

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

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Rickettsial diseases have affected the military throughout history. Efforts such as those of the Joint U.S. Typhus Commission near the beginning of World War II and of military researchers since have reduced the impact of these diseases on U.S. and Allied forces. Despite the postwar development of effective antibiotic therapies, the newly emerging antibiotic-resistant scrub typhus rickettsial strains of the Asian Pacific region mandate continued research and surveillance. Similarly, tick-infested training areas in the United States and similar exposure abroad render the spotted fevers and the ehrlichioses problematic to deployed troops. The military continues to work on countermeasures to control the arthropod vectors, as well as actively participating in the development of rapid accurate diagnostic tests, vaccines, and improved surveillance methods. Several rickettsial diseases, including epidemic typhus, scrub typhus, the ehrlichioses, and the spotted fevers, are reviewed, with emphasis on the military historical significance and contributions.

Introduction

Certain rickettsial diseases have had a significant impact on military operations throughout history. Before the middle of the 20th century, when effective antibiotics were introduced, military morbidity and mortality rates attributable to diseases such as epidemic typhus and scrub typhus were high. As far back as 429 B.C. and the plague of Athens, as described by Thucydides, typhus was thought to have killed the Athenian general Pericles and caused the downfall of Athens during the Peloponnesian War.¹ There are reports in which the destruction of whole armies occurred. Arguably the most famous example is the destruction of the Grand Army of Napoleon following the 1812 withdrawal from Russia, which was attributed, in large part, to typhus and a "lousy" Russian winter. Because symptoms of these diseases often resemble those of other febrile illnesses, such as malaria and leptospirosis, rickettsioses are difficult to diagnose even today (Table I). Consequently, rickettsioses are often underreported or misdiagnosed and their true impact is underestimated. The purpose of this report is to describe the military significance of the rickettsioses and to outline the contributions made by military researchers to minimize the risk to military operations and personnel (Table II). These contributions have also greatly benefited the general public, both in the United States and globally.²

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Epidemic Typhus

Epidemic typhus (also known as louse-borne typhus, camp fever, ship fever, jail fever, and others) is an acute febrile illness of humans. The causative agent, *Rickettsia prowazekii*, contaminates the feces of its vector, the human body louse, *Pediculus humanus*. Aerosolized dried feces can be inhaled to induce infection, but the rickettsiae-laden feces are primarily self-inoculated through scratching of the site of the louse bite.³ Fever and headache occur 8 to 12 days following exposure, typically accompanied by generalized maculopapular rash, which spreads from the body trunk to arms and legs. Untreated infection results in approximately 20% mortality rates, increasing to 50% or more among older, immunosuppressed, or otherwise weakened patients. Recrudescence typhus or Brill-Zinsser disease, often consequent to immunosuppression, other weakening illnesses or war-time stress, and confined living conditions, can appear decades after the initial disease episode and be spread in lousy naive populations, thus causing major epidemics. In addition to this human reservoir, a zoonotic reservoir, the southern flying squirrel (*Glaucomys volans*), has been implicated in sporadic human cases in the eastern United States.⁴

U.S. Military Significance

U.S. military experience might have begun as early as the American Revolution. In his historical account of the rickettsial diseases, prominent rickettsiologist and military medical historian Ted Woodward suggested that the war might have been prolonged because of the typhus-induced illness of up to one-third of the New York Army of Major General Nathaniel Greene just before a confrontation with British forces in 1776.⁵ During and immediately after World War I, epidemic typhus was a major public health problem, with millions of cases in Eastern Europe and Russia, killing hundreds of thousands of civilians and soldiers. For example, one-third of the Red Army doctors contracted typhus and 20% of them died, despite knowledge of the body louse as a disease carrier.^{5,6} However, primarily because of effective delousing strategies, there were only 47 cases and 3 deaths among U.S. troops.^{7,8}

At the beginning of World War II (soon after the attack on Pearl Harbor), the Joint U.S. Typhus Commission, consisting of Army, Navy, and Public Health Service members, was established by executive order, in anticipation of threats posed primarily by epidemic typhus.⁹ Concern was justified, because during and immediately after the war hundreds of thousands of cases, with up to 10% mortality rates, appeared in civilian populations in Egypt, French North Africa, Naples, Germany, Japan, and Korea.^{3,10} Despite the massive civilian outbreaks, implementation of Typhus Commission recommendations, which included command-enforced cleanliness, application of dichlorodiphenyltri-

TABLE I
PRINCIPAL AGENTS AND CHARACTERISTICS OF SELECTED MILITARILY IMPORTANT RICKETTSIOSES

Disease Agent	Disease ^a	Mode of Transmission (Primary Vector)	Geographic Distribution
<i>Rickettsia prowazekii</i> ^b	Epidemic typhus	Infected human louse feces (human body louse, flying squirrel flea)	Worldwide
<i>Rickettsia prowazekii</i>	Brill-Zinsser disease	Recrudescence of latent <i>R. prowazekii</i> from previous infection (no vector)	Worldwide
<i>Rickettsia typhi</i>	Murine (endemic) typhus	Self-inoculation (scratching bite site) of rat flea feces (Oriental rat flea)	Worldwide
<i>Orientia tsutsugamushi</i>	Scrub typhus	Bite of infected larval mite or "chigger" (Trombiculid mite)	Afghanistan, Pakistan, and India to Siberia, China, southwestern Pacific Islands, Southeast Asia, northern Australia
<i>Rickettsia rickettsii</i> ^b	RMSF, Brazilian spotted fever	Tick bite	North and South America
<i>Rickettsia conorii</i>	Boutonneuse fever, Mediterranean spotted fever	Tick bite	Mediterranean littoral to India, Africa
<i>Rickettsia caspii</i>	Astrakhan spotted fever	Tick bite	Astrakhan, Russia
<i>Rickettsia sibirica</i>	North Asian (Siberian) tick typhus	Tick bite	Siberia, Armenia, Pakistan, Northern China
<i>Rickettsia japonica</i>	Oriental spotted fever	Tick bite	Southwest Japan
<i>Rickettsia australis</i>	Queensland tick typhus	Tick bite	Australia
<i>Rickettsia africae</i>	African tick bite fever	Tick bite	Sub-Saharan Africa
<i>Rickettsia sharonii</i>	Israeli tick typhus	Tick bite	Israel
<i>Rickettsia akari</i>	Rickettsialpox	Mite bite	Northeastern United States, Korea, Ukraine, Croatia
<i>Rickettsia honei</i>	Flinders Island tick typhus	Tick bite	Flinders Islands, Tasmania
(TT-118)	Thai tick typhus	Tick bite	Thailand, Malaysia
<i>Rickettsia felis</i>	Cat flea typhus	Cat flea bite	Western and southwestern United States
<i>Ehrlichia canis</i>	Canine ehrlichiosis, tropical canine pancytopenia	Tick bite	Southeast Asia, southwestern United States, Venezuela
<i>Ehrlichia chaffeensis</i>	HME	Tick bite	Americas, Europe, Thailand
<i>Anaplasma phagocytophilum</i>	HGE	Tick bite	United States, Europe
<i>Neorickettsia sennetsu</i>	Sennetsu fever	Unknown	Japan, possibly Malaysia
<i>Coxiella burnetii</i> ^b	Q fever	Inhalation of infectious aerosol, tick bite	Worldwide
<i>Bartonella quintana</i>	Trench fever	Infected louse feces into skin, rodent contact	United States, Mexico, Europe, Africa, Middle East, China, Japan, Bolivia

^a There are currently no Food and Drug Administration-licensed rickettsial vaccines.

^b Listed as a Select Agent (bioterrorism/biowarfare threat).

chloroethylene (DDT), immunization with the Cox-type vaccine, and other preventive measures, resulted in only 104 cases and no deaths among U.S. forces.

During the Korean conflict, there were approximately 32,000 cases and 6,000 deaths caused by epidemic typhus among South Korean soldiers and civilians.¹¹ However, disease incidence diminished in that population as control measures used in World War II were implemented, and only a single case was

reported among U.S. troops.¹² In addition to DDT dusting and administration of the Cox-type vaccine, spraying with newer insecticides proved effective.

No cases were reported during the Vietnam conflict, but tens of thousands of epidemic typhus cases have been reported since 1993. These recent outbreaks have been reported in several African countries, including Rwanda, Ethiopia, Nigeria, and Zaire, and in Central and South American countries including

TABLE II

TIME LINE OF SELECTED KEY U.S. MILITARY CONTRIBUTIONS IN THE INVESTIGATION, DIAGNOSIS, PREVENTION, AND TREATMENT OF RICKETTSIAL DISEASES

Dates	Description	Associated Military Unit	Reference
1948-1949	First antibiotic treatment of scrub typhus and murine typhus (chloramphenicol); first antibiotic prophylaxis for scrub typhus (chloramphenicol)	WRAIR ^a /IMR/University of Maryland	27, 104
1963	First specific test for serodiagnosis of scrub typhus (indirect fluorescent antibody test)	WRAIR	105
1969	First infected trombiculid mite colonies established for repellent, human vaccine, and prophylaxis challenge studies	USAMRU-Malaysia	106
1977-1980	Doxycycline prophylaxis trials to prevent scrub typhus	NAMRU2-Taiwan USAMRU-Malaysia	107, 108
1986	First field-deployable kit using indirect immunoperoxidase test for serodiagnosis of scrub typhus, spotted fever, and typhus group rickettsiosis	USAMRU-Malaysia	94
1990	First scrub typhus DNA sequencing (56-kd gene)	WRAIR	109
1990	First use of PCR to diagnose a human rickettsiosis (typhus)	NMRI	110
1990	First use of PCR to detect scrub typhus rickettsiae in host blood	WRAIR/NMRI	111
1996	FDA-cleared dipsticks for scrub typhus and typhus	NMRI/Integrated Diagnostics	2, 32
1996	Identification/isolation of first antibiotic-resistant <i>Orientia tsutsugamushi</i> strains	AFRIMS-Bangkok ^b	28
1997	Patented, r56, 56-kd scrub typhus recombinant protein (vaccine candidate/diagnostics)	NMRI	112
1999-2004	"Real-time" quantitative PCR for epidemic typhus, scrub typhus, and other rickettsioses (Perkin-Elmer TaqMan)	NMRI/USUHS	32, 113
2003	Microarrays used to distinguish pathogenic from nonpathogenic epidemic typhus strains	NMRI	114

^a Name changed from Army Medical Department and Graduate School, 1955. FDA, Food and Drug Administration; IMR, Malaysian Institute For Medical Research; USUHS, Uniformed Services University of the Health Sciences.

^b In 1959, the Bangkok SEATO Medical Research Laboratory became the AFRIMS in collaboration with the Royal Thai Army.

Mexico and Peru.² Typical of disease facilitated by crowded conditions, a major outbreak in Burundi was associated with refugee camps during the civil war.¹³

U.S. Military Contributions

The successful prevention and control of this potentially devastating disease can be attributed to military medicine and research. Working in Cairo from 1943 to 1945, the Typhus Commission developed the protocols that successfully controlled the Egyptian outbreak. Subsequent implementation of those protocols in other war-torn countries in the European theater resulted in significant control of the disease. This productive collaboration with the Egyptian Ministry of Health in typhus control evolved into the current Naval Medical Research Unit 3 (NAMRU-3) in Cairo.¹⁴ At the Naval Medical Research Institute (NMRI), Dr. Emilio Weiss first demonstrated that antibiotic resistance could be acquired by *R. prowazekii*¹⁵ and later helped to develop the Renografin density gradient method used to purify rickettsial agents internationally.¹⁶ There is no Food and Drug Administration-licensed typhus vaccine because the World War II Cox vaccine no longer meets modern standards. An effective modern subunit vaccine was developed at NMRI in the early 1980s (G. Dasch, personal communication). Military scientists also developed improved animal models, serodiagnostic assays, and molecular diagnostic and identification assays in that decade. Finally, protective immune responses to this agent were demonstrated to be dependent on specific antibody and cellular immunity, including natural killer and cytotoxic and helper T cell dependent-mechanisms.¹⁷

In addition to the now-commonplace, but laboratory-based,

polymerase chain reaction (PCR) systems, newer "real-time" nucleic acid detection systems that use state-of-the-art technologies are presently available. These include the Idaho Technologies LightCycler, the Cepheid Smart Cycler, and the Perkin-Elmer TaqMan, which are being used to specifically detect and/or quantify epidemic typhus rickettsiae.¹⁸

Because *R. prowazekii* is considered a "threat agent," novel DNA microarray technologies are currently being developed within the Department of Defense Military Infectious Disease Research Program for epidemic typhus, among other infectious agents. These novel assays will detect host responses to the agent very early in the infection through amplification of RNA and rapid sequencing for simultaneous multiagent identification (A. Richards, personal communication).

Summary and Key U.S. Military Contributions

The degree of successful control of epidemic typhus throughout the 20th century represents a prime example of the effectiveness of military-led medical research. However, many of the measures used, such as ground and vegetation treatments with chlorinated hydrocarbons and personnel dusting with DDT, are no longer permitted, and the requisite equipment for mass de-lousing is not available. Field sanitation, frequent bathing, and newer preventive treatments such as permethrin impregnation of uniforms and use of standard repellents such as *N,N*-diethyl-*m*-toluamide (DEET) facilitate louse control. However, exposure to lousy conditions will continue to threaten U.S. military personnel involved in increasingly frequent humanitarian deployments, and *R. prowazekii* remains on the U.S. government's Select Agent list because of its capacity for aerosol infections

and its weaponization in the former Soviet Union. The key U.S. military contributions include (1) the use of DDT to prevent epidemic typhus (World War II), (2) Typhus Commission protocols developed for the control of typhus (World War II), (3) the first use of PCR to diagnose a human rickettsiosis (typhus), (4) microarrays used to distinguish pathogenic from nonpathogenic typhus, and (5) real-time quantitative PCR for epidemic typhus and other rickettsioses (Perkin-Elmer TaqMan).

Murine Typhus

Murine typhus (also known as endemic, flea-borne, shop, or urban typhus) is an acute, zoonotic, febrile illness of humans. The etiological agent, *Rickettsia typhi*, is transmitted primarily through the bite of an infected rat flea (*Xenopsylla cheopis*) or through self-inoculation by scratching of the feces-contaminated site of the flea bite.¹⁹ Compared with epidemic typhus, with which it shares common symptoms, it has a case fatality rate of less than 4% but can require prolonged hospitalization. Fever typically begins 6 to 10 days following exposure and lasts 9 to 15 days.²⁰ The macular or maculopapular rash presents on the body trunk and is not as extensive as that found in epidemic typhus.²¹ Treatment typically consists of courses of the tetracyclines. The disease is reportedly found worldwide and has most recently been reported in the United States, Greece, Kuwait, Indonesia, Vietnam, Thailand, and Australia.^{6,19} It is often associated with port cities or coastal areas. Maxcy first distinguished murine typhus from epidemic typhus by its epidemiology in the 1920s, and it was distinguished from scrub typhus or rural typhus by Fletcher in 1926, working at the Malaysian Institute for Medical Research in Kuala Lumpur.²²

U.S. Military Significance

Before World War II, the U.S. military did not distinguish among cases of "typhus."¹⁹ However, during the war 787 cases of murine typhus and 15 fatalities, mostly overseas, were reported. Of these, 497 cases occurred within the continental United States (CONUS) and 34 in the China-Burma-India theater of operations. There were no cases of murine typhus reported during the Korean conflict, but several serological investigations performed during the Vietnam conflict suggested that it was a common cause of fevers of unknown origin (FUOs). For example, it was second only to malaria as a cause of FUO among U.S. military personnel assigned primarily in urban areas, on bases and cantonments.¹⁹ One study of Air Force personnel at Cam Ranh Bay suggested that as many as 30% of FUOs were attributed to murine typhus when malaria was excluded.²³ The Armed Forces Epidemiology Board reported that 15% of the FUOs in the Vietnam conflict could be attributed to murine typhus.²⁴ In a more recent epidemiological investigation in Indochina, Navy LCDR A. Corwin and others at NAMRU-2 in Jakarta reported 36% predeployment seroreactivity to *R. typhi* among Indonesian military personnel conducting peacekeeping operations in Cambodia.²⁵

U.S. Military Contributions

Military scientists at both Walter Reed Army Institute of Research (WRAIR) and NMRI provided the first detailed protein evidence supporting the species differentiation of *R. prowazekii*

and *R. typhi* and identified the antigens responsible for their immunogenicity. NMRI scientists demonstrated that, although these agents can be differentiated, they share many attributes, and modern diagnostic antigens and vaccines against them can be based on similar principles. Dipstick tests and enzyme-linked immunosorbent assays based on purified typhus antigens developed in military laboratories have been important for improved global surveillance of these rickettsial infections in many countries.

Summary and Key U.S. Military Contributions

Because murine typhus was not distinguished as a separate entity until the middle 1920s, it is difficult to ascertain its earlier impact on the military. However, its distribution throughout the world and episodic impact in later conflicts of the 20th century are apparent. U.S. military-led research efforts have identified diagnostic, treatment, and prevention strategies, rendering this sometimes fatal disease less of a military threat today. The key U.S. military contributions include (1) the first antibiotic treatment of murine typhus (with chloramphenicol), (2) a Food and Drug Administration-approved murine typhus dipstick antibody assay, and (3) a field-deployable kit using an indirect immunoperoxidase assay for serodiagnosis of murine typhus.

Scrub Typhus

Scrub typhus (also known as mite-borne typhus or tsutsugamushi disease) is an acute, zoonotic, febrile illness of humans. The etiological agent, *Orientia tsutsugamushi*, is transmitted through the bite of infected, free-living, larval, *Leptotrombidium* spp. mites, or chiggers, which function as both vectors and reservoirs. Fever typically begins 7 to 10 days (but up to 21 days) following the bite and is accompanied by a maculopapular rash, headache, and lymphadenopathy, with common central nervous system involvement.¹⁹ Frequently a pathognomic focal lesion or eschar is produced at the bite site. Mortality rates vary greatly but, if the disease is unrecognized or untreated, mortality rates of up to 60% have been reported.²⁶ The severity of disease is dependent on the strain of *Orientia* and the patient's immune status. Treatment typically consists of courses of tetracycline antibiotics or, less frequently, chloramphenicol.^{6,27} Although fatalities are rare among treated cases, there have been reports in recent years of antibiotic-refractory strains and deaths among otherwise appropriately treated cases.²⁸ Distribution of scrub typhus occurs in an approximately 13,000,000-km² triangular area of the Asian Pacific rim, which encompasses the islands of the southwest Pacific Ocean to northern Australia and extends from southern and eastern Asia to Korea and the Kamchatka littoral.²⁹ The disease displays a very diverse ecology, ranging from mountainous environments to semiarid plateaus and to the most familiar tropical climates, wherever vector trombiculid mites can be found.

U.S. Military Significance

A late 19th century reference noted a third century A.D. description of a disease in China with symptoms similar to those of scrub typhus and its associated vector.²² The disease was described in Japan in 1810 and first appeared in Western medical literature in 1878, in a published letter from a medical mission-

ary in Japan. Soon thereafter, Baelz and Kawakami described a similar disease in Japan that they called "flood-fever."²²

British forces in Burma reported early indications of the military impact of scrub typhus in 1932 and 1934,³⁰ and Japanese field artillery troops training near Mount Fuji, Japan, experienced outbreaks in 1934.³¹ Since then, several hundred military cases have been reported in and near Camp Fuji among U.S. Army soldiers (1946–1948 and 1953), Japanese ground defense forces (1959–1982), and U.S. Marines (1981–1983 and 2000–2001).^{2,32}

The military impact of the sporadic Mount Fuji outbreaks pales in comparison to that of the cases reported among both Allied and Japanese troops during World War II in the Pacific region, where scrub typhus proved to be the most significant rickettsial disease of the war for Allied forces. In that preantibiotic era, the disease sometimes caused more casualties than actual combat,³³ with mortality rates sometimes exceeding 27%. There were more than 20,000 cases among Japanese forces and 16,000 cases among Allied troops, including more than 7,300 cases and 331 deaths among U.S. troops. Toward the end of the war, preventive practices recommended by the Typhus Commission were implemented by unit commanders, which effectively reduced the rates of infection. These activities included clearing areas proximal to troop encampments and impregnating uniforms with repellents. Intensive investigations by U.S. military researchers under the auspices of the Typhus Commission identified the diverse epidemiology and ecology of the disease and its vectors,³⁴ as well as the antigenic diversity of *Orientia*.

The impact on U.S. and United Nations military personnel was minimal during the Korean conflict, with only eight cases being reported. However, reports of scrub typhus in civilian populations began to appear in the middle 1980s.³⁵ In response to the threat of this disease and Korean hemorrhagic fever to deployed troops, an overseas laboratory of the WRAIR, the U.S. Army Medical Research Unit (USAMRU) in Korea, was activated in Seoul from 1990 to 1993. The investigators reported scrub typhus to be of greater prevalence than Korean hemorrhagic fever.³⁶ Subsequently, epidemiological investigations and public health reports identified scrub typhus as a significant reemerging civilian health problem.

During the Vietnam conflict, the mission-compromising influence of scrub typhus reemerged, first appearing in 1962,³⁷ but, because of recognition and appropriate antibiotic treatments, there were no U.S. military fatalities attributable to scrub typhus. In 1972, however, the Armed Forces Epidemiology Board reported that 20 to 30% of FUOs, once malaria was excluded, were attributable to scrub typhus. All three U.S. services conducted investigations during the conflict, in attempts to understand the etiology and epidemiology of these FUOs. Reports of the 9th Medical Laboratory indicated that scrub typhus was the primary cause of FUOs in 1969 (18%), the year of greatest U.S. troop involvement in the conflict.³⁸ One after-action report in 1967 described 16 cases in a 34-man combat reconnaissance platoon 2 weeks after deployment inside a hotly contested but mite-infested area.³⁹

More recent investigations, primarily conducted by Allied and U.S. military epidemiologists, demonstrated the distribution of scrub typhus pathogens and potential risks to deployed forces. LTC G. Brown and colleagues at USAMRU-Malaysia found that

5% of British and New Zealand soldiers training in Malaysia showed 4-fold or greater increases in antibody titers to *O. tsutsugamushi* during their deployment.⁴⁰ In 1997, LCDRA. Corwin and colleagues (at NAMRU-2, in Jakarta) reported 8% seroprevalence of *O. tsutsugamushi* among a cohort of Indonesian military personnel before a deployment.²⁵ In 2003, LCDR A. Richards and colleagues, also of NAMRU-2 in Jakarta, described serological evidence of infection with *O. tsutsugamushi* (9.4%, $N = 53$) among residents of Gag Island, Indonesia.⁴¹

U.S. Military Contributions

Because of the serious impact of scrub typhus on U.S. and Allied forces during World War II, military research efforts to control this disease have been ongoing and continue to the present day. These efforts have focused on treatment, diagnosis, and prevention with antibiotic prophylaxis, vaccine development, and vector control. One of the most important and dramatic success stories of military medical research involved the first clinical trials of active scrub typhus treatment using the newly developed antibiotic chloromycetin (chloramphenicol), made by Parke-Davis (Detroit, Michigan). These trials, funded by the U.S. Army under the aegis of the Armed Forces Epidemiology Board, started in 1948 outside Kuala Lumpur, Malaya.^{42,43} The investigations were led by Drs. Joe Smadel, Ted Woodward, and Herb Ley of the U.S. Army Medical Department Research and Graduate School (later the WRAIR), in collaboration with the Malaysian Institute for Medical Research and others. The studies resulted in the first successful treatment of active human scrub typhus, which stopped fatalities and terminated fevers within 30 hours. Also associated with the study was the first successful antibiotic treatment of murine typhus (two cases) and typhoid fever (two cases). The early treatment trial successes quickly led to antibiotic prophylaxis trials, in which treated volunteers exposed in hyperendemic areas did not become infected while receiving therapy.⁴² This research led to the nomination of the research team for the 1948 Nobel Prize. The final team of researchers, arriving in 1953, established a long-term collaboration with the Institute for Medical Research and the Malaysian government that became the USAMRU-Malaysia. Although it worked with several infectious diseases until it closed in 1989, the unit primarily focused research on the epidemiology, ecology, diagnosis, and control of scrub typhus.²² Later investigations at USAMRU-Malaysia and NAMRU-2 in Taiwan (later relocated to Jakarta) independently demonstrated the value of doxycycline prophylaxis in the prevention of scrub typhus infections.

A U.S. military physician working at the U.S. Army Component, Armed Forces Research Institute of Medical Sciences (AFRIMS), an overseas component of the WRAIR, and Thai associates found that up to 15% of certain Thai scrub typhus patients did not respond to appropriate antibiotic therapy.²⁸ Isolates were used in *in vitro* and animal studies at AFRIMS and NMRI to confirm resistance.^{29,44,45} In 1998, NMRI was renamed the Naval Medical Research Center (NMRC). In a later study, investigators working from 1996 to 1998 at NAMRU-2, in Jakarta, observed breakthroughs among Indonesian troops who were deployed to Laos, Vietnam, and Cambodia and were receiving doxycycline prophylaxis for malaria.^{42,46} Preliminary studies conducted by those investigators at AFRIMS and NMRC suggested azithromycin and rifampin to be effective alternatives for

use against the putative resistant strains.^{6,44,47} In the past 15 years, military scientists at AFRIMS, NAMRU-2, WRAIR, and NMRI have continued the outstanding investigations on scrub typhus and *Orientia* begun at USAMRU-Malaysia. They have contributed to advances in the understanding of the innate and specific immune responses, cellular immunity and pathology, antigenic properties, genetics, and vector biology of *Orientia*.

Efforts to develop an effective scrub typhus vaccine in the 1940s using yolk sac-propagated rickettsiae⁴⁸ or in the 1970s at WRAIR using purified cell culture-derived organisms yielded vaccines that were protective against homologous murine challenges but fared poorly with heterologous challenges.⁴⁹ The overall early failure of the scrub typhus vaccine efforts was attributed primarily to the multiplicity of antigenic strains.⁴³ Recent military research advances include the development of a polyvalent subunit protein vaccine and corresponding DNA vaccine for scrub typhus, the first use of recombinant antigens for serodiagnostic assays for scrub typhus, including rapid tests, and rapid molecular methods for detection and genetic typing of *Orientia* in clinical and animal samples. Increased evidence of marked variation in the clinical presentation of scrub typhus has been associated with detailed evidence of a large amount of genetic and antigenic diversity among *Orientia* isolates and the presence of *Orientia* in novel mite vectors and ecological settings. Very recent work on the effectiveness of recombinant protein (56 kd) and DNA vaccine candidates in mouse and monkey models, conducted at the NMRC, appears promising (A. Richards, personal communication). In addition to permitting controlled repellent studies, the infected trombiculid mite colonies maintained at AFRIMS were developed to provide controlled vector challenges to evaluate such vaccines.

Because the prodromal period for the rickettsioses can extend up to 2 weeks, the blood supply could be adversely affected if blood was drawn for transfusion from infected, vector-exposed donors, especially field-deployed troops, during that period. The infected blood could contain rickettsiae that would be potentially lethal if transfused into weakened recipients. In response to field evidence of this problem, Army, Navy and Air Force investigators at the NMRI, WRAIR, and Walter Reed Army Medical Center performed joint-service studies in which the survivability of the rickettsiae in fresh and frozen blood was proved.⁵⁰ These researchers examined innovative new technologies, including leukoreduction filtration for removal of rickettsiae and sterilization using psoralens.^{51,52}

Summary and Key U.S. Military Contributions

Scrub typhus had the greatest impact of the rickettsioses during World War II. Recent reports of antibiotic resistance suggest that it may be problematic to U.S. forces deployed to Asian Pacific areas. Military-led research, which so dramatically and successfully introduced antibiotic therapy in the late 1940s, identified this potential problem and is now at the forefront in vaccine development and rapid detection. The key U.S. military contributions include (1) the first use of antibiotics for the treatment and prophylaxis of scrub typhus, (2) the first field-deployable kit using an indirect immunoperoxidase assay for serodiagnosis of scrub typhus, (3) the first published DNA sequence of a rickettsia (56-kd *Orientia* gene), (4) the first identification and isolation of *Orientia* strains resistant to standard

antibiotic therapy, and (5) patented, r56, 56-kd, recombinant scrub typhus protein (vaccine candidate and diagnostic aid).

Ehrlichioses

Ehrlichial disease is often an otherwise-nonspecific fever noted after tick exposure. Fever, headaches, myalgias, rigors, and malaise are typical findings. Unlike with rickettsial pathogens, rash is less common and of variable description (thus the designation of human monocytic ehrlichiosis [HME] as "spotless fever"). The case fatality rate is less than 5% for these infections, and prompt treatment with doxycycline is generally effective within 24 to 48 hours. Diagnosis is generally serologic; an acute titer of more than 80 suggests acute infection, with a 4-fold difference between acute and convalescent titers. Ehrlichiae have a worldwide distribution and are significant in human and veterinary medicine.^{2,53}

The ehrlichiae show many structural, growth, and genetic differences from rickettsiae. HME caused by *Ehrlichia chaffeensis* and human granulocytic ehrlichiosis (HGE) caused by *Anaplasma phagocytophilum* are transmitted via inoculation by a tick bite. Unlike the rickettsiae, the ehrlichiae are not maintained transovarially in ticks but must be acquired during larval or nymphal feeding on infected animal reservoirs. The vector of HME is *Amblyomma americanum*, the "Lone Star" tick, which is distributed through the mid-Atlantic states, the South, and parts of the Midwest, particularly Oklahoma and Missouri. In the United States, the vectors of HGE are *Ixodes scapularis* and *Ixodes pacificus*, which are also vectors of Lyme disease (and *Babesia microti* for *I. scapularis*); therefore, HGE shares the geographic range of the Lyme pathogen. These pathogens are transmitted in the season corresponding to tick activity, from about April to October but with variations according to the locality. It is clear from numerous serosurveys that the ehrlichial infections are often asymptomatic or subclinical. Recognized infections are more often recognized among male subjects, with outdoor occupations also representing higher risk.^{54,55} The first *Ehrlichia* species to gain recognition was *Anaplasma marginale*, which was recognized by Thaler in 1910 as the cause of a significant illness in cattle worldwide.⁵⁶ Subsequent discoveries were also in veterinary medicine, i.e., *Cowdria ruminatum* in 1925, *Ehrlichia canis* in 1935, *Ehrlichia phagocytophila* in 1940, *Neorickettsia helminthoeca* in 1950, *Ehrlichia equi* in 1969, and others. The first recognized human infection from ehrlichiae was in Japan in 1954, caused by *Neorickettsia sennetsu* (formerly *Rickettsia* or *Ehrlichia sennetsu*), the etiological agent of sennetsu fever. The first discovery of human ehrlichial infection in the United States was in 1986. A critically ill patient was noted to have a rickettsia-type syndrome, with cytoplasmic inclusions in peripheral mononuclear cells. Subsequent evaluation by the Centers for Disease Control and Prevention (CDC) suggested ehrlichiae as the cause and demonstrated a positive serological response to *E. canis*. In retrospect, many epidemiological and clinical features of the patient's illness were not consistent with *E. canis* infection.

U.S. Military Significance

The first U.S. military impact of ehrlichiae was recognized in military working dogs in the Vietnam conflict. Hundreds of dogs

were infected with canine ehrlichiosis (also known as tropical canine pancytopenia), manifested as a chronic wasting syndrome with pancytopenia, hemorrhage, and high mortality rates.^{53,57,58} Two hundred twenty U.S. military dogs died of this infection between 1968 and 1970, with a larger number being euthanized.

Among military personnel, the greatest impact of the ehrlichioses, like the spotted fever group rickettsiae (SFGR), has been associated with training or deployments in tick-infested areas within CONUS and overseas. In 1990, investigators isolated a novel pathogen from a febrile soldier from Fort Chaffee, Arkansas, which was subsequently named *E. chaffeensis*.⁵⁹ In 1992, clinicians noted an unusual cluster of illnesses resembling an ehrlichial disease in northwest Wisconsin and Minnesota. By 1993, subsequent evaluation by researchers showed an *Ehrlichia* species causing granulocytic morulae, which was phylogenetically similar to *E. equi* and *E. phagocytophila*.⁶⁰ Notably, "Bullis fever," a previously unknown illness, caused an outbreak of more than 1,000 cases of a nonspecific febrile syndrome at Camp Bullis, Texas, during World War II.^{61,62} Despite intensive investigation, the cause was never fully elucidated. The organism was shown to be a previously unknown rickettsial pathogen transmitted by *A. americanum*. These cases occurred more than a decade before the first recognition of human ehrlichiosis (in Japan). However, it now seems most likely that Bullis fever was actually HME.

U.S. Military Contributions

The U.S. military has funded, collaborated in, and conducted investigations of several ehrlichioses, especially since the era of the Vietnam conflict. Many of these contributions are associated with the detection and control of the diseases through epidemiological investigations and the development of clinical treatment regimens to minimize their severity. For example, tetracycline therapy, based on investigations at the Saigon Laboratory, WRAIR, resulted in a 50% response rate among infected military working dogs.⁶³ Further studies were conducted at WRAIR to investigate the etiology and pathology of canine ehrlichiosis and the microbiology of *E. canis*.⁵³

At the University of Illinois, U.S. military-funded collaborative studies involving U.S. Army scientists working in the veterinary laboratory of Dr. M. Ristic or in collaborations with his laboratory had many successes. As a result, the first successful *Ehrlichia*-infected cell lines were developed.⁶⁴ In vitro propagation of these ehrlichiae, including *N. sennetsu*, provided antigen for the indirect immunofluorescence assays used to screen human and animal sera for exposure to the agents.⁶⁵ These findings included the first evidence of a relationship between *E. canis* and *N. sennetsu*.⁶⁶ Research performed by CPT G. Lewis in that laboratory with *E. equi* showed the narrow host range of that veterinary pathogen, compared with other ehrlichiae.^{67,68} Further studies there defined the ultrastructural features and in vivo target cells.⁶⁹ Using clinical samples collected during human FOU studies conducted in Malaysia at USAMRU-Malaysia from 1978 to 1983, scientists in Dr. Ristic's laboratory discovered the first serological and isolation evidence of *N. sennetsu* outside Japan.⁷⁰⁻⁷² In vitro studies conducted at USAMRU-Malaysia in 1985 provided evidence of endothelial cell involvement in human infection.⁷³

Military contributions to the study of the human ehrlichiae

have more recently focused on *E. chaffeensis*, because a number of large military facilities are located and a variety of military training activities occur within the range of this pathogen.⁷⁴ Notably, the organism was first correctly identified in a U.S. Army soldier by U.S. Army and CDC researchers.⁵⁹ Two early seminal studies were also performed in military facilities. The first study (by CDC investigators) in 1989 examined an outbreak of HME among U.S. Army reservists exposed to ticks during a training exercise.⁷⁵ The study showed that 9 of 74 (12%) seroconverted to *E. chaffeensis*, but none was hospitalized and most had minimal or no symptoms. This was among the first reports demonstrating that ehrlichiosis is frequently mild or asymptomatic among normal hosts. A larger serosurvey conducted by U.S. Army and CDC researchers among U.S. Army soldiers from various locations who were training at Fort Chaffee revealed a significant rate of seroconversion to *E. chaffeensis*. Approximately two-thirds of these seroconversions were asymptomatic.⁷⁶ These two studies helped clarify the epidemiology of HME, which was previously thought to be more frequently severe. Similar results were obtained from a smaller serosurvey performed at Quantico, Virginia, among U.S. Marine Corps officer trainees.⁷⁷ However, HME and HGE can infrequently present with severe or fatal illness, which has been observed among military personnel (B. Hale, personal communication). Notably, a more recent serosurvey from Fort Chaffee, performed by CDC researchers, showed that 15% of 1,067 National Guard troops had significant antibody titers to either SFGR antigens or ehrlichiae. Of the much smaller number with paired sera, 5 of 93 seroconverted to ehrlichiae and 33 seroconverted to SFGR antigens.⁷⁸ Almost one-third of seroconverters reported symptoms consistent with a tick-borne illness. A notable recent outbreak of ehrlichiosis and spotted fever among trainees at Fort Chaffee shortly after a blood bank drive resulted in a multistate recall of blood products.⁷⁹ No transmissions occurred, but the incident serves as a reminder of the potential risk of transmission of ehrlichiae via blood transfusion.

A retrospective serosurvey of more than 10,000 banked serum specimens by U.S. Navy investigators showed a nearly 4% positivity rate for HGE, revealing low but notable previous exposure history for military members.⁸⁰ WRAIR and NMRC researchers have participated in a number of serosurveys that have expanded the known geographic distribution of ehrlichiae in Europe, Africa, Asia, and other locations.⁸¹ The U.S. Army Center for Health Promotion and Preventive Medicine has also been active in this area, expanding knowledge of the tick vectors of ehrlichiae.⁸²

Summary and Key U.S. Military Contributions

These earlier and more recent outbreaks, often investigated solely by military epidemiology teams, underscore the ongoing risks of the ehrlichioses among operational forces, including the canine units, which are increasingly frequently deployed in training, peacekeeping, or other actions in hostile environments. In the absence of licensed vaccines, other prevention and vector-control measures remain the only protection for these forces. The key U.S. military contributions include (1) treatment regimens to protect military working dogs from lethal canine ehrlichiosis, (2) collaborative development of cell culture isolation and indirect immunofluorescence assay techniques, (3) initial isolation of *E. chaffeensis*, (4) multiple epidemiological in-

vestigations to determine causes of outbreaks in CONUS and outside CONUS, and (5) development of tick-repellent systems to reduce risks to deployed forces.

Spotted Fevers

Rocky Mountain spotted fever (RMSF) is the most commonly reported and most severe rickettsial disease found in the United States. The etiological agent, *Rickettsia rickettsii*, which is considered the most virulent of the SFGR, is transmitted by the bite of the *Dermacentor variabilis* tick, which is found primarily in the eastern half of the United States, and *Dermacentor andersoni*, in the western half. RMSF has also been reported in South and Central America, where it is transmitted by *Amblyomma cajennense*.² RMSF is an important rickettsial disease for the U.S. military, given its prevalence in areas where military training takes place.

Mediterranean spotted fever (also known as boutonniere fever, Indian tick typhus, Marseilles fever, or Kenya tick typhus) is caused by *Rickettsia conorii* and is transmitted by the bite of *Rhipicephalus sanguineus* dog ticks in the Mediterranean, although other vectors are known. The agent is maintained by transovarial passage in the ticks. This disease is endemic in southern Europe, North and West Africa, India, Pakistan, Israel, Russia, Georgia, and the Ukraine. Astrakhan spotted fever and Israeli tick typhus are caused by rickettsial agents classified in the *R. conorii* complex but may differ in vector hosts and clinical severity.² This rickettsial disease has possible importance, given the potential for deployments and training in areas where this disease is endemic.

Other common spotted fevers include Siberian tick typhus, rickettsial pox, Oriental spotted fever, Australian tick typhus, African tick bite fever, Flinders Island tick typhus, Asian or Thai tick typhus, and cat flea typhus.² African tick bite fever (also known as African spotted fever) is recognized as an emerging health problem for local populations and international travelers to sub-Saharan Africa. Seroepidemiological studies suggest that it may be the most widespread rickettsiosis in the world.^{83,84} The disease is caused by *Rickettsia africae*, a recently identified SFGR transmitted by unguulate ticks of the *Amblyomma* genus in rural sub-Saharan Africa and the French West Indies.⁸⁵ By 2000, high seroprevalence of anti-*R. africae* antibodies and clinical African tick bite fever had also been noted in the population of Guadeloupe in the French West Indies but nowhere else in the western hemisphere. Numerous new spotted fever rickettsial agents have been discovered in the past decade, with the widespread use of molecular tools, but their importance in human disease is currently uncertain.

U.S. Military Significance

The risk of spotted fever rickettsioses, as well as potential exposure to other tick-transmitted agents, is an important consideration for the training and operations of our military in the United States and abroad. Numerous studies by U.S. military and other government investigators have dramatized these risks. In 2000, 846 ticks removed from soldiers at Fort A.P. Hill, Virginia, revealed a prevalence of *Rickettsia amblyommii*.² Warner et al.⁶⁰ described an outbreak of tick bite-associated illness among military personnel following a field training exer-

cise at Fort Chaffee, Arkansas. Ticks bit 75% of the personnel who participated in the training exercise, and one-half of these personnel were seropositive for *Rickettsia rickettsii*, which shares SFGR antigens with *R. amblyommii*. In examining more than 3,000 ticks at Fort Chaffee, Arkansas, during May, August, and November 1990, Kardatzke et al.⁸⁶ found that 4.8% were positive for SFGR and 0.3% were positive for ehrlichiae. Stromdahl et al.⁸² determined the prevalence of infection in ticks submitted to the Human Tick Kit Program of the U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Grounds, Maryland. They found that 15% of the *A. americanum* DNAs produced amplicons of the expected size for *E. chaffeensis*, 26 (12%) of 222 produced amplicons indicating *Borrelia burgdorferi*, and 2% appeared to be infected with both. Four percent of *D. variabilis* ticks submitted to the program were PCR positive for SFGR. Restriction fragment length polymorphism analysis revealed that all cases involved *Rickettsia montanensis*, an agent not known to cause disease among humans. One hundred twenty-seven *D. variabilis* ticks from Monroe County, Wisconsin, were evaluated for *B. burgdorferi* and 14 (11%) were positive. Five (21%) of 24 *I. scapularis* ticks were positive for *B. burgdorferi* and one was positive for the agent causing HGE.⁸⁰ Another SFGR, *Rickettsia parkeri*, was previously observed in the Gulf Coast tick, *Amblyomma maculatum*, and was considered to be nonpathogenic. However, the first human case was reported for a patient evaluated at the Naval Medical Center at Portsmouth.⁸⁷

The prevalence of tick-borne disease in military forces has also been evaluated overseas. In 1992, Mediterranean spotted fever was observed in a 36-year-old male soldier who returned home after deployment to Somalia.⁸⁸ Sirisanthana, working in collaboration with AFRIMS and NMRI investigators, described the first human cases of SFGR in Thailand.⁸⁹ Arguably the most significant recent impact of a SFG rickettsiosis occurred in January 1992 in a unit of the U.S. Army 82nd Airborne Division conducting a training mission in Botswana. A WRAIR-deployed epidemiology consultant team determined that 50% of the unit had become infected with *R. africae* from exposure to indigenous ticks.⁹⁰ This agent is present in most *Amblyomma hebraeum* and *Amblyomma variegatum* ticks and commonly causes seroprevalence rates of more than 70% in populations in disease-endemic areas in sub-Saharan Africa; it is a common cause of infections among visitors to savannah areas in this region, where the tick is common. In the summer of 2000, a U.S. Navy Environmental and Preventive Medicine Unit-5 field exercise was conducted on the island nation of Antigua, West Indies. In collection from cattle at the abattoir in the capital St. Johns, adult specimens of the African tick vector *Amblyomma variegatum* and also of *Boophilus microphilus* were obtained. Ticks were crushed, and filter blots were sent to the CDC for DNA extraction. Rickettsial DNA was detected by PCR for two targets, with 84% of the *A. variegatum* being positive for *rOmpA* and 52% for *gltA* primers. None of the *B. microphilus* tested PCR positive, even those from animals carrying positive *Amblyomma*. Further work with restriction fragment length polymorphism and DNA sequencing confirmed the agent as *R. africae*. Although no human cases were seen, DNA markers for *R. africae* were commonly detected in this collection of *A. variegatum* ticks on the island of Antigua. Identification of the agent of African tick bite fever from Antigua

by a U.S. Navy-CDC effort portended further spread of this emerging pathogen through the Caribbean Sea region and into North America.⁹¹

U.S. Military Contributions

Department of Defense investigators have significantly contributed to the study of spotted fevers, including the development of diagnostic techniques and devices. Woodward et al.⁹² used an immunofluorescence technique with skin biopsies from patients with RMSF, on days 4 and 8 of illness. *R. rickettsii* organisms were identified earlier than if serological testing had been used. Kelly et al.,⁹³ working at USAMRU-Malaysia, found that the indirect immunoperoxidase test for SFGR correlated well with the indirect immunofluorescence assay. Because the indirect immunoperoxidase test did not require a fluorescence microscope, the investigators were able to field-test kits for specific serodiagnostic testing for the rickettsioses in several clinical laboratories throughout Malaysia and Southeast Asia.⁹⁴ A dot-blot enzyme immunoassay for serological confirmation of illness attributable to *R. conorii* was developed and evaluated in close partnership between a civilian small business and military scientists at the AFRIMS, the NMRI, and WRAIR. In one evaluation of that assay, CPT L. Broadhurst of WRAIR and colleagues, using samples collected consequent to a SFGR outbreak among U.S. troops deployed to Botswana, found that the dipstick test read positive in more of the probable cases (62%) than did the IgG (16%) and IgM (55%) indirect immunofluorescence assays.⁹⁵ In another study, PCR amplification of DNA to detect *R. rickettsii* and *R. typhi* in experimentally infected adult *D. variabilis* ticks and *X. cheopis* fleas was demonstrated. PCR amplification of DNA provides a rapid, sensitive, highly specific assay for detection of rickettsial infection in arthropod vectors.⁹⁶

Cross-reactivity of sera can be a problem in serological testing. Sera of patients suffering from Mediterranean spotted fever were evaluated by immunoblotting, to assess cross-reactivity. A prevalence of IgM antibodies to *Proteus* OX-19, *Proteus* OX-2, the *Rickettsia* typhus group, *Legionella pneumophila* serovars 4 and 5, *Legionella bozemannii* Wiga, and *Legionella micdadei* Tatlock was found. Western blot assays confirmed that the antibodies were directed against the lipopolysaccharide. There is a common cross-reacting epitope among *L. bozemannii* Wiga, *R. typhi*, and *Proteus* OX-19 but cross-reacting antibodies to *L. micdadei* and OX-2 were different and independent. A misdiagnosis could be made on the basis of this IgM cross-reaction.⁹⁷

The military has also assisted with several research projects looking at genetic differences and molecular biology of different rickettsiae. Quantitative analyses of variations in the injury of endothelial cells elicited by 11 isolates of *R. rickettsii* were performed to help understand the molecular basis for the varied cellular injury caused by different isolates.⁹⁸ Radulovic et al.⁹⁹ isolated, cultivated, and partially characterized the ELB agent, now named *Rickettsia felis*, associated with cat fleas. This agent is a rickettsia but differs from *R. typhi*, *R. prowazekii*, and *R. rickettsii* in its 17-kd gene sequence. The 16S rRNA sequence also differs from those of *R. typhi* and *R. rickettsii*. *R. felis* is a potential cause of human murine typhus-like illness. The ability to propagate this agent in larger quantities has allowed for greater study. Eremeeva et al.¹⁰⁰ described the Western blot analysis of heat shock proteins of Rickettsiales and other eubacteria. Heat shock proteins of four *Rickettsia* species, three

Bartonella species, *Orientia tsutsugamushi*, and 17 other eubacterial species were characterized with an enhanced chemiluminescence Western blotting technique, with antibodies raised against recombinant heat shock proteins from *Escherichia coli* and purified GroES from *R. typhi*. Cianciotto et al.¹⁰¹ detected *mip*-like sequence and Mip-related proteins within the family Rickettsiaceae. The Mip surface protein enhances the ability of *Legionella pneumophila* to infect macrophages and protozoa. The investigators used *mip*-specific probes and low-stringency Southern hybridizations to detect DNA sequences homologous to *mip* within *Coxiella burnetii* and *Rochalimaea quintana*. They also used specific anti-Mip antisera and immunoblot analysis to detect Mip-related proteins within these bacteria, as well as within *Rickettsia* and *Ehrlichia* species. Dasch and Jackson¹⁰² provided a genetic analysis of isolates of the SFGR belonging to the *R. conorii* complex. They were able to demonstrate that the 120-kd cytoplasmic gene could be used to differentiate isolates of *R. conorii*, *R. africae*, and Israeli tick typhus. These serotypes show genetic variations of this gene. Shirai et al.,¹⁰³ working at the WRAIR, experimentally infected cotton rats (*Sigmodon hispidus*) with *R. rickettsii*, in their examination of the role of mammals in the ecology of RMSF. The Sheila Smith strain of *R. rickettsii* was nonpathogenic for cotton rats. The short period of rickettsemia in the cotton rat suggests that this mammal is probably not an important reservoir. Cotton rats infected with the Sheila Smith strain (virulent) still developed rickettsemia after reinfection with the same strain, even with high antibody titers.

The first modern vaccine for RMSF was developed by Army scientists at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). Unfortunately, although this Renografin density gradient-purified, duck-cell vaccine was an effective immunogen, its high content of lipopolysaccharide caused reactogenicity among vaccinees. A subunit vaccine based on the strategy developed at NMRI for typhus rickettsiae alleviated this problem. Scientists at WRAIR and the USAMRIID made important contributions to understanding the genetics of mouse resistance to rickettsialpox and to the nature of cellular immune mechanisms responsible for protective and cross-protective immunity to SFGR.

Quantitative real-time assays for SFGR are in development at the NMRC (A. Richards, personal communication). The Department of Defense has selected one of these technologies, the Idaho Technologies Ruggedized Advanced Pathogen Identification Device (RAPID), for its fieldable Joint Biological Agent Identification and Diagnostic System. This system for identification of multiple biological agents of operational concern will include capabilities to detect the etiological agents of Q fever, typhus, and scrub typhus.

Summary and Key U.S. Military Contributions

Tick-borne infections among training or deployed troops are not just theoretical risks but have actually occurred, with very high infection rates. No licensed vaccines are available, but the SFGR are easily treatable if recognized. Therefore, training and rapid disease diagnosis, using newer, faster, diagnostic techniques, are essential. Prevention (through the use of repellents and uniform impregnation) and education are required to reduce the risk to the military of SFGR infection. The key U.S. military contributions include (1) the first modern vaccine for

RMSF, developed by Army scientists at USAMRIID; (2) rapid SFGR detection in sectioned skin lesion biopsies and tick hemolymph by direct fluorescent antibody staining; (3) dipstick tests for *R. conorii* for human serodiagnosis; (4) indirect fluorescent antibody and indirect immunoperoxidase assays for disease serodiagnosis; and (5) multiple epidemiological investigations in CONUS and outside CONUS to determine troop risk attributable to tick-borne exposure.

Summary and Key U.S. Military Contributions

The rickettsioses are often ignored or forgotten by the military planners between major deployments. However, these diseases have played a significant historical role, sometimes compromising U.S. military missions. Because rickettsial vaccines and tick repellants have often been of low U.S. public health interest, in contrast to surveillance efforts, military investigators deployed to overseas and CONUS-based military medical research laboratories have made very substantial contributions to medical research on these agents. Because rickettsial diseases are underreported and often unrecognized throughout the world, increased worldwide epidemiological surveillance for these and other infectious diseases continues to be of importance, particularly because novel agents and vectors continue to be discovered. To date, there are no Food and Drug Administration-licensed rickettsial vaccines. Using traditional threat assessment approaches such as the field-deployable, U.S. Army epidemiology consultant teams and the newly emerging technologies, military and civilian investigators together are better able to rapidly identify new reservoirs and foci of infection, before they adversely affect missions. Today, novel health hazards posed by antibiotic-resistant scrub typhus and genetically modified rickettsiae and the bio-threat posed by rogue nations or terrorists potentially deploying such Select Agents as *R. rickettsii*, *R. prowazekii*, and *Coxiella burnetii* are of concern to U.S. military and public health officials. Military and, increasingly, collaborative civilian commercial research efforts are addressing these concerns. These efforts include developing effective, safe, longer-lasting repellents, a scrub typhus vaccine, and rapid infectious disease or threat agent detection systems, such as the RAPID. These diseases will not be eliminated; therefore, our military must be prepared for them.

The key U.S. military contributions include (1) development of vector control protocols (e.g., habitat clearing, DDT, repellents, and uniform impregnation), (2) first-ever successful treatments of the rickettsioses by antibiotic treatments of humans and working dogs, (3) first-ever successful antibiotic prophylaxis protocols (chloramphenicol and doxycycline), (4) development/validation of accurate serodiagnostic tests for several of the rickettsioses (indirect immunofluorescence, indirect immunoperoxidase, enzyme-linked immunosorbent, dipstick, and wicking assays), (5) vaccine development and evaluations (RMSF, typhus, scrub typhus, and DNA vaccines), (6) first-ever DNA sequencing of rickettsial pathogens, (7) first detection/evaluation of antibiotic resistance in rickettsiae, (8) risk assessment through a plethora of prospective and retrospective seroepidemiological investigations, (9) development of rickettsial agent detection systems using developing technologies (PCR, real-time RAPID, and microarrays), and (10) infected vector colonies for vaccine validation and repellent evaluations.

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