

History of U.S. Military Contributions to the Study of Parasitic Diseases

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U.S. military researchers have made major contributions to the discovery, diagnosis, treatment, and prevention of a number of parasitic diseases. We review the paramount U.S. military contributions to the understanding of leishmaniasis, filariasis, schistosomiasis, trypanosomiasis, gastrointestinal parasites, intestinal capillariasis, and angiostrongyliasis.

Introduction

Military investigators have been responsible for numerous advancements in the diagnosis and treatment of parasitic diseases worldwide. U.S. military clinicians have a large collective experience in tropical medicine, primarily because of the occupational exposures of internationally deployed military forces. In this article, we discuss the seminal contributions of military medicine to the study of parasitic diseases.

Leishmaniasis

Leishmaniasis is a sand fly-borne parasitic disease that causes a diverse group of clinical diseases, including cutaneous, mucocutaneous, and visceral manifestations. The disease is endemic in 88 countries, encompassing all continents except Australia and Antarctica. Two million new cases (1.5 million cutaneous and 500,000 visceral) occur annually.^{1,2} The significance of leishmaniasis is rising because of an increase in travel in the public sector, military deployments to disease-endemic areas, and factors that modulate the pathogenicity of the disease, such as immunodeficiency states (e.g., human immunodeficiency virus). Human infections occur several weeks to months following a female sand fly bite (generally *Phlebotomus* spp. in the Old World and *Lutzomyia* spp. in the New World). The clinical presentation depends on the specific *Leishmania* species (more than 20 cause human disease) and host factors. Diagnosis is typically established by demonstrating amastigotes in biopsy specimens, although novel diagnostic techniques are being developed.³ Therapy typically involves a pentavalent antimonial agent in the form of sodium stibogluconate (Pentostam; GlaxoSmithKline, Brentford, United Kingdom) or meglumine antimoniate (Glucantime; Rhône-Polenc, Paris, France); despite the efficacy of these agents, significant side effects have led to the evaluation of additional treatments for this parasitic disease.

Military physicians from around the world contributed to the historical descriptions of leishmaniasis. Although leishmaniasis

had existed for hundreds of years (e.g., Moche pottery in Peru), the first report of parasites in skin lesions was made by British Major D.D. Cunningham in 1885. Further descriptions are attributed to the Russian Army physician Peter Fokitsch Borovsky (1898). James Homer Wright is usually credited with the first clinical description for an Armenian patient treated at the Massachusetts General Hospital (1903).^{4,5} The disease was named after British Colonel W.B. Leishman, who described the organism in 1903 while examining the spleen during autopsy of a soldier with visceral leishmaniasis (VL) acquired at Dum Dum, India (hence, the name "Dumdum fever"). In the same year, a second British military physician, Colonel C. Donovan, linked the clinical disease to parasites recovered from splenic puncture of a living patient. The organisms have subsequently been referred to as Leishman-Donovan bodies.⁶

U.S. Military Significance

During World Wars I and II, thousands of soldiers contracted leishmaniasis.^{7,8} Among U.S. forces, there were 1,000 to 1,500 cases of cutaneous leishmaniasis (CL) (*Leishmania tropica*), with nearly one-half of the cases occurring in the Middle East near Ahvaz, Iran, in a 3-month period (November 1943 to January 1944), with an estimated incidence rate of 1.9 cases per 1,000 soldiers.^{9,10} Sporadic cases occurred in Latin America, Panama, and North Africa.¹¹⁻¹⁴ There were 50 to 75 cases of VL (*Leishmania donovani*) among personnel stationed in the Mediterranean theater (North Africa, Italy, and the French Riviera) and India. The diagnosis was often initially unrecognized, resulting in lengthy delays in treatment; 30 of these patients were subsequently admitted to a hospital in North Carolina for diagnosis and therapy.^{10,11} Only a single death attributable to kala-azar acquired during World War II was reported among U.S. soldiers.¹¹

During the 1990-1991 Persian Gulf War, 20 cases of CL attributable to *Leishmania major* and 12 viscerotropic cases attributable to *L. tropica* were described in association with deployments to Saudi Arabia, Kuwait, and Iraq.^{15,16} The occurrence of viscerotropic disease was unexpected; kala-azar had been only sporadically observed in Saudi Arabia, secondary to *L. donovani*.⁷

Outside of wartime, U.S. military members remain at risk for leishmaniasis because they often train in disease-endemic areas, most notably Central America.^{17,18} Many cases were acquired at the now-closed U.S. Army Jungle Training Operating Center in Panama, as well as in French Guiana.^{5,19} The first case involving a military member in this region was reported in 1944.¹² Sporadic cases were noted from the 1940s to the 1960s.²⁰⁻²² Outbreaks occurred in Panama, including 10 cases among 1,300 U.S. Marines in 1973²³ and 10 cases in a U.S. Army unit of 627 soldiers in 1980.¹⁷ Between 1977 and 1984,

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more than 70 cases occurred, with a sharp increase in 1984–1985.^{24–27} Before recent Southwest Asia deployments, 85% of recent U.S. military cases were New World CL, with a total of 400 cases being diagnosed and treated at Walter Reed Army Medical Center (WRAMC) from 1969 to 2000.²⁸ The military and economic impacts of *Leishmania* cases are significant; one study estimated a cost of \$17,000, with an average of 92 lost workdays per case.¹⁹ Another study reported 2 person-years of lost duty time during a period when a different type of antimony was given.²¹ A 2004 estimate for a 20-day antimony outpatient treatment course was \$11,000 (D. West, personal communication).

Recent deployments to the Middle East (Iraq, Afghanistan, and Kuwait) for Operations Iraqi Freedom and Enduring Freedom have resulted in numerous leishmaniasis cases among U.S. military members.^{29–31} An initial report describing 22 cases of CL was published in October 2003; all patients were treated with a 20-day course of intravenous sodium stibogluconate therapy.²⁹ An updated report in April 2004 reported 522 parasitologically confirmed cases of CL; all cases tested to date ($N = 176$) were *L. major*.³⁰ In addition, two VL cases, probably attributable to *L. donovani*, occurred among U.S. Army Special Forces soldiers, who presented with fevers, hepatosplenomegaly, and cytopenias many months after returning from Afghanistan.³¹ This emphasizes that military members returning from deployments to the Middle East or Central America with consistent clinical findings should be evaluated for leishmaniasis.³²

U.S. Military Contributions

Epidemiology

U.S. military research on leishmaniasis has included understanding vector biology and disease transmission. Across the globe, U.S. military entomologists and biologists conduct sand fly surveys regarding *Leishmania* carriage and distribution of various species.^{33–35} Walter Reed Army Institute of Research (WRAIR) scientists assisted in describing the first known cases of CL attributable to *L. tropica* and *L. major* in Kenya.^{36,37} Walton et al.^{38,39} characterized the first “fast-growing” strain of *Leishmania mexicana* causing espondia and a unique strain of the *L. mexicana* complex causing diffuse CL, while working in the Canal Zone. Other contributions included the development of a computer-generated map, using satellite-sensing equipment to predict the geographic and seasonal distributions of the sand fly vector, *Phlebotomus papatasi*.⁴⁰

Hoogstraal et al.^{34,41,42} at U.S. Naval Medical Research Unit (NAMRU)-3 first described the epidemiology of leishmaniasis in the Sudan; cases in Kenya had been previously noted.⁴³ Their investigations ascertained the geographical distributions of *Phlebotomus orientalis* (the primary sand fly transmitter of disease), the rodent reservoir (*Arvicanthis niloticus luctuosus*, the grass rat), and the *Leishmania* parasite (*L. donovani*) and helped institute appropriate control programs in the region.^{34,41} Similarly, researchers at NAMRU-3 were instrumental in determining that the cause of an outbreak of leishmaniasis among Sinai-based multinational force and observers was attributable to the construction of a dam, which altered the ecology of the area, including rodent and sand fly populations.⁴⁴

More recently, WRAIR researchers discovered an outbreak of leishmaniasis in hunting dogs within the United States.⁴⁵ In addition, approximately 65,000 sand flies were collected with

light traps in Iraq during Operation Iraqi Freedom; 1.4% tested positive with a fluorogenic polymerase chain reaction (PCR), and some of the geographic locations of the vector *P. papatasi* were determined.²⁹ Scientists at WRAIR maintain active sand fly colonies and provide live material for research purposes (P. Lawyer, personal communication).

The potential for transmission through blood transfusions, following the viscerotropic cases in Operation Desert Shield, was identified by observing the persistence of viable inoculated *Leishmania* for 15 days in blood units held under blood bank storage conditions.⁴⁶ WRAIR investigators found that both *L. tropica* and *L. donovani* could be transmitted via blood in BALB/c mice for up to 25 days after donation. In addition, a new method to detect infected cells using flow cytometry was developed.⁴⁷

Pathogenesis

Temperature sensitivity has been shown by U.S. Army researchers to correlate with disease manifestations.⁴⁸ An in vitro temperature-dependent promastigote model was created, which demonstrated that *Leishmania* species causing VL were more tolerant of warmer temperatures.⁴⁹

Clinical Manifestations

WRAMC physicians first reported a viscerotropic form of *L. tropica* among veterans of the Persian Gulf War.¹⁵ Of the 12 reported cases that were disseminated to visceral organs, none had classic symptoms of kala-azar; four patients were afebrile, three did not have hepatosplenomegaly, and none had significant cytopenias or hypergammaglobulinemia. Instead, symptoms consisted mainly of a mild nonspecific illness characterized by malaise, cough, abdominal pain, and diarrhea; one case was asymptomatic. In addition, a prolonged incubation period was described, with one case occurring 2 years after the last possible exposure.^{15,50} The cases emphasize the need for awareness of the varied presentations of leishmaniasis.⁵¹

U.S. military researchers helped define the incubation period and salient clinical characteristics of CL and VL.^{10,11,26,27,52} Investigations by two groups separated by 25 years (four cases in 1974 and seven cases in 2000) showed that CL has a propensity to develop in areas of trauma (Köebner phenomenon); similar findings were noted in mice inoculated with leishmaniasis at WRAIR.^{53,54}

Diagnosis

Definitive diagnosis is made by direct visualization of the *Leishmania* parasites (nucleus with kinetoplast) in tissue biopsy, touch preparation, skin scraping, or autopsy specimens.⁵⁵ WRAIR is one of two College of American Pathologists-certified leishmaniasis diagnostic laboratories in the United States. Investigators at WRAIR have spent two decades perfecting culture media to grow *Leishmania* parasites,⁷ and biopsies or scrapings from cutaneous lesions are culture-positive in 75% of cases.^{26,28} To enhance diagnostic capability, inoculation of specimens into golden hamsters can be performed.⁷ VL can be diagnosed with bone marrow (60–85% sensitive), spleen (nearly 100% sensitive), liver, or lymph node aspiration or biopsy samples.^{2,11} Researchers at the U.S. Army Medical Research Center in Kenya found that quantification of parasite density is useful in deter-

mining therapeutic responses and noted that *Leishmania* can be isolated from a variety of specimens, including urine and nasal secretions.^{56,57}

The Armed Forces Institute of Pathology (Washington, DC) evaluated the sensitivities of a wide range of diagnostic stains and found that the Brown-Hopps stain was superior.⁵⁸ Despite novel staining methods, diagnosis may be difficult in low-parasite burden infections; Giemsa staining has a sensitivity of approximately 40%.¹ WRAIR has developed genus-specific monoclonal antibodies for use in immunofluorescent antibody tests, which increase the sensitivity of finding parasites in fresh or fixed tissues (reported sensitivity of 100%, compared with 56% with culture).⁵⁹ Another monoclonal antibody has been developed that can detect as few as one organism per low-power field on Formalin-fixed tissues and yet maintain excellent specificity and sensitivity.⁶⁰

The WRAIR laboratory offers *Leishmania* diagnosis by culture, PCR, and rK39 antigen testing (P. Weina, personal communication). PCR can be used for diagnosis and speciation; Wortmann et al.⁶¹ developed a real-time fluorogenic PCR (LEIS.P1) that was specific for a conserved region of the rRNA gene of *Leishmania*. PCR detection was both timely (less than 24 hours with fresh tissue) and accurate. PCR technology is also useful for VL diagnosis using blood and urine specimens.^{62,63} WRAIR has assisted in studies of a kinesin-related antigen, rK39, in an enzyme-linked immunosorbent assay (ELISA) dipstick test for the early diagnosis of VL.⁶⁴ Serum testing, including indirect immunofluorescent antibody testing using promastigotes, has also been extensively studied.⁶⁵

Viscerotropic leishmaniasis represented a special diagnostic challenge, because specific immune responses (e.g., serum antileishmanial antibody titers) were lower than those among patients with classical VL.¹⁵ Researchers from WRAIR and others cloned and characterized two immunodominant antigenic fragments of *L. tropica*, Lt-1 and rLt-2, which could be useful in diagnostic testing.⁶⁶ Finally, WRAIR investigators examined exoantigens produced by *L. donovani* parasites, which might be useful for both diagnosis and monitoring of responses to therapy; ELISAs can detect these antigens in blood, saliva, urine, and tissues, with a reported loss of detection with successful therapy.⁶⁷⁻⁶⁹

The Montenegro skin test is used to determine infection with *Leishmania* and may be helpful for naive populations, such as deployed U.S. military personnel, to establish incidence data. Unfortunately, this test is not currently approved by the Food and Drug Administration and thus is unavailable. U.S. Army physicians are currently developing a new preparation for skin testing.⁷⁰

Treatment

Treatment for leishmaniasis has steadily evolved over the past century. The use of local therapies, including ethyl chloride spray, acid berberine sulfate, and roentgen radiation, and systemic therapy with neostam (stibamine glucoside) for CL were common during World War II.¹⁰ VL cases were treated with the trivalent compound Fuadin (stibophen) or a pentavalent antimonial agent (neostam, neostibosan, or stibanose).¹¹

The current therapy for leishmaniasis (most New World cases and more extensive Old World CL) includes the pentavalent antimonial agents, which have been used since the 1940s.⁷¹

Since 1978, the Army Surgeon General has maintained an Investigational New Drug protocol for sodium stibogluconate (Pentostam), with a centralized leishmaniasis treatment center at WRAMC and more recently at Brooke Army Medical Center. The biochemical mechanisms of the antileishmanial activity of antimony were largely unknown until Berman et al.⁷² showed effects on macromolecular synthesis, purine nucleotide synthesis (decreased ATP and GTP levels), and energy metabolism resulting from inhibition of glycolysis. In addition, WRAIR researchers developed in vitro models, including a radiorespirometric microtechnique, for rapid drug-susceptibility studies. The use of these models has shown that poor therapeutic responses may be related not to parasite resistance but instead to pharmacodynamic or host immunological factors.^{73,74}

U.S. military scientists have been instrumental in defining the efficacy as well as the dosage and duration of antimonial therapy for leishmaniasis.⁷⁵ Oster et al.⁷⁶ showed that once-daily therapy with 600 mg/day for 10 days was more efficacious than three divided doses or continuous therapy with the same total amount for American CL. Another landmark finding was that 20 mg/kg/day was superior to 10 mg/kg/day (100% and 76% success rates, respectively), without additional toxicity; this led to the recommendation of Pentostam at 20 mg/kg/day for 20 days in the treatment of American CL.^{25,77} U.S. military clinicians working with Guatemalan researchers showed that short-course antimonial therapy (10 days) might be effective in treating CL.⁷⁸ A similar study conducted among military personnel returning to the United States with American CL (predominantly *Leishmania panamensis*) also demonstrated that a 10-day course of Pentostam was therapeutically equivalent and less toxic than a 20-day course.⁷⁹

Investigators at NAMRU in Peru were the first to study Pentostam for the treatment of mucosal leishmaniasis; they found that only 30% of patients were cured, which suggested that alternate therapies are needed.⁸⁰ Aronson et al.²⁸ showed that the efficacy of Pentostam among U.S. military beneficiaries (96 patients, 1989–1996) was 91% for CL and 93% for visceral/viscerotropic disease; success rates were 97% after two or three treatment courses. Current therapy of CL acquired in the Middle East includes intravenous Pentostam therapy for 10 or 20 days, with the longer course being used for large lesions, facial, ear, and some joint lesions, and cases with poor clinical response at 10 days.

U.S. military clinicians have played a significant role in defining the adverse events of Pentostam. In a report by Aronson et al.,²⁸ 28% interrupted therapy because of medication side effects, although the effects were generally reversible. Ninety-seven percent of patients experienced chemical pancreatitis, 67% elevations in liver function findings, 58% arthralgias and myalgias, 44% hematological suppression, and 9% rash. In another study, military clinicians found that 98% of patients developed pancreatitis (elevation of amylase or lipase levels) during antimonial therapy but continued therapy and/or rechallenge was usually well tolerated.⁸¹ Chulay et al.⁸² characterized electrocardiographic changes during Pentostam therapy; 54% of patients developed electrocardiographic changes, which were correlated with both the daily dose and the duration of therapy. The most common findings included T wave flattening or inversion and prolongation of the QT interval. WRAIR inves-

tigators helped determine that the cause of a cluster of cardiotoxicity cases was attributable to a high-osmolarity lot of antimony.⁸³ Intriguing side effects of antimonial therapy, such as herpes zoster reactivation and CD4⁺ cell count decreases, were first formally described by WRAMC clinicians.⁸⁴

Given the side effect profiles of antimonial agents, as well as the cost (approximately \$100 per 100-mL vial) and increasing resistance, particularly in India (with cure rates as low as 35%), new agents for *Leishmania* treatment are needed.⁷¹ U.S. military investigators showed that liposomal amphotericin B had efficacy against *Leishmania* in animal models and that the drug concentrated in reticuloendothelium, where the visceral parasites reside.^{85,86} Taking the next step, U.S. military members, working with other investigators worldwide, showed high VL cure rates (97%) with a total dose of AmBisome of 15 mg/kg, divided into five injections.⁸⁷⁻⁸⁹ Failure of AmBisome therapy for CL has been cited by WRAMC clinicians and may be attributable to low dermal penetration.⁹⁰ Oral agents such as azoles, allopurinol, and miltefosine have also been studied by U.S. military researchers, in collaboration with other groups.^{7,91-96}

In addition, the U.S. military has evaluated topical antimicrobial agents for CL therapy. Paromomycin (15%) plus gentamicin (0.5%) in a patented base (WR279396) was shown to be effective in an animal model.⁹⁷ A human phase II trial showed that this compound decreased the time to healing of *L. panamensis* CL, compared with a placebo (35 days and 56 days, respectively); overall cure rates were similar between the groups.⁹⁸ In an effort to further define the role of topical drugs, a study by WRAIR workers and other investigators found no advantage of adding topical paromomycin/methylbenzethonium chloride treatment to standard antimonial therapy.⁹⁹

WRAIR investigators have contributed to the search for new antileishmanial agents for decades, and screening of nearly 100,000 compounds in a hamster model led to the recognition of a class of quinoline compounds with activity 700 times greater than that of antimonial agents.^{8,100,101} The more recent development of an axenic amastigote system for drug screening represents another important step toward finding novel drugs.¹⁰² Researchers continue to be involved in each step of drug development, from in vitro and animal studies to human clinical trials.⁷

Prevention

Successful prevention depends on human behaviors and control of local reservoirs and sand fly vectors.¹⁸ The U.S. military was instrumental in developing a new lotion formulation of *N,N*-diethyl-*m*-toluamide (DEET) with effectiveness for up to 12 hours.²⁶ U.S. military and Columbian researchers demonstrated that permethrin-impregnated uniforms decreased the occurrence of leishmaniasis by 75% (12% vs. 3% infection rate) during a 6-week period of exposure.¹⁰³ The cost of treating uniforms has been estimated at 15 cents per month. Despite continued efforts in personal protective measures, surveys suggest that compliance was poor in the past, which resulted in both sporadic cases and outbreaks among military personnel.^{26,104}

Understanding the vector has led to changes in military activities in an effort to decrease the number of *Leishmania* cases. Dichlorodiphenyltrichloroethane (DDT) was shown in experiments to reduce the insect vector, leading to improved control.^{101,105,106} Other preventive strategies include reducing ani-

mal reservoirs. U.S. military scientists showed that cyfluthrin (a pyrethroid insecticide), when applied to animal burrows and around campsites, reduced sand fly populations and human infection rates.¹⁰⁷ The use of chlorpiricin to reduce rodent populations during World War II led to a reduction in case rates from 70% to 0.4%.¹⁰ Finally, using insecticides against the arthropod vectors in areas where troops camped reduced case rates.¹⁰⁸

Summary of Key U.S. Military Contributions

The U.S. military (1) contributed to the epidemiology and prevention of leishmaniasis, (2) gained the largest collective experience in diagnosing and treating leishmaniasis cases within the United States, (3) performed pivotal exploration in leishmaniasis diagnostic tests, and (4) contributed to antileishmanial treatment through drug screening and clinical trials.

Filariasis

Filariasis is a mosquito-borne nematode infection endemic to Southeast Asia, Africa, and South America. Humans are infected by the bite of anopheline or culicine mosquitoes and may develop an acute febrile syndrome with lymphangitis/cellulitis, tropical pulmonary eosinophilia, and/or chronic lymphangitis/elephantiasis (usually after multiple infections).¹⁰⁹ The lymphatic filarial species of major human importance are *Wuchereria bancrofti* and *Brugia malayi*.

U.S. Military Significance

During World War II, U.S. service members were commonly medically evacuated from the Pacific theater, especially Samoa, because of filarial fever.¹¹⁰ Nearly 40,000 U.S. troops were exposed to filariasis during their time in the South Pacific, with 14,000 to 16,000 troops having clinical evidence of infection; some battalions were devastated, losing 70% of their manpower to filariasis.¹¹¹⁻¹¹³ A special treatment center in Klamath Falls, Oregon, was established to rehabilitate these service members.¹¹² U.S. service members were occasionally infected with filariae during the Korean and Vietnam conflicts.^{110,114,115} Soluble-antigen fluorescent antibody test results were positive for 15% of field soldiers in South Vietnam, but the impact on battle readiness was much less apparent than in World War II.¹¹⁶

U.S. Military Contributions

NAMRU-2 surveyed the Indonesian archipelago, describing the distributions of both major and minor filarial species among human and animal inhabitants. Joesoef and Cross¹¹⁷ summarized the major human surveys describing more than 150,000 screened subjects, with an overall prevalence of 10.8% for microfilaremia (*B. malayi* more than *W. bancrofti* more than *Brugia timori*). In the context of these surveys, Partono et al.¹¹⁸ developed the technique of administering one provocative dose of 100 mg of diethylcarbamazine (DEC) to patients to facilitate the diagnosis of bancroftian and brugian filariasis; before this discovery, it was often necessary to draw blood for microscopic examination in the middle of the night, which was an inconvenience for both patients and physicians.

Discovery of new animal reservoirs and new filarial species was a natural result of examining countless blood smears. At-

mosoedjono et al.¹¹⁹ discovered new mammalian reservoirs for *B. malayi* and *Brugia pahangi* in Indonesia. Purnomo and colleagues contributed to the taxonomic literature by identifying several new species of animal (frog and bat)^{120,121} and human (*B. timori*)¹²² filariae. To further investigate the life cycle of the newly described *B. timori*, Purnomo et al.¹²³ developed a vector model using *Aedes togoi*. The nearly commensal relationship between human and filarial parasites led researchers to investigate the origin of an apparent lack of immune response against the parasite. Piessens et al.¹²⁴ discovered that patients were not innately unresponsive to parasite antigens but filarial disease involved suppressor T cells targeted to parasite antigens.

Preclinical trials for new drugs or vaccines were not initially possible because of the complex life cycle of the filarial parasites. In response, Riberu et al.¹²⁵ and Franke et al.¹²⁶ developed a technique to maintain microfilariae (*B. malayi* and *W. bancrofti*) in culture. Cross et al.^{127,128} established the first animal model (silver leaf monkey, *Presbytis cristatus*) for the study of bancroftian filariasis; numerous previous trials using cats, Mongolian jirds, hamsters, and other primates had failed to successfully sustain the infection. The U.S. military conducted studies investigating potential treatment regimens. Baird et al.¹²⁹ demonstrated the efficacy of ivermectin against *W. bancrofti* in vitro. Kurniawan et al.¹³⁰ used the silver leaf monkey model to demonstrate the efficacy of ivermectin and DEC against *Wuchereria kalimantani* (naturally occurring *Wuchereria* in monkeys). Partono et al.¹³¹ demonstrated that repeat DEC therapy was an adequate means to control filariasis (*B. timori*). Furthermore, they established that low-dose weekly DEC treatment was capable of eradicating filarial infection from six villages in Flores, Indonesia.¹³²

Summary of Key U.S. Military Contributions

The U.S. military (1) established in vitro culture for microfilariae, (2) developed the provocative dose of DEC for diagnosis, (3) discovered the first animal model for *W. bancrofti*, (4) provided evidence that survival of filarial parasites is promoted by parasite-driven suppression of host immunity, and (5) established that filarial eradication programs can be safe and effective.

Schistosomiasis

Schistosomes are trematodes (flukes) that primarily infect the portal or vesicular plexus of veins, producing hepatosplenic (*Schistosoma mansoni* and *Schistosoma japonicum*) or urinary (*Schistosoma hematobium*) schistosomiasis.¹⁰⁹ Humans are infected when they come in contact with snail-infested freshwaters of the African, American, and Asian continents. The cercariae penetrate intact skin, transform into schistosomulae, and migrate to their respective end organs via the systemic circulation. Acute infection leads to a febrile syndrome (Katayama fever) accompanied by lymphadenopathy, hepatosplenomegaly, and diarrhea. Wayward migration (especially through the central nervous system) may induce significant morbidity and death. Inflammation stimulated by ova-secreted proteins and granuloma formation leads to primary tissue destruction and end-organ damage.

U.S. Military Significance

From the time of the Napoleonic conquests, schistosomiasis has proved to be a significant medical adversary for troops exposed to freshwater in tropical environs.¹³³ Several hundred British and Australian troops became infected in Egypt and the Middle East during World War I.¹³⁴ During World War II, more than 1,500 British and African troops became infected in Nigeria.¹³³ Perhaps the most spectacular outbreak was among U.S. service members during the liberation of the Philippine Islands, when hundreds of troops were infected during the invasion of Leyte.¹³³ During World War II, a high prevalence of active schistosomiasis was found among Puerto Rican nationals applying for enlistment into the U.S. Army; potential recruits were rejected on the basis of positive stool examination findings. In addition, medical historians suspect that *S. japonicum* infection was the cause of "Dapeco fever" among prisoners of war and guards in the Davao penal colony (southern Mindanao, Philippines).¹³³ Sixty-nine troops reportedly suffered from schistosomal dermatitis during operations in the Mekong Delta during the Vietnam conflict,¹³⁵ but there was no evidence that U.S. service members were infected with human schistosomes.¹³⁶

U.S. Military Contributions

U.S. military researchers made significant discoveries regarding the epidemiology of schistosomiasis. Carney et al. contributed to the knowledge of the vectors and animal reservoirs for *S. japonicum* in Indonesia,¹³⁷ as well as new geographic locations for mammalian schistosomes.¹³⁸ Cross¹³⁹ performed the initial studies of the life cycle and transmission of *S. japonicum* via the snail host *Oncomelania hupensis lindonensis*.

Key observations regarding schistosome-induced hepatic fibrosis were noted by researchers at NAMRU-3 in Cairo. Mansour et al.^{140,141} investigated the pathogenesis of hepatic fibrosis in *S. mansoni* infections in an in vitro/murine model (fibroblast stimulation by a T-cell subset and inhibition of collagen production by mononuclear cells), whereas Dunn et al.¹⁴² noted reversal of fibrosis in a rabbit model via increased collagenolysis. Improvement in fibrosis was also noted after treatment with praziquantel in a murine model.¹⁴³

Recovery of parasite ova is critical in establishing the diagnosis of schistosomiasis and, given the intermittent and/or low-grade passage of ova, this can present a challenge. In an attempt to improve the recovery of ova, Weller and Dammin¹⁴⁴ compared two methods, acid-ether centrifugation and zinc sulfate flotation, and ultimately concluded that the acid-ether method was superior. A "rectal scraper," developed by the U.S. Army Medical Laboratory in San Juan, was also developed to improve the recovery of *S. mansoni* ova.¹³³

Many pivotal clinical observations were made by U.S. military researchers regarding schistosomiasis. Renal dysfunction was characterized among patients with hepatosplenic schistosomiasis with *Salmonella* bacteremia, including the relationship with massive proteinuria,^{145,146} histopathological changes of the kidney,¹⁴⁷ and amyloidosis.¹⁴⁸ In addition, Mansour et al.¹⁴⁹ noted the association of elevated γ -glutamyltranspeptidase levels among patients with late stages of hepatosplenic schistosomiasis. With regard to urinary schistosomiasis, Lehman et al.¹⁵⁰ characterized the clinical, microbiological, and radiographic profiles among patients with urinary schistosomiasis, and

Laughlin et al.¹⁵¹ demonstrated a greatly increased prevalence of bacteruria among patients with *S. hematobium*.

Evidence that end-organ dysfunction was reversible was established by researchers at NAMRU-3. Clinical improvement of obstructive uropathy attributable to *S. hematobium* and advanced hepatosplenomegaly attributable to *S. mansoni* was possible with a single dose of praziquantel.^{152,153} In addition, various praziquantel regimens were evaluated for the treatment of *S. mansoni*.^{154,155}

McCarthy et al.¹⁵⁶ and Hyams et al.¹⁵⁷ noted an association between receiving injections for schistosomiasis therapy and contracting hepatitis B. In addition, researchers at NAMRU-3 established the safety and immunogenicity of recombinant hepatitis B vaccination for patients with *S. mansoni*¹⁵⁸ and infants born to mothers with *S. mansoni*.¹⁵⁹

Preventing service members from entering cercaria-infested water proved to be a challenge to commanders and preventive health units. A number of molluscicides were evaluated by the U.S. Army in an attempt to eliminate the snail host necessary to complete the schistosome life cycle.¹⁶⁰ Additional findings included evidence that chlorination to 1 ppm rendered cercaria-infested water safe for drinking and bathing,¹³³ whereas water with salinity less than 1.5% might still be potentially infectious.¹⁶¹ Hunter et al.¹⁶² determined that even waters appearing free of snails might be infected with cercariae from snails 1 mile away. U.S. military members at NAMRU-3 demonstrated that topically applied niclosamide lotion provided some protective efficacy to those occupationally exposed to *S. mansoni*.¹⁶³

Summary of Key U.S. Military Contributions

The U.S. military (1) devised techniques for increasing diagnostic yield for schistosome ova, (2) proved that advanced hepatosplenomegaly and obstructive uropathy are reversible with praziquantel therapy, (3) established the safety and efficacy of a hepatitis B recombinant vaccine among patients with schistosomiasis, and (4) established the necessary salinity and chlorination to neutralize cercariae.

Trypanosomiasis

African trypanosomiasis is caused by the flagellated protozoa *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, which are transmitted to humans via the bite of the tsetse fly (*Glossina* spp.). *T. rhodesiense* occurs primarily in East Africa, with an acute course of fever, rash, carditis, or early central nervous system disease. *T. gambiense* occurs in West Africa and has a more chronic course. In the first stage, fevers and adenopathy (Winterbottom's sign) predominate; in the later stage, meningoencephalitis-phase neurological signs and progressive somnolence ("sleeping sickness") develop.¹⁶⁴

Trypanosoma cruzi is the etiological agent of human American trypanosomiasis and is transmitted to humans by reduviid bugs. American trypanosomiasis may present as an acute disease, characterized by a local chagoma and Romana's sign of painless swelling of the periocular tissues. Several years after the initial infection, individuals may develop cardiomyopathy, gastrointestinal mega-syndromes, and peripheral nerve involvement.¹⁶⁵

U.S. Military Significance

African trypanosomiasis among human patients was initially recognized in 1902, when the organism was noted in the blood of a British sailor returning from Gambia, thus the name *T. gambiense*.¹⁶⁴ Epidemics of sleeping sickness were a major obstacle to the exploration and colonization of Africa by Europeans in the early 1900s. Although trypanosomiasis was a common problem in native populations during World War II, the disease did not pose a major problem of military significance to U.S. forces, with only approximately 14 diagnosed cases of this infection.¹⁶⁶ Recent evidence suggesting that African trypanosomiasis may be reemerging in the Democratic Republic of the Congo, with prevalence rates of more than 60% in some villages, raises concerns that the disease could affect military operations in that area.^{167,168} Furthermore, treatment of infected soldiers could be problematic, given the limited number of available drugs and emerging resistance.¹⁶⁹

American trypanosomiasis among human patients was initially recognized in 1909 by Carlos Chagas, who discovered the etiological agent, clinical presentation, insect vectors, and reservoir hosts.¹⁶⁵ Although historically Chagas' disease has not had a significant impact on U.S. military operations, a survey in a disease-endemic area of Brazil found a 28.4% prevalence of Chagas' disease among Brazilian military recruits.¹⁷⁰⁻¹⁷³

U.S. Military Contributions

In 1973, the U.S. Army Medical Research Unit (USAMRU) was established in Kenya to pursue research concerning African trypanosomiasis initiated at WRAIR. USAMRU has concentrated on the epidemiology and clinical spectrum of African trypanosomiasis in the Lambwe Valley of Kenya, whereas research at WRAIR described the expression of variant surface glycoproteins on individual trypanosomes during antigen switching and identified potential new drugs from their stockpile of more than 200,000 compounds.¹⁷⁴⁻¹⁹⁵ Research on *T. cruzi* at WRAIR has been directed toward potential drug therapies.¹⁹⁶⁻²⁰¹

USAMRU investigators significantly contributed to the epidemiological, clinical, and diagnostic treatment of African trypanosomiasis during their work in the Lambwe Valley from 1973 to 1989. Studies by Welde and coworkers were published in a special issue of the *Annals of Tropical Medicine and Parasitology* in 1989.¹⁷⁴⁻¹⁸⁹ Their research included evaluation of various serological tests for diagnosis of trypanosomiasis, studies on factors contributing to disease transmission, tsetse fly control measures, and studies of the disease in cattle and goats. USAMRU investigators also described the initial presentations of primary sleeping sickness among 209 patients, providing in-depth descriptions of clinical symptoms, physical examination results, and laboratory findings.¹⁸⁰ They found that cerebrospinal fluid (CSF) values were abnormal for nearly 60% of patients with primary sleeping sickness, indicating early parasite invasion of the CSF. Their findings explained the high relapse rate among individuals treated with suramin on the basis of clinical symptoms alone (without CSF analysis), because suramin does not penetrate the central nervous system and hence would be ineffective among patients with early central nervous system disease.¹⁸⁰ This finding supported the change in clinical practice to include lumbar puncture procedures in all cases.

Trypanosomiasis is considered a “neglected” disease concerning drug development. Only four new drugs were approved between 1975 and 1999 for treatment of trypanosomiasis, i.e., benznidazole and nifurtimox for Chagas’ disease and eflornithine and pentamidine for African trypanosomiasis.²⁰² WRAIR testing of potential drug classes that might be effective against African trypanosomiasis from 1979 to 1982 contributed to efforts to find new classes of drugs with antitrypanosomal activity to be evaluated as potential new trypanocidal drugs, but none has been approved to date.^{190–194} Using a mouse model, more than 300 compounds from the WRAIR inventory were tested for suppressive activity against *T. cruzi*.^{196–201} More than 50 drugs were determined to have trypanocidal activity equal to or greater than that of nifurtimox, the current drug of choice for the treatment of American trypanosomiasis. Classes of compounds found to have significant suppressive activity included 7-amin-quinolines, phosphonium salts, 8-aminoquinolones, anticancer compounds (i.e., cyclohexamide), thiosemicarbazones, and the antidepressant protriptyline. These classes may be further evaluated to find new drug therapies for American trypanosomiasis.²⁰³

Summary of Key U.S. Military Contributions

The U.S. military (1) conducted studies in the Lambwe Valley of Kenya on the epidemiology, clinical manifestations, and control of African trypanosomiasis, (2) discovered the expression of variant surface glycoproteins on trypanosomes during antigen switching, (3) demonstrated the utility of routine CSF analysis in African trypanosomiasis to determine the optimal therapy, and (4) conducted drug studies at WRAIR for African and American trypanosomiasis.

Gastrointestinal Parasites

Human intestinal parasites have been reported throughout the world. More than one-half of the world’s population is harboring one or more worms, and millions have intestinal protozoan infections.²⁰⁴

U.S. Military Significance

U.S. military personnel deployed overseas are often assigned to areas where intestinal parasitoses are highly endemic. Although most of these infections are well tolerated and have little effect on military operations, it is important that potential problems be identified and medical officers informed of their existence. Hookworm infections were found among a number of U.S. military personnel deployed to Grenada and Vietnam.²⁰⁵ Amebiasis attributable to *Entamoeba histolytica* has been a problem in U.S. military operations; many acquired the disease while serving in Korea or Vietnam. The large number of potential cases of amebiasis reported at the U.S. Naval Hospital in Da Nang, Vietnam, caused a great deal of concern. An investigation of 900 admissions to the hospital for treatment of diarrhea found that less than 4% of the patients had *E. histolytica* infections, and it became clear that overdiagnosis was common. *Ascaris lumbricoides*, *Giardia lamblia*, and hookworm, however, were found for some patients.²⁰⁶

U.S. Military Contributions

Colonel Bailey K. Ashford discovered hookworm (*Ancylostoma duodenale*), a frequent cause of anemia, in Puerto Rico in

1899. His discovery led to worldwide therapeutic campaigns to cure this important parasitic disease.^{207–209} U.S. Army physician C. Craig’s work on amebiasis at the turn of the 20th century is noteworthy, particularly his development of serological tests, including the complement-fixation test, to aid in the diagnosis of this disease.²¹⁰ In addition, he contributed to the understanding of the clinical and pathological findings for amebiasis.^{211,212} U.S. Army Captain E. Vedder is credited with demonstrating that emetine is a powerful amebicide in vitro and that it is useful in the treatment of amebic dysentery.²¹³

Since the 1950s, research scientists at NAMRU-2 have been studying diseases in Southeast Asia and have produced a great deal of information on parasitic diseases. Widespread biomedical surveys were conducted to determine the prevalence and distribution of intestinal parasites in Indonesia and the Philippines. More than 40,000 stool samples were collected in these areas, establishing that *A. lumbricoides*, *Trichuris trichuria*, and hookworms were the most common parasitic infections. These data were based on examination of single stool specimens; however, if more than one stool specimen had been examined, then 99% of the populations surveyed would have been shown to have been infected.^{214,215}

NAMRU-2 developed and evaluated an indirect hemagglutination serological test for amoebic liver abscesses. Examination of more than 500 patients demonstrated sensitivity and specificity of 99% and 95%, respectively. Epidemiological studies conducted in Indonesia and the Philippine Islands found serological evidence of exposure to *E. histolytica* for 14% of Indonesians and 7% of Filipinos; stool examinations for the parasite showed prevalence rates of 8% and 5%, respectively.²¹⁴

The trematodes *Prosthodendrium molenkampi* and *Phaneropsolus bonnie* are intestinal parasites endemic to Southeast Asia. Investigators at the Armed Forces Research Institute of Medical Sciences in Bangkok first described the life cycle of these trematodes, showing that parasites were acquired when dragonfly naiad were eaten uncooked.²¹⁶ In addition, scientists from the Armed Forces Research Institute of Medical Sciences conducted extensive studies on the epidemiology of cyclospora (*Cyclospora cayetanensis*) infections in Nepal.²¹⁷ They also determined that the treatment of choice for the condition is trimethoprim-sulfamethoxazole.²¹⁸

Summary of Key U.S. Military Contributions

The U.S. military (1) discovered that hookworm was a frequent cause of anemia, (2) performed biomedical surveys of intestinal parasitoses in Southeast Asia, (3) determined the seroepidemiology of amebiasis in Indonesia and the Philippines, (4) developed a serological test for diagnosis of amebiasis, (5) described the life cycle of two intestinal trematodes in Asia, and (6) determined the treatment for cyclosporiasis.

Intestinal Capillariasis

Capillaria philippinensis is an intestinal parasite that was first reported from the Philippines. Infection occurs through ingestion of raw freshwater fish. The parasite infects the small intestine, producing diarrhea and malabsorption, with eventual death for up to one-third of untreated patients.

U.S. Military Significance

Because of early concern regarding infection of troops (two U.S. military bases were in Luzon at the time), investigations were conducted to determine the life cycle and route of transmission of the parasite. When it was discovered that infection occurs from eating raw fish, concern diminished, because this practice is uncommon among U.S. military personnel.

U.S. Military Contributions

Researchers at NAMRU-2 were largely responsible for the description of intestinal capillariasis. They carried out extensive studies in the Philippines on the epidemiology, means of transmission, pathology, diagnosis, and treatment and were responsible for providing conclusive documentation of this parasitosis.

U.S. military researchers determined the life cycle of the parasite; it is acquired through ingestion of uncooked freshwater and brackish-water fish that harbor the larvae. NAMRU-2 investigators also described the disease manifestations of intestinal capillariasis, which include borborygmi, abdominal pain, weight loss, and diarrhea. Laboratory findings include low levels of total protein, potassium, carotene, and calcium, because of malabsorption. After a few months without treatment, patients become emaciated and develop pallor and anasarca. Death may occur because of electrolyte loss and heart failure; intercurrent bacterial septicemia also may contribute.²¹⁹ More than two thousand patients have been reported in Northern Luzon in the Philippines. The parasite has also been found to be endemic in Thailand, and there are reports of cases in Korea, Japan, Taiwan, India, Iran, and Egypt.²²⁰

Treatment in the early years of study involved the use of thiabendazole, antidiarrheal agents, potassium, and a high-protein diet. U.S. military researchers showed the effectiveness of mebendazole (200 mg twice per day for 1 month in relapse cases and for 2 weeks in new cases) for the treatment of intestinal capillariasis, and this drug replaced thiabendazole.²²¹ Further studies defined the drug regimen of choice as 200 mg of albendazole twice per day for 10 days; relapses are rare.²²² Without the continuous efforts by NAMRU-2 over several years, little would have been known about the disease, the means of transmission, and treatment.

Summary of Key U.S. Military Contributions

The U.S. military (1) discovered the life cycle of *C. philippinensis* and the mode of transmission, (2) described the epidemiology and clinical aspects of intestinal capillariasis, and (3) determined the standard treatment for intestinal capillariasis.

Angiostrongyliasis

Angiostrongylus cantonesis, the rat lung worm, is the most common cause of eosinophilic meningitis. The natural hosts are rats, with the parasite being found in pulmonary vessels. The intermediate hosts are usually terrestrial and aquatic mollusks, particularly snails, but infection has been found in a number of paratenic hosts, such as planaria, freshwater prawn, and land crabs. Humans acquire infection by eating infected intermediate or paratenic hosts. Larvae are released, penetrate the intestines, and are carried to the central nervous system. Humans often develop an eosinophilic meningitis with headache, stiff neck,

paresthesias, nausea, vomiting, and paralysis of the internal rectus muscles. Specific diagnosis is difficult unless larvae are found in the eye or CSF; eosinophilic meningitis and a history of eating undercooked mollusks are suggestive.²²³

U.S. Military Significance and Contributions

Angiostrongyliasis was noted among U.S. Marines stationed in Okinawa who ate raw terrestrial snails. NAMRU-2 carried out extensive basic studies on *Angiostrongylus* infection in Southeast Asia. Studies on strains of the parasite, from Taiwan, the Philippines, Thailand, Indonesia, Vietnam, Okinawa, and Malaysia, led to a finding of a new species, *Angiostrongylus malaysiensis*.²²⁴⁻²²⁶ NAMRU-2, in collaboration with Taiwan investigators, also carried out extensive epidemiological investigations on the island.

NAMRU-2 also developed an ELISA for the diagnosis of angiostrongyliasis.²²⁷ The test was used to diagnose three U.S. Marines stationed in Okinawa who had eaten the land snail *Achatina fulica* while participating in survival training. The U.S. Marine Corps survival training manual advises members not to eat raw snails in Okinawa or elsewhere.²²⁸

Summary of Key U.S. Military Contributions

The U.S. military (1) discovered a new species, *A. malaysiensis*, (2) studied the epidemiology of angiostrongyliasis in Taiwan, and (3) developed an immunological test for angiostrongyliasis.

Conclusions

Diseases such as leishmaniasis, filariasis, and schistosomiasis caused thousands of infections among U.S. soldiers during World Wars I and II; more recently, leishmaniasis has affected military members during the Persian Gulf War and Operation Iraqi Freedom. The U.S. military community has contributed much and will continue to play a paramount role in parasitic disease research. Key contributions made by the U.S. military in the study of parasitic diseases over the past 100 years include the following: (1) provided nationwide expertise in the diagnosis and treatment of leishmaniasis, (2) performed pivotal exploration for leishmaniasis diagnostic tests, (3) contributed to antileishmanial treatment through drug screening and clinical trials, (4) developed the provocative dose of DEC for diagnosis of filariasis, (5) discovered the first sustainable animal model for *W. bancrofti*, (6) established the safety and efficacy of a hepatitis B recombinant vaccine among patients with schistosomiasis, (7) established the necessary salinity and chlorination to neutralize cercariae, (8) conducted studies on the epidemiology, clinical manifestations, and control of African trypanosomiasis and demonstrated the utility of CSF analysis to determine the optimal therapy, (9) discovered hookworm, *A. duodenale*, (10) described the prevalence and distribution of intestinal parasitic infections in Southeast Asia and developed a serological test for amebiasis, (11) determined treatment for cyclosporiasis, (12) provided detailed information and established treatment of a new disease, intestinal capillariasis, caused by *C. philippinensis*, and (13) described a new species of nematode, *A. malaysiensis*.

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