine antimoniate, 89% were cured, compared with 72% of antimony recipients [24]. In patients from Saudi Arabia with *L. major* infection, a placebo-controlled trial of fluconazole (200 mg q.d. for 6 weeks) healed 59% of recipients at 3 months, compared with 22% in the control group, with a modestly shorter time to healing in the azole group [25]. Great interest has been raised regarding oral miltefosine, but no trials involving OWCL have been published, and the data for patients with NWCL have been variable, with a placebo-controlled trial showing 91% efficacy in Colombia and 53% in Guatemala [26].

Liposomal amphotericin B has been approved by the FDA for the treatment of visceral leishmaniasis but not for cutaneous disease. Our experience with use of this agent for treatment of cutaneous leishmaniasis is unimpressive [27]. We recently used it as salvage therapy for *L. major*–associated facial lesions that were unresponsive to pentavalent antimony, in combination with imiquimod, with inadequate results. Our concern is that the relatively low dermal levels that can be achieved may contribute to a poor treatment response. Because of its toxicity, we reserve conventional amphotericin deoxycholate for salvage therapy and note that there is limited information to guide dose or duration for treatment of cutaneous leishmaniasis, although 0.5–1 mg/kg per day for 14–30 days has been suggested.

The pentavalent antimonials—generally sodium stibogluconate (Pentostam; GlaxoSmithKline) and meglumine antimoniate (Glucantime; Specia Rhone Poulenc)—have been used successfully for treatment of leishmaniasis for more than half a century. In the United States, these agents must be used under an investigational new drug protocol, because they have not been submitted to the FDA for approval. Intralesional injection of pentavalent antimony (until the base of the lesion blanches) is internationally used, particularly if the lesions are small and few in number. There is no current mechanism in the United States to allow use of this method of delivery, which is painful, requires multiple doses, and has various regimens noted with regard to dosing intervals and numbers of injections. In the United States, sodium stibogluconate is available for parenteral use under a protocol from the Centers for Disease Control and Prevention drug services (telephone 404-639-3670) for civilian use and for military use (via the Walter Reed Army Medical Center, Washington, D.C. [telephone 202-782-1663], and Brooke Army Medical Center, San Antonio, TX [telephone 210-916-5554]). The military protocols are for persons who are current military health care beneficiaries or who acquired infection in the course of military duties. They require patients to receive outpatient treatment at 1 of the 2 medical centers noted above. There are a limited number of published studies of parenteral pentavalent antimony for the treatment of OWCL [28, 29]. Although sodium stibogluconate has predictable associated toxicities (mainly chemical and sometimes clinical pancreatitis, increased liver function test values, electrocardiographic changes, mild marrow suppression, reactivation of herpes virus infections, rash, headache, arthralgias, myalgias, and fatigue), they are reversible once medication is removed, and treatment clearly accelerated healing [30].

We recommend follow-up of patients for $\geqslant$6 months. Photographic documentation before and after therapy can be very helpful to assess treatment response. Patients are advised that relapse is possible, generally in the first 2 months, and they should observe their scars for lack of healing, increase in size, scabbing, induration, and ulceration. We advise against elective surgery or receipt of tattoos until 12 months of observation

have passed, because trauma can activate disease even in remote sites [31].

VISCERAL LEISHMANIASIS

Visceral leishmaniasis is a major public health problem in Iraq, usually attributed to *L. infantum* infection, with a case rate in 2001 of 10.9 cases per 100,000 persons [32]. The greatest impact in areas of endemicity involves children aged <5 years, suggesting that immunologically naive adult Americans would be at risk. The Baghdad/central Iraq area was reported to have the highest rates of transmission, but since 1991, the range has expanded to include the more southern Thi Qar, Muthanna, Missan, and Basrah governates [32]. Because parasitemia in asymptomatic blood donors has been found to persist at low levels [33], the donation of blood from Americans visiting Iraq has been deferred for 1 year, and all patients in whom any type of leishmaniasis has been diagnosed have been recommended for permanent deferral [34].

To date, 4 soldiers (2 of whom were deployed to Afghanistan and 2 of whom were deployed to Baghdad, Iraq) have been parasitologically confirmed to have visceral leishmaniasis [35]. All patients had fever, hepatosplenomegaly, cytopenia, and hypergammaglobulinemia. In the 2 Afghanistan-acquired cases, the incubation periods were 3 and 14 months, and parasites were found with granulomatous inflammation only in the liver (the results of bone marrow biopsies were negative); *L . infantum/L. donovani* complex was identified by PCR primers in 1 case. In the Iraq-acquired cases (one patient had been in Iraq for 11 months, and the other had left Iraq 7 months earlier), a bone marrow biopsy revealed parasites; one patient was determined to be infected with *L. infantum/L. donovani*. The results of an rK39 serological test (Kala azar detect; InBios) were positive for all patients, and the serum samples sent to the Centers for Disease Control and Prevention (Atlanta, GA) for *Leishmania* immunofluorescent antibody testing yielded titers of 1:1024 or greater.

Visceral leishmaniasis can be visualized and/or cultured from tissue samples, such as spleen, bone marrow, liver, and lymph node specimens. The highest yield (>95%) is for aspirates of the spleen, although there is a risk of hemorrhage, whereas the safer bone marrow aspirate has a sensitivity of 60%–85% [36]. Antibody detection with the direct agglutination test has been variably reported, and immunofluorescent antibody serological testing has a specificity of 70%–89% [36]. Antigen detection in urine specimens is being evaluated. In Iraq, among 40 children at the An Nasiriyah Women's and Children's Hospital, using consensus review of bone marrow biopsy as the reference standard, the rK39 serological test identified all cases of visceral leishmaniasis with a specificity of 100% (P.J.W., unpublished data).

There are additional recent options for the treatment of visceral leishmaniasis. The FDA has approved liposomal amphotericin (AmBisome, Gilead [Nexstar]; 3 mg/kg q.d. for days 1–5, then boosted at days 14 and 21) for the immunocompetent host with visceral leishmaniasis. Conventional amphotericin B has a cure rate of >95% but is more toxic. Other lipid-associated amphotericin formulations have been studied and have shown good effect but have not received FDA approval. The pentavalent antimonials—for example, sodium stibogluconate (Pentostam; GlaxoSmithKline; 20 mg/kg q.d. for 28 days)—have been effective, although regional resistance (up to 65% of new cases in Bihar, India) has been noted [37]. Miltefosine is marketed in India and is an oral antineoplastic agent (a phosphocholine analogue) that was found to be effective for the treatment of visceral leishmaniasis in India, with a reported cure rate of 95% [38]. Aminosidine (paromomycin) is an aminoglycoside that acts synergistically with the pentavalent antimonials, and when administered parenterally, it was effective for treatment of visceral leishmaniasis in Sudan, Kenya, and the United Kingdom [39].

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