Armed Forces Pest Management Board
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Tick-Borne Diseases: Vector Surveillance and Control

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AFPMB Technical Guides

This is one of a series of Technical Guides (TGs) published by the Defense Pest Management Information Analysis Center (DPMIAC), Armed Forces Pest Management Board (AFPMB). The AFPMB is a directorate within the Office of the Deputy Under Secretary of Defense for Installations and Environment that recommends policies and procedures, provides guidance, and coordinates the exchange of information related to pest management throughout the Department of Defense (DoD). As a unit of the AFPMB, DPMIAC collects, analyzes and disseminates published and unpublished information on arthropod vectors and pests, natural resources, and environmental biology important to the DoD. Other DPMIAC products include country- or region-specific Disease Vector Ecology Profiles (DVEPs). All TGs and DVEPs, as well as DPMIAC's database of some 250,000 articles and books on pest management and medical zoology, are available at the AFPMB Web site, http://www.afpmb.org/lrs.

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Inquiries, comments or suggestions for improving TGs may be directed to the Chief, DPMIAC, at (301) 295-7476, FAX (301) 295-7473.

Mention of specific insecticides, equipment items, or other materials herein does not constitute official endorsement by the AFPMB or DoD.

Acknowledgments

1. Introduction

a. Background

Ticks are among the most important of all arthropod vectors of disease. Worldwide, ticks are second only to mosquitoes in the number of diseases they transmit to humans. In the United States, where ticks are responsible for more human disease than any other arthropod group, the severity of disease transmission has been increasing, both in terms of incidence and the number of pathogens transmitted. For example, Lyme disease, or Lyme borreliosis, first recognized from a cluster of Connecticut cases in the mid-1970s, is now the most commonly reported arthropod-borne illness in the United States, accounting for over 23,000 cases in 2002. Moreover, the principal vector of this disease in the eastern United States, *Ixodes scapularis*, has also been implicated in the transmission of babesiosis and human granulocytic ehrlichiosis. In most parts of the world, tick-borne diseases are potentially serious health threats to troops, civilian employees, and residents at military installations. Direct effects on the mission (troop morbidity) as well as indirect effects (illness in dependents or DoD civilian personnel and related health care costs) can be lessened through aggressive public education, surveillance, and prevention/control programs, together with prompt diagnosis and treatment.

b. Purposes of this Guide

The purposes of this guide are fourfold:
1. To familiarize preventive medicine personnel with the various diseases transmitted by ticks in the United States.
2. To provide surveillance protocols to evaluate current and potential threats of tick-borne diseases at military installations.
3. To describe methods for personal protection against tick-borne diseases.
4. To describe reliable control alternatives, both chemical and nonchemical, for reducing populations of tick vectors.

c. Specific Objectives

This TG provides specific directions and guidance to Pest Management Professionals, including:
1. Determining whether known or suspected tick vectors of disease are present on military installations.
2. Determining the infection rate in these vectors.
3. Ascertaining the presence of other tick species on installations, and evaluating their role in disease epidemiology.
4. Collecting sera from mammal hosts of tick vectors, and determining the prevalence of tick-borne pathogens in these sera.
5. Identifying installation habitats that pose a risk of human exposure to ticks.
6. Assessing the potential for spread of tick-borne disease agents on installations, based on an analysis of suitable habitats, vector ticks and wild mammal hosts.
7. Providing tick-borne disease education for installation personnel and dependents.
8. Gathering information on the epidemiology of tick-borne diseases in humans, pets, and other animal hosts.

9. Recommending or performing prevention/control measures based on surveillance and environmental data, installation mission, and other relevant factors.

2. Basic Tick Biology

a. Classification

Ticks are members of the arthropod class Arachnida, subclass Acari, order Parasitiformes. Many ticks resemble spiders in that both groups lack visible abdominal segmentation. However, in spiders the mouthparts are inserted anteriorly on a structure called the cephalothorax, which comprises the fused head and thorax. Legs are also borne on the cephalothorax, which is connected to the abdomen by a narrow pedicel. In contrast, the mouthparts of ticks form a discrete anterior gnathosoma or capitulum, and that part of the body on which the legs are inserted (the podosoma) is broadly joined to the portion of the body behind the legs (the opisthosoma) to form the idiosoma (the "body") of the tick. Within the Parasitiformes, ticks belong to the suborder Ixodida, which contains a single superfamily, the Ixodoidea, that is divided into two major families, Argasidae and Ixodidae, and the rare family Nuttalliellidae, with a single African species. The family Argasidae, or soft ticks, consists of about 183 species worldwide. Adult argasids lack a dorsal sclerotized plate or scutum, their integument is leathery and wrinkled, their mouthparts are not visible from above, and they show no obvious sexual dimorphism. The family Ixodidae, or hard ticks, contains some 683 species. As adults, ixodids exhibit prominent sexual dimorphism: the scutum covers the entire dorsum in males, but in females (and immatures) the scutum is reduced to a small podonotal shield behind the capitulum, thereby permitting great distention of the idiosomal integument during feeding.

b. Life History

All ticks have a six-legged larval stage, one or more eight-legged nymphal stages, and an eight-legged adult stage. Ixodid (hard) ticks have a single nymphal stage, while argasids (soft ticks) may have as many as eight. In both families, all stages and both sexes feed on blood. Hard ticks generally take one blood meal per stage, followed by molting, and adult females oviposit and die following the blood meal. Males feed little, if at all, and die after mating. Soft ticks feed intermittently, and adult females may feed and oviposit several times.

Ticks have evolved a great variety of host relationships but are commonly classified as "one-host," "two-host," "three-host," or "many-host" species. One-host ticks complete all feeding and molting on a single animal, usually a large-sized, wandering host (sheep, cow, horse, deer, antelope, buffalo, etc.). This strict host specificity is uncommon, being seen in only about a dozen tick species (mostly ixodids), though these may be of great economic importance. In two-host ticks, the molt from larva to nymph occurs on the first host, which is usually a small mammal or bird, and the engorged nymph drops to the ground, where it molts to the adult stage. The adult must then find a second, usually much larger host. Again, there are only about a dozen two-host ticks, almost all of them ixodids, including several associated with livestock. All other ixodid ticks are three-host species that detach after engorging at each life stage, with molts taking place off the animal. A variety of hosts may be utilized by each life stage, increasing the likelihood that the tick will acquire pathogens, but larvae and nymphs usually feed on smaller

hosts than adults. Many-host ticks are typically argasids, which feed on a number of different animals during their life cycle, the adults feeding several times.

Copulation takes place following the last molt, after which the female engorges and produces eggs, which are laid on the ground or in some sheltered location. Female hard ticks lay their eggs in one large batch, often numbering several thousand, then die. Female soft ticks lay multiple smaller egg batches, from less than a hundred to a few hundred at a time. Ticks are extremely hardy and can survive long periods of environmental stress. Some species have been known to survive unfed for many years, and all life history stages can tolerate long periods of submersion in water. Ticks have also developed a number of adaptations to limit water loss, and soft ticks in particular are resistant to desiccation, often inhabiting xeric environments.

c. Behavior

Hard ticks, and a few species of soft ticks, seek their hosts by climbing vegetation, either grass or brush, and waiting for a suitable animal to pass. In a behavior known as questing, the first pair of legs is extended and used to grasp the host when contact is made. The height at which questing takes place determines the size, and therefore the species, of host selected. Questing heights vary between tick species and among the different life stages of any given species. In contrast, most soft ticks inhabit caves, dens, stables, and other places used by potential hosts. Typically, soft ticks secrete themselves by day in loose soil or cracks and crevices, attacking their host at night, usually while it is asleep. They crawl to the host, engorge in a few minutes or hours, and then return to their hiding place. All ticks orient to potential hosts in response to products of respiration. Carbon dioxide is especially attractive, even from a distance, and this behavioral characteristic enables surveillance studies to be conducted with dry ice.

d. Vector Potential

Several characteristics of ticks make them outstanding vectors of pathogenic agents. Their wide host range and tendency to feed on several hosts during their life cycle ensures ample opportunity to acquire and transmit pathogens. Their hardiness and longevity enable them to survive long periods of unfavorable environmental conditions. They have a high reproductive potential, ensuring maintenance of large populations and a high frequency of host-vector contact. They also feed slowly and, in the case of ixodids, attach to hosts for relatively long periods, which allows sufficient time for pathogen acquisition and transmission, as well as tick dispersal by migrating or wandering hosts. In the United States, ixodids are responsible for transmitting the majority of human tick-borne diseases: babesiosis, Colorado tick fever, ehrlichiosis, Lyme disease, Rocky Mountain spotted fever, and tularemia. While argasids do not directly transmit these diseases to humans, they may be involved in maintaining pathogen cycles among reservoir hosts, as in the case of Colorado tick fever. In addition, soft ticks of the genus *Ornithodoros* are the vectors of tick-borne relapsing fever.

3. Diseases Transmitted by Ticks in the United States
a. Babesiosis (CHPPM Fact Sheet)
Causative agent and distribution. Human babesiosis is an uncommon infection caused by several species of blood-invading, malaria-like protozoans in the genus *Babesia* that collectively

are known as piroplasms. The disease is found mainly in temperate regions of the Northern Hemisphere and was first recognized in humans in 1957, with the report of a case from the former Yugoslavia. The first American case was described in 1968. In the United States, babesiosis occurs chiefly along the northeast coast and in the upper Midwest, where the agent is *B. microti*. Additional types of *Babesia* that are associated with human disease in limited areas of the United States are isolate WA1 (known from the West Coast) and isolate MO1 (detected in Missouri). Occasional cases have been reported as far south as Mexico. In Europe, *B. divergens* is chiefly responsible for human disease, with cases reported from France, Ireland, Scotland, Spain, Sweden, Russia, and the former Yugoslavia. Human infections with less well characterized isolates of *Babesia* have been reported from China, Taiwan, Egypt, the Canary Islands, and South Africa. Other *Babesia* species cause illness in animals.

Symptoms. Seroprevalence studies indicate that most human infections are asymptomatic. Manifestations of symptomatic disease are similar to those of malaria, but without periodicity, and include a gradual onset of malaise, anorexia and fatigue, followed by fever, chills, profuse sweating, headache, and generalized myalgia. Symptoms usually occur one to four weeks after tickbite, though most patients have no recollection of tick attachment. The clinical spectrum ranges from mild, self-limited illness to life-threatening disease with jaundice, hemolytic anemia, renal failure, and hypotension. Abnormalities resulting from hemolysis and liver dysfunction include persistent anemia, decreased levels of hemoglobin, hemoglobinuria, thrombocytopenia, icterus, and elevated levels of serum hepatic aminotransferase and lactate dehydrogenase. The disease is most severe, and sometimes fatal, in elderly, asplenic, or immunocompromised persons. Co-infection with *Borrelia burgdorferi*, causal agent of Lyme disease, has been documented and may increase the severity of both diseases.

Vectors and transmission. In the northeastern and midwestern United States, *B. microti* is transmitted primarily during summer months by nymphs of the blacklegged (or deer) tick, *Ixodes scapularis*. Along the Pacific Coast, where *I. scapularis* does not occur, the vector is the closely related western blacklegged tick, *I. pacificus*. Other species of North American *Ixodes* also may be involved. In Europe, the principal vector of *B. divergens* is the castor bean or sheep tick, *I. ricinus*, though *I. trianguliceps* is also known to transmit infection.

Reservoirs. Human babesioses are passed transstadially but not transovarially in vector ticks; therefore, tick larvae can only acquire infection by feeding on reservoir hosts. In the northeastern United States, the white-footed mouse, *Peromyscus leucopus*, and meadow vole, *Microtus pennsylvanicus*, are competent reservoirs of *B. microti*, readily infecting larvae of *I. scapularis*. Reservoirs for isolates WA1 and MO1 are unknown. In Europe, cattle serve as reservoir hosts for *B. divergens*.

b. Colorado tick fever
Causative agent and distribution. Colorado tick fever (CTF), or mountain fever, is an acute viral dengue-like illness caused by an *Orbivirus* in the family Reoviridae. First described in 1850, CTF is the only common tick-transmitted viral disease in North America, occurring in areas above 1525 m throughout the mountain West, including Alberta and British Columbia, Canada, the various mountain chains of California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington and Wyoming, and the Black Hills of South Dakota. Between 200 and 300 cases are reported annually in the United States, mostly from Colorado, but the actual yearly incidence is probably much higher. Victims are usually people engaged in

recreational activities in mountain pine forests, forested canyons, or along rivers and streams in narrow, cultivated mountain valleys.

Symptoms. CTF is frequently biphasic. After an incubation period of 3-6 days, the first phase of illness generally begins with abrupt onset of fever, chills, severe headache, eye pain, photophobia, myalgias, sore throat and nausea. These symptoms persist for 5-8 days and, after a brief remission, recur within 3 days in about 50% of patients. In the course of infection, a transient petechial or macular rash may appear. Hematologic abnormalities include leukopenia and thrombocytopenia. The virus develops in most internal organs and may give rise to rare complications, such as encephalitis, aseptic meningitis, hemorrhage, pericarditis, orchitis, atypical pneumonitis, and hepatitis. Encephalitis and severe bleeding are usually seen only in children. Though sometimes depicted as a mild illness, convalescence can be prolonged, and some patients take several weeks to fully recover. Mortality is rare, generally less than 0.2% of reported cases.

Vectors and transmission. The primary vector is the Rocky Mountain wood tick, *Dermacentor andersoni*, whose range is coextensive with that of the disease. Other tick species from which the virus has been isolated include *D. albipictus, D. occidentalis, D. parumapertus, Haemaphysalis leporispalustris,* and *Otobius lagophilus*. Cases have been reported from March to November but most occur from late May to early July, when adult and nymphal ticks are active.

Reservoirs. CTF is passed transstadially in vector ticks, but transovarial transmission has not been demonstrated. Unfed nymphs can overwinter the virus, but the chief reservoirs are various small mammals, especially the golden-mantled ground squirrel, *Spermophilus lateralis*, deer mouse, *Peromyscus maniculatus*, bushy-tailed woodrat, *Neotoma cinerea*, least chipmunk, *Tamias minimus*, Uinta chipmunk, *T. umbrinus*, Nuttall's cottontail rabbit, *Sylvilagus nuttallii*, and porcupine, *Erethizon dorsatum*.

c. **Ehrlichiosis** (CHPPM Fact Sheet)

Causative agent and distribution. Ehrlichioses are caused by several species of minute (0.2-1.5µ), gram-negative, pleomorphic coccobacilli called ehrlichiae that belong to the family Anaplasmataceae. Typically, ehrlichiae infect the cytoplasm of circulating leukocytes via phagocytosis, thus adversely affecting the host's immune system. Until the late twentieth century, the only species known from humans was *Neorickettsia sennetsu*, the agent of sennetsu fever, a rare, mononucleosis-like illness that appears to be confined to Japan and Malaysia. Human ehrlichiosis in the United States was first diagnosed in 1986 in a 51-year-old Detroit native who had been exposed to ticks in rural Arkansas. In 1990, the agent of this disease was isolated from the blood of a U.S. Army reservist at Fort Chaffee, Arkansas; the next year, it was described as a new species, *Ehrlichia chaffeensis*, or human monocytic ehrlichiosis (HME). To date, this infection has been reported from all states except South Dakota, North Dakota and West Virginia, but its greatest annual incidence is in the southeastern and south-central states, particularly Arkansas, Missouri and Oklahoma. In 1994, a third type of human ehrlichiosis, termed human granulocytic ehrlichiosis or HGE, was reported in 12 patients from Minnesota and Wisconsin, two of whom died. Subsequent research revealed that the agent of HGE is identical to organisms that cause ehrlichiosis in horses (*E. equi*) and in cattle and sheep (*E. phagocytophilum*), and in 2001 the organism was reclassified as *Anaplasma phagocytophilum*, or human granulocytic anaplasmosis (HGA), a disease now known from 17 states, mostly in the

Northeast and upper Midwest. Recently, a small number of HGE/HGA cases have also been linked to *Ehrlichia ewingii*, the agent of canine granulocytic ehrlichiosis.

        Symptoms. In the United States, ehrlichiosis caused by *E. chaffeensis* and *A. phagocytophilum* ranges from illness so mild that no medical attention is sought to severe, life-threatening or fatal disease. After an incubation period of 1-21 (mean 7) days, ehrlichiosis usually presents as a nonspecific febrile illness that resembles Rocky Mountain spotted fever except that a maculopapular or petechial rash develops in only 20-33% of patients, versus over 80% of patients with Rocky Mountain spotted fever. Moreover, the rash caused by ehrlichiosis occurs on the palms of the hands or soles of the feet in less than 5% of cases. Characteristic clinical features are high fever and headache, but other common symptoms include malaise, myalgia, nausea, vomiting and anorexia. Severe and sometimes fatal complications may ensue, most notably acute renal failure, encephalopathy, and respiratory failure. Common abnormalities, which are transient and most pronounced 5-7 days after onset, include leukopenia, absolute lymphopenia, thrombocytopenia, elevated levels of serum hepatic aminotransferase and, rarely, neutropenia or cerebrospinal fluid pleocytosis. On average, patients with ehrlichiosis are older than those with Rocky Mountain spotted fever, and males are infected more often than females. About 75% of cases are reported from May through July, and approximately 90% of patients have a history of tick exposure in the three preceding weeks. Sennetsu fever is characterized by sudden onset, with fever, chills, headache, malaise, muscle and joint pains, sore throat and sleeplessness. Generalized lymphadenopathy with tender, enlarged nodes is common. Lymphocytosis, with postauricular and posterior cervical lymphadenopathy is suggestive of infectious mononucleosis. This is a generally benign disease; no fatalities have been reported.

        Vectors and transmission. *Ehrlichia chaffeensis* and probably *E. ewingii* are transmitted by the lone star tick, *Amblyomma americanum*, whose range encompasses the Southeast and south-central states, where most cases of HME, and of HGE caused by *E. ewingii*, are reported. The vectors of *A. phagocytophilum* are the blacklegged tick, *Ixodes scapularis*, in the eastern United States and the western blacklegged tick, *I. pacificus*, along the Pacific Coast. Cases of sennetsu fever are associated with swampy areas, where trematode flatworms are suspected vectors.

        Reservoirs. White-tailed deer, *Odocoileus virginianus*, appear to be natural reservoir hosts for *E. chaffeensis* and may serve as a source of infection for *A. americanum* in all life history stages. Deer, elk, and wild rodents are thought to be likely reservoirs of *A. phagocytophilum*, though birds have also been suggested. Dogs may be a reservoir of *E. ewingii*, but the natural history of this disease is poorly understood. The reservoir of sennetsu fever is unknown.

        d. Lyme disease (CHPPM Fact Sheet)

        Causative agent and distribution. Lyme disease, also known as Lyme borreliosis, tick-borne meningopolyneuritis and Bannwarth's syndrome, is the most common vector-borne infection of humans in the temperate Northern Hemisphere, including North America, Europe, and northern Asia. In the United States, Lyme disease was first recognized in the mid-1970s in the vicinity of Old Lyme, Connecticut; since then, it has been reported from all states except Montana, though over 90% of confirmed cases have occurred along the Northeast coast (southern Maine to northern Virginia), in the upper Midwest (especially Wisconsin and Minnesota), and in northern California. The etiologic agent, the spiral-shaped bacterium *Borrelia burgdorferi*, was not discovered until 1981, but cases of this disease have been

described in the European medical literature since 1883. Worldwide, six genomic groups are currently recognized: 1) *B. burgdorferi* sensu stricto, 2) *B. garinii*, 3) *B. afzelii*, 4) *B. japonica*, 5) *B. lusitaniae*, and 6) *B. valaisiana*. All United States isolates to date have been *B. burgdorferi* sensu stricto, whereas in Europe most isolates have been either *B. garinii* or *B. afzelii*. Antigenic differences between these groups may explain some of the variation observed in principal clinical manifestations in infected persons in the United States versus those in other parts of the world. Reports of a Lyme-like syndrome in Australia, South America, and the tropical portions of Africa and Asia are frequently published, but most are based on dubious serologies and none have implicated a vector.

Symptoms. Lyme disease has been called "the great imitator" because of its protean manifestations. Within 1-3 weeks of tickbite, a characteristic macular dermatitis called erythema migrans (EM), formerly known as erythema chronicum migrans (ECM), develops at the site of tick attachment—but only in somewhat over 60% of cases. Most patients have just one EM, but 25-50% may experience multiple lesions; in either case, the rash gradually expands to a width of between several inches and a foot or more. Because an EM is flat and produces no sensation, it may not be noticed if it is located on a part of the body that is difficult to see. Vague flu-like symptoms (low-grade fever, headache, fatigue, arthralgias, myalgias, and regional lymphadenopathy) may precede or accompany EM formation, but asymptomatic infections also occur. Without antibiotic treatment, the EM and associated symptoms sometimes disappear in 3-4 weeks. Untreated patients may show no further signs of illness or they may develop late-stage disseminated Lyme disease from one to several months afterward. Disseminated disease is characterized by neurologic abnormalities (including the clinical picture of aseptic meningitis, encephalitis, chorea, cerebellar ataxia, cranial neuritis with facial palsy, motor or sensory radiculoneuritis and myelitis; 15-30% of patients), cardiac abnormalities (including atrioventricular block, acute myopericarditis or cardiomegaly; less than 10% of patients), and musculoskeletal complaints, especially arthritis of the large joints (about 60% of patients), but these manifestations apparently vary regionally with strains of the spirochete and may also be dependent on immunogenetic factors. In some patients, neurologic and/or arthritic symptoms may become chronic and debilitating.

Vectors and transmission. All known primary vectors of Lyme disease are members of the tick subgenus *Ixodes*, genus *Ixodes*. In North America, these are the blacklegged tick, *I. scapularis*, in the East and upper Midwest (the northern form of *I. scapularis* was formerly known as *I. dammini*), and the western blacklegged tick, *I. pacificus*, on the West Coast. In the Old World, the so-called castor bean or sheep tick, *I. ricinus*, extends across Western Europe into European Russia, where its range overlaps that of *I. persulcatus*, the principal vector throughout Palearctic Asia, including Japan and Taiwan. Various other *Ixodes* that seldom bite humans or are not members of subgenus *Ixodes* may serve as enzootic or maintenance vectors of *B. burgdorferi*, e.g., *I. (I.) dentatus* in eastern North America, *I. (I.) spinipalpis* (including its junior subjective synonym *I. neotomae*) in western North America, and *I. (Partipalpiger) ovatus* in Japan. As well, certain ticks in other genera, such as the lone star tick, *Amblyomma americanum*, by virtue of their abundance, wide distribution and lack of host specificity, may occasionally become infected with and transmit *B. burgdorferi* to humans, even though experimental evidence indicates that they are inefficient vectors. Spirochetes have also been detected in mosquitoes, deer flies and horse flies in both the northeastern United States and Europe, though the role of insects in the transmission of *B. burgdorferi* appears to be negligible. In North America, most cases of Lyme disease result from the bites of nymphal ticks, which are

chiefly active during late spring and summer and are often unnoticed because of their small size. In Asia, however, adults of *I. persulcatus* are most often involved in transmitting borreliae to humans. It should be noted that simultaneous infections with *B. burgdorferi* and *Babesia microti* (the agent of babesiosis) and/or *Anaplasma phagocytophilum* (the agent of human granulocytic ehrlichiosis/anaplasmosis) have been observed in ticks, and there is evidence that two or even three of these organisms may be transmitted during a single tick bite.

   Reservoirs. Rodents, insectivores, other small mammals, and even birds maintain spirochetes within their tissues for prolonged periods, if not for life, and readily infect larval ticks that feed on them. Infection is then passed transstadially to nymphs and adults. In North America, the white-footed mouse (*Peromyscus leucopus*), dusky-footed woodrat (*Neotoma fuscipes*) and California kangaroo rat (*Dipodomys californicus*) are important reservoir hosts, while field mice and voles in the genera *Apodemus* and *Clethrionomys* are the chief reservoirs across Eurasia. The white-tailed deer (*Odocoileus virginianus*) of eastern North America, which are so important as hosts of adult *I. scapularis*, are incompetent as reservoirs of borreliae. Less than 1% of unfed tick larvae have been found infected with *B. burgdorferi*, indicating that transovarial transmission is of little consequence in the maintenance of Lyme disease in nature.

   e. **Powassan encephalitis** ([CHPPM Fact Sheet](#))
   Causative agent and distribution. Powassan (POW) encephalitis is a serious though uncommon tick-borne viral encephalitis or illness caused by a *Flavivirus* in the family Flaviviridae. The virus was first isolated from an encephalitis patient in 1958 in the town of Powassan, Ontario, Canada, and is now known to be focally present in Canada, the northeastern United States (New York, New Jersey, New England), and Russia, though incidence is dependent upon the seasonal activity of vector ticks. Most cases have been reported from rural or forested areas, where the greatest risk of transmission occurs from June to September. Children and adult males are more likely to be infected because these groups often enter tick habitats.

   Symptoms. POW virus may cause no symptoms or only mild illness in some individuals, or it may enter the central nervous system, producing encephalitis and meningitis similar to what is seen in the more serious mosquito-borne encephalitides. Symptoms begin suddenly, 7-14 days post-infection, and include headache, fever, nausea and vomiting, stiff neck, and sleepiness. Severe infections cause labored breathing, tremors, disorientation, stupor, seizures, coma, spastic paralysis, and death. POW encephalitis is often associated with long-term illness and permanent brain damage; the case fatality rate ranges from 0.3- 60%, the highest rate among the arboviruses. Infection, whether inapparent or overt, confers immunity.

   Vectors and transmission. In North America, POW virus has been isolated from *Ixodes cookei* (the presumed primary vector and a specific parasite of the woodchuck or groundhog, *Marmota monax), I. marxi, I. spinipalpis* and *Dermacentor andersoni*, all of which can acquire the virus at any life history stage and remain infective for life. In Russia, the virus has been isolated from *Haemaphysalis neumanni, I. persulcatus,* and *D. silvarum*, as well as from mosquitoes.

   Reservoirs. Ticks, with possible transovarial passage of virus, and mammals. Woodchucks are excellent reservoirs, as is the snowshoe hare, *Lepus americanus*, which amplifies the virus and populations of vector ticks. Antibodies to POW virus have been detected in 38 wild and 5 domestic mammal species.

f. Rocky Mountain spotted fever ([CHPPM Fact Sheet](#))

Causative agent and distribution. Rocky Mountain spotted fever (RMSF), known over its vast range by many other names (tick fever, tick-borne typhus fever, black fever, black measles, New World spotted fever, North American tick typhus, Mexican spotted fever, Tobia fever, São Paulo fever), is the most frequently reported rickettsial disease in the United States. RMSF is the prototype of the spotted fever group of rickettsiae and is caused by *Rickettsia rickettsii*, a small (1-2μ long, 0.3μ wide), pleomorphic, obligately intracellular parasite that multiplies freely in the cytoplasm and occasionally in the nuclei of host cells. Though first described in 1872 from residents of the Bitterroot, Snake and Boise River valleys of Montana and Idaho, this disease is endemic throughout the continental United States, southern Canada, and western and central Mexico. Infection also occurs in Costa Rica, Panama, Colombia, Argentina and Brazil. In the United States, most cases are now acquired in the mid- and south Atlantic and south-central states, especially within a triangular area extending from southern New Jersey and the Carolinas westward to eastern Oklahoma (currently, North Carolina and Oklahoma report the highest incidence of RMSF). Most infections arise from exposure in rural or suburban environments, but urban foci also exist, as in some parks and vacant lots of New York City.

Symptoms. The incubation period, 2-5 days in severe infections and 3-14 in milder cases, is followed by abrupt moderate to high fever, malaise, deep muscle pain, severe frontal and occipital headaches, chills, conjunctival injection, and vomiting. The most characteristic and constant symptom is a maculopapular rash that appears from the second to the fifth day after the onset of other symptoms on the wrists, ankles and, less commonly, the back, later spreading to all parts of the body. Rickettsiae multiply in the epithelial linings of capillaries, smooth muscle of arterioles, and other blood vessels; therefore, death may occur at any time during the acute clinical phase (9-15 days after onset of symptoms) as a result of disseminated intravascular coagulation caused by widespread rickettsia-induced vasculitis. Sequelae in recovered patients may include cardiac abnormalities, loss of motor coordination, paralysis, and loss of fingers or toes due to gangrene. In the absence of antibiotic therapy, the case fatality rate is 15-20%, but even today 2-5% of patients die, chiefly because of misdiagnosis or failure to seek treatment.

Vectors and transmission. *Rickettsia rickettsii* is transmitted by the bite of an infected tick or by contamination of abraded skin with crushed tick tissues or feces. The proportion of infected ticks in nature is generally small (1-5%), and most human cases stem from the bites of adult ticks in late spring and summer (nymphs occasionally transmit infection). In eastern North America, the principal vector is the American dog tick, *Dermacentor variabilis*, which parasitizes domestic dogs, with the result that most victims are women and children (introduced Pacific Coast populations of *D. variabilis* are not known to transmit *R. rickettsii*). In western North America, the chief vector is the Rocky Mountain wood tick, *D. andersoni*, adults of which are associated with large game animals and cattle; consequently, most victims are men engaged in agricultural or recreational activities. Throughout its North American range, *Amblyomma americanum* apparently does not play a significant role in the transmission of RMSF, but in Latin America *A. cajennense* is the principal vector of this disease.

Reservoirs. Ticks are the primary reservoirs, maintaining infection by transovarial and transstadial passage. Many other tick species help maintain RMSF in nature, especially *Haemaphysalis leporispalustris*, which transmits infection between rabbits throughout North America. Though *R. rickettsii* has been isolated from numerous small and medium-sized mammals, including members of the genera *Didelphis, Microtus, Peromyscus, Sigmodon,*

Spermophilus and *Tamias*, none of these hosts appear to develop the prolonged rickettsemia necessary to infect feeding ticks.

g. Southern tick-associated rash illness ([CHPPM Fact Sheet](#))

Causative agent and distribution. Southern tick-associated rash illness (STARI), also called Master's disease or southern Lyme disease, is a Lyme-like infection presumptively caused by a newly recognized spirochete bacterium named *Borrelia lonestari*, which has only recently been successfully isolated in culture. Unlike classic Lyme disease, STARI appears to be confined to the southeastern and south-central United States. The current incidence of infection is unknown.

Symptoms. Persons living in or with a history of traveling through the southeastern or south-central United States may experience an erythema migrans (red, expanding rash with central clearing, indistinguishable from the EM of Lyme disease), accompanied by mild flu-like symptoms, such as fatigue, headache, stiff neck and, occasionally, fever. Late sequelae or disseminated disease are rare. A case definition of STARI has not yet been developed.

Vectors and transmission. Unlike Lyme disease, which is transmitted by the bite of the blacklegged tick, *Ixodes scapularis*, STARI is associated with the bite of the lone star tick, *Amblyomma americanum*, whose range is essentially coterminous with the area in which most cases of STARI have been reported: the Southeast and south-central United States. There is a spring-summer seasonality in case reports. The relative rarity of this disease is underscored by research indicating that live spirochetes have been observed in only 1-5% of *A. americanum* tested.

Reservoirs. White-tailed deer, *Odocoileus virginianus*, appear to be naturally infected with *B. lonestari*, but whether deer also serve as reservoir hosts is unclear.

h. Tick-borne relapsing fever ([CHPPM Fact Sheet](#))

Causative agent and distribution. Tick-borne relapsing fever is a systemic spirochetal infection caused by about 14 strains or species of *Borrelia* that are chiefly distinguished by their area of first isolation and/or vector, rather than by inherent biological differences. Each spirochete "species" develops in and is transmitted by a particular species of argasid tick in the genus *Ornithodoros*. There is great variation in strain pathogenicity, and some strains are highly enzootic, rarely infecting humans. Moreover, strains isolated during a relapse often show antigenic differences from those obtained during the initial episode. Morphologically and physiologically, relapsing fever borreliae resemble *B. burgdorferi* but tend to be smaller, ranging from 9-15µ long and 0.2-0.5µ wide. Tick-borne relapsing fever is essentially worldwide in distribution, except for Australia, New Zealand, and Oceania. In its various entities, the disease has been reported from Africa, the Near East, central and southern Asia, Eastern Europe and the Mediterranean, and the western United States and Canada south into Central and South America. In the United States, the disease was first recognized in 1915 and is now known from foci in 11 states (Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Texas, Utah, Washington, and Wyoming), although most cases have been reported from California and Washington.

Symptoms. Most patients do not report a history of tickbite, but an inconspicuous 2-3 mm pruritic eschar may develop at the bite site. Following an incubation period of about 7 days (range 4-18), tick-borne relapsing fever begins abruptly with high fever, chills, tachycardia, throbbing headache, myalgia, arthralgia, abdominal pain, and malaise. Nausea, vomiting, and

diarrhea also may be experienced. Neurologic involvement occurs in 5-10% of cases. A transitory petechial, macular or papular rash develops in 4-50% of cases, usually as the primary fever subsides. Common abnormalities include leukocytosis, an increased erythrocyte sedimentation rate, and thrombocytopenia. In untreated cases, the primary fever lasts 3-6 days, followed by an afebrile interval of about 8 days (range 3-36). Untreated victims can experience 3-10 relapses (range 0-13), with febrile periods followed by afebrile intervals. Generally, the severity of illness decreases with each relapse. Mortality is rare (case fatality rate 0-10%) and usually limited to infants, the elderly, or those physiologically incapacitated, but pregnant patients may experience spontaneous abortion or transplacental transmission.

Vectors and transmission. In the western United States and Canada, *Ornithodoros hermsi* transmits *Borrelia hermsii* in forested mountain habitats, generally at elevations over 900 m but sometimes at lower altitudes. In xeric lowlands from Kansas to Mexico, *O. turicata* transmits *B. turicatae* and, rarely, *O. parkeri* transmits *B. parkeri*. Elsewhere, *O. rudis* and *O. talaje* (both now also classified in the genus *Carios*) transmit *B. venezuelensis* and *B. mazzottii*, respectively, in Central and South America, while *O. tholozani* vectors *B. persica* in the Near and Middle East, and *O. moubata* transmits *B. duttonii* in Africa. Additional vectors and their associated spirochetes are *O. erraticus* (large variety) and *B. hispanica* in southwestern Europe and North Africa; *O. erraticus* (small variety) and *B. crocidurae, B. merionesi, B. microti,* and *B. dipodilli* in Africa, the Near East, and Central Asia; *O. asperus* and *B. caucasica* in the Caucasus Mountains of the former Soviet Union southward to Iraq; and *O. tartakovskyi* and *B. latyschewii* in Central Asia and Iran. Tick-borne relapsing fever is a highly focal infection, often associated with rustic mountain cabins or remote caves where, in the absence of humans, ticks of either sex and in all active stages transmit the disease between small mammals, especially rodents. People become infected when they occupy such shelters, usually during summer months but also at other times of year. Transmission is by the bite of ticks or their infectious coxal gland fluids, from which spirochetes can pass into bite wounds or penetrate unbroken skin.

Reservoirs. Long-lived (10 years or more) ornithodorine ticks serve as persistent reservoirs, with transovarial and transstadial passage of borreliae, though the extent of transovarial transmission varies among tick species. Once infected, ticks remain so for life. Rodents serve as natural sources of infection for ticks; however, humans are the reservoir host of *B. duttonii*.

i. **Tick paralysis** (CHPPM Fact Sheet)

Causative agent and distribution. Tick paralysis is believed to be caused by a variety of proteinaceous toxins, specific to different tick species, that are secreted into the host along with other salivary compounds during tick feeding. In humans and animals, paralytic toxins either block the release of acetylcholine at the synapses or inhibit motor-stimulus conduction. Generally, only female ticks cause paralysis, and they must be attached to a host for several (4-7) days before they begin secreting the toxin in their saliva. This malady has chiefly been reported from North America, Europe, Asia, South Africa, and eastern Australia. Historically, the greatest number of cases has occurred in North America, with highest incidence along the border between British Columbia, Canada, and the states of Washington, Idaho and Montana. In the eastern United States, cases have been reported from seaboard areas of Virginia, the Carolinas and Georgia, but there are also records from Kentucky, Tennessee, Mississippi and Oklahoma.

Symptoms. This affliction is characterized by an ascending flaccid paralysis. In humans, it usually begins in the legs, with muscle weakness and loss of motor coordination and sensation.

Paralysis gradually progresses to the trunk, with loss of coordination in the abdominal muscles, back muscles, and eventually the intercostal muscles of the chest. Paralysis of the last-named muscle group is especially serious because it can lead to respiratory failure. Ultimately, the victim may be unable to sit up or move either arms or legs, and chewing, swallowing and speaking may become difficult. The condition progresses rapidly, and death may occur 24-48 hours after onset of symptoms. Recorded mortality rates are 10-12%. Diagnosis simply involves finding an embedded tick, usually at the nape of the neck or in the scalp. After removal of the tick, symptoms generally resolve within hours or days, which suggests that the tick toxin is either rapidly excreted or metabolized. However, if paralysis is advanced, recovery can take weeks or months. No drugs are available for treatment.

   Vectors and transmission. Worldwide at least 46 ixodid and argasid species in 10 genera have been implicated in cases of tick paralysis involving humans and domestic or wild animals. However, in North America only five tick species – *Dermacentor andersoni, D. variabilis, Amblyomma americanum, A. maculatum* and *Ixodes scapularis* – are known to cause paralysis in humans. In the Pacific Northwest, most cases occur during the spring and early summer, coincident with the adult activity period of *D. andersoni*. In the eastern United States, cases of tick paralysis in dogs and occasionally in humans have been associated with bites of *D. variabilis*, but in California this tick apparently produces paralysis only in dogs. Interestingly, even in regions with a high incidence of tick paralysis, only a portion of the female tick population appears to be able to cause this condition. Paralysis ticks often attach at the nape of the neck, where they may be concealed by long hair; for this reason, most victims are girls. Among adults, men engaged in outdoor activities are more likely than women to be affected.

   Reservoirs. There are no reservoirs associated with tick paralysis, since the affliction does not entail the passage or maintenance of an infectious agent.

  j. Tularemia (CHPPM Fact Sheet)

   Causative agent and distribution. Tularemia, or rabbit fever, is a zoonosis caused by the pleomorphic, gram-negative aerobic bacterium *Francisella tularensis*, which exists in different strains called biovars, two of which occur in North America: a highly virulent, sometimes fatal form associated with lagomorphs, sheep and ticks (biovar *tularensis*), and a less virulent and apparently waterborne form associated with muskrats, water rats, beavers, voles and, in Japan, rabbits (biovar *palaearctica*). Biovar *tularensis* is known only from North America, but biovar *palaearctica* appears to occur throughout the temperate Northern Hemisphere. A third biovar, *mediaasiatica*, has been proposed for the strain from Central Asia. Worldwide, human cases of tularemia have been reported from the Arctic south to Mexico, Venezuela, Turkey, Israel and Iran.

   Symptoms. Clinical presentation depends mainly on the route of inoculation and virulence of the strain. The most common manifestations of tick-borne tularemia are ulceroglandular disease (about 80% of cases), characterized by an ulcer at the site of tickbite with painful regional lymphadenopathy (usually inguinal or femoral), and glandular disease, characterized by regional adenopathy without ulceration. A typhoidal form, with fever, chills, headache, abdominal pain and prostration, but without skin involvement or adenopathy, can also be tick-borne. Other forms of tularemia – oculoglandular, primary pneumonic, and primary oropharyngeal – are presumably not tick-borne. The incubation period is related to strain virulence and size of inoculum, the usual time frame being 3-5 days, with a range of one day to two weeks. Classical tularemia is characterized by the sudden onset of fever, chills, headache,

myalgia, malaise and fatigue. The severity of illness is highly variable, ranging from mild, afebrile, self-limited disease to rare cases of fulminant septic shock; secondary pneumonia, mild hepatitis, and pharyngitis are common complications. Mortality in uncomplicated tularemia is 1-3% with antimicrobial treatment; typhoidal tularemia and secondary pneumonia are associated with increased morbidity and mortality (5-10%).

       Vectors and transmission. Modes of transmission of tularemia to humans are varied and include inoculation of skin, conjunctivae or oropharyngeal mucosa with infected blood or tissues while skinning, dressing or performing necropsies on animals; bites of ticks, fleas, deer flies and mosquitoes; handling or ingestion of insufficiently cooked meat of infected rabbits or hares; drinking contaminated water; inhalation of dust from soil, grain or hay contaminated by infected rodents; handling contaminated animal pelts or paws; and, rarely, bites of animals (cats, dogs, coyotes, skunks, hogs, squirrels) whose mouths were presumably contaminated from eating infected rabbits. In North America alone, *F. tularensis* has been isolated from at least 13 species of ixodid ticks (1 *Amblyomma*, 5 *Dermacentor*, 2 *Haemaphysalis*, 5 *Ixodes*), though the three major vectors appear to be *A. americanum* in the southeastern and south-central United States, *D. andersoni* in the West, and *D. variabilis* in the eastern and central states and parts of the Northwest. Each year, 150-300 cases are reported in the United States, chiefly in Arkansas, Missouri and Oklahoma. Cases occur year-round but peak during the fall and winter rabbit-hunting season and during the summer, when people are outdoors and ticks or other vectors are abundant. Most adult victims are male.

       Reservoirs. Ixodid ticks and numerous wild animals, especially rabbits (*Sylvilagus* spp.), hares (*Lepus* spp.) and rodents, serve as reservoirs of tularemia, though rabbits and hares may experience high mortality rates during epizootics. Important rodent reservoirs include species in the genera *Arvicola, Castor, Microtus, Mus, Ondatra* and *Spermophilus*. Transstadial passage of *F. tularensis* occurs in vector ticks, but claims that transovarial transmission also occurs have not been confirmed.

4. Tick Surveillance
a. General

Whether for biodiversity studies or disease surveillance, there are several standard methods that can be used to determine the number and types of ticks in a given area, including tick drags, tick flags, tick walks, dry ice (carbon dioxide, CO_2) traps, and tick collections from the bodies, burrows or nests of host animals. Whichever method is chosen, it should be applied consistently to ensure that different data sets are statistically comparable. Also, different tick species and life stages are collected disproportionately by the various methods, so it may be necessary to use more than one method in order to develop a complete picture of an area's tick fauna.

b. Tick Drags, Tick Flags, and Tick Walks (Image)

Tick drags, in which a piece of cloth is passed over or around areas where ticks are questing, collect representative samples of the ixodid ticks present and more or less mirror the actual exposure that a person might experience in a given area. This technique is manpower-intensive, yielding few ticks in areas of low to moderate tick density. However, it is ideal as a quick "spot check" of tick activity and is particularly useful when surveying for adult *Ixodes scapularis*, which may take up to an entire day to move toward dry ice traps. A tick drag can be made from

a sheet of soft white material, such as muslin or flannel, and is usually about 1 m long by 1 m wide. Stapling a 1.2 m dowel to the leading edge serves to keep the sheet spread open as it is pulled over vegetation and small obstacles. A 2-m cord is attached at both ends of the dowel to form a loop, which is used to pull the drag. A second dowel is sometimes attached to the trailing edge of the drag, but this may become ensnared in dense vegetation or may limit the cloth's ability to conform to the contours of the area being surveyed.

When ticks are questing close to the surface of the ground or in dense vegetation, a tick flag will sometimes produce better results than a drag. A flag is made by attaching a piece of cloth to a stick or dowel so that it resembles a flag. The flag is then waved back and forth under, in and around vegetation or leaf litter, taking advantage of those areas where ticks are most likely to quest for their preferred host. For example, because *I. scapularis* immatures chiefly infest small mammals, they are more likely to be picked up by low flagging than by dragging over the tops of plants. Also, immature *I. scapularis* tend to remain in the leaf litter around a dry ice trap, rather than climb onto the trap itself, and can therefore best be collected by flagging the trap area.

Of course, anyone pulling a drag is, in effect, engaged in a tick walk. Ticks attaching to a person walking in a prescribed area provide the best estimate of the tick threat to humans. To conduct a tick walk, wear white, 100% cotton clothing (pants and shirt, or coveralls, and socks) to highlight any ticks encountered. Pants should be bloused into socks or boots. If live ticks are required for disease testing, repellent should not be used, but openings in outerwear should be sealed with tape to prevent tick entry and attachment.

Regardless of the tick survey method employed, select multiple sites representing the different installation habitats. If any one habitat type accounts for more than half the outdoor activities taking place on an installation, perform 50% of the tick surveillance in that habitat, and select the remaining sites according to the estimated extent of human activity in them. Note that some habitats will contain more ticks than others. Highest tick densities can generally be correlated with areas of high mammal activity, such as animal trails or bedding sites. Edge habitats, where forests open to fields, trails or clearings, often have the highest tick concentrations.

Sampling should be done during different times of day to improve the chances of collecting at peak tick activity periods. Early morning may not be a good time for tick collection because of overnight dew and low temperatures, which reduce tick activity. Late morning from 1100-1200 and late afternoon from 1500-1630 are often better periods for surveys. Note basic weather conditions on a data sheet. When dragging, a fixed distance (e.g., 100 m) can be used to standardize samples. If the same area is to be dragged repeatedly, engineer's flagging can be used to mark the beginning and end of the drag line. Cloth or clothing should be examined for ticks every 10 paces or so. Carry break-proof plastic collection vials (e.g., cryovials), each labeled with sample number, site location, and date. Remove adult and nymphal ticks from cloth with forceps or tweezers, or use a 2-inch wide piece of tape or lint roller that can be marked with the date, time, location, collector's name, and drag distance. Adhering the tape to the inside of a sealable plastic bag containing a piece of moist (not dripping wet) paper towel will keep ticks alive for weeks if stored in a cool location. A piece of waxed paper can be placed over the adhesive surface, allowing it to be folded on itself. A blade of grass will provide sufficient moisture for ticks in vials.

c. Carbon Dioxide Trapping ([Image])

For many tick species, dry ice traps yield the most specimens in terms of labor expended. This technique capitalizes on the ability of ticks to sense CO_2 and move toward its source. Sensitivity to CO_2 varies among tick species. Thus, *Amblyomma americanum* is attracted to CO_2 to a greater degree than *Dermacentor variabilis*, even though more specimens of the latter can be collected by this method than by dragging, per man-hour. *Ixodes scapularis* is also attracted to CO_2, but since 12 to 24 hours of trapping may be required to attract these relatively slow-moving ticks, dragging or small mammal checks are generally considered better methods for sampling this species. To construct a CO_2 trap, simply place some dry ice in a vented, insulated container and set the container in the center of a sheet or board on the ground. If the trap will not be monitored, tape can be attached, sticky side out, on the perimeter to capture attracted ticks. A half pound (0.23 kg) of dry ice will last about 2 hours at 80^oF (27^oC) in an insulated container. Many variations on this theme have been developed, including traps designed to collect ticks over a 7-day period using a 12-kg block of dry ice, and traps with tubing to sample argasid ticks in burrows or tree cavities. Where dry ice is not available, gas cylinders or chemical generation may be used as a CO_2 source for traps. To generate CO_2 chemically, mix 128 g of sodium bicarbonate (baking soda) with 88 g of dry succinic acid in a 1.0-liter chamber having a ¼-inch (7 mm) hole in its side. Insert a 0.5-liter cup with a 1/32-inch (0.75 mm) hole in its bottom into the chamber. Activate the CO_2 generator by filling the cup with water.

Setting more than one CO_2 trap at each sampling site will provide a more reliable estimate of the actual size of the tick population. To adjust for differences in the time that traps have operated at various sites, calculate a trap index (TI) for each site. The TI is obtained by adding the total tick counts from all traps at one site and dividing by the number of traps (usually 3), yielding the average catch for the site. This number is then divided by the time, in hours, that the traps were operating. For example, assume that 3 traps were set up at a site at 0800 hrs. and ticks were counted at 1000 hrs. The tick counts recorded were 47, 13, and 30. Since a total of 90 ticks were trapped by the 3 traps in 2 hours, the resulting TI would be 15 ticks per trap hour [(90/3)/2]. Studies of *A. americanum* have shown that when all tick stages are counted, the number of ticks found on a CO_2 trap per hour approximates the number that would be expected to attach to a human who remained in that spot for an hour. Threshold TIs can be used to trigger tick control programs and are determined by combining information from all sources and evaluating the nature and frequency of human use in a given area. On sites used briefly or infrequently, more ticks may be tolerated than in heavily used recreation areas. In some cases, such as picnic grounds in a Lyme-endemic area, it may be reasonable to use a threshold TI as low as 1. On the other hand, in a recreational fishing area, a TI of 1 for a single adult *D. variabilis* (easily seen and removed) would generally not signal the need for control; rather, personal protection would be emphasized.

d. Host Trapping and Examination

Host trapping is perhaps the best method of assessing local tick populations, particularly if host nests can be sampled and their contents extracted with Berlese-Tullgren funnels. In the case of burrowing mammals (e.g., field mice), far more ticks will be found in nests than on the host

animals themselves. When planning fieldwork that will involve examination of wild hosts, preparations should include the following:

1. Review the Centers for Disease Control and Prevention publication "Methods for Trapping and Sampling Small Mammals for Virologic Testing" (TG 40) and "Protection from Rodent-borne Diseases with Special Emphasis on Occupational Exposure to Hantavirus" (TG 41) to determine whether resources exist to make small mammal collections and to minimize hantavirus exposure.

2. Contact the installation Environmental Science or Preventive Medicine Office for preliminary coordination and to determine what other personnel should be contacted (e.g., natural resources, security, veterinarians, game wardens, game control, range control).

3. If possible, obtain maps (e.g., installation training or terrain analysis maps, both 1:50,000) and geographic information system vegetation data.

4. Solicit recommendations for sampling areas from the personnel listed above based on potential for tick contact or known problem spots.

5. Obtain state trapping permits or determine whether trapping can be conducted under pre-existing permits issued to the installation.

6. If sampling deer, determine the duration of the hunting season, days when most deer are harvested, and the locations of deer check stations.

7. Schedule sampling dates and prepare a letter of notification for the wildlife biologist, game warden, or other designated point of contact.

8. Schedule time for presentation of tick-borne disease educational material to installation personnel who will be assisting with tick sampling or who need such information for news releases.

9. Assemble all necessary supplies.

Deer are most efficiently examined at check stations during the hunting season, using the following procedures:

1. Wear rubber gloves (e.g., disposable surgical gloves or snug plastic work gloves) and coveralls, or other protective clothing, while collecting ticks.

2. Use fine forceps, preferably with rounded tips and mildly serrated tines, to collect ticks from the head/neck and ano-genital areas. Spend 5 minutes in each of these two areas, recording the number of ticks collected within each time period. If more time is available, collect more ticks, giving priority to unengorged specimens. If no ticks are seen in a given area, the remaining time may be spent examining other parts of the deer's body. Record each collection area separately (area, number of ticks, amount of time). It is helpful to work with a large white enamled tray, from which ticks may be transferred to collection vials. A fine-tipped (000 or 0000) artist's brush is useful for picking small ticks from the tray.

3. Try to keep ticks intact during removal. Intact specimens are needed for species identification, and live specimens are required for pathogen isolation. Ticks may be difficult to remove if deeply embedded, so it may at times be necessary to cut the surrounding host skin, removing both skin and attached ticks.

4. Place ticks in labeled, screw-cap, plastic vials that have each been humidified with a piece of grass. When using vials with a water-absorbent base (e.g., plaster of Paris), put several drops of water in each vial to lightly moisten them. Do not place more than 10 fully engorged ticks in a vial; instead, split the sample among as many vials as needed. Large numbers of engorged females tend to increase mortality in a vial, thus destroying the sample.

5. Print the sample number on the label or use preprinted computer labels.
6. Store tick vials at room temperature. If ticks are being stored for several days prior to shipment, it may be necessary to conserve water by placing the specimens in a refrigerator. Before refrigeration, the vials should be tightly sealed and placed inside a closed plastic bag containing a moistened paper towel. Do not freeze the specimens.
7. Ship ticks as soon as possible if pathogen analyses are to be performed.
8. Adhere to established protocols when collecting blood from deer for serological testing.

Domestic animals often come in contact with tick-infested habitats and may therefore play an important role in transmitting vector-borne diseases to humans. Clearly, surveillance of domestic animals may assist in determining whether tick-borne disease is present. With respect to Lyme disease, dogs may be considered "sentinel" animals because they are at greater risk of tick infestation than humans, are compliant and easily sampled, and have a pronounced antibody response to the spirochete infectious agent. Moreover, since dogs frequently develop asymptomatic disease that can lead to lameness, their owners are often motivated to have their animals tested, and most military bases have veterinary support, which can serve to coordinate on-base and off-base studies. When dogs are brought in for serological testing, tick collections should also be made. Collecting ticks from pets like dogs, cats and birds can sometimes be facilitated with a lint roller, which is moved against the grain of the fur or feathers, as close to the skin as possible. This technique will not work for long-haired animals and will not remove embedded ticks (Appendices).

e. Tick Identification (Interactive Program for Teaching Tick Morphology)

Using dichotomous keys, tick nymphs and adults can usually be determined to species simply by removing them from their collection tubes, patting them down with a tissue so that they are moist but not wet (thereby eliminating glare), and viewing them through a stereoscopic microscope capable of magnifying images 60-90 times. As a general rule, only larval ticks are mounted on microscope slides. Because larvae are routinely examined by phase contrast microscopy, which requires a high degree of transparency, their opaque internal tissues are first macerated by immersing the larvae in lactophenol, an acid corrosive that does not weaken the integument as do basic corrosives (e.g., potassium hydroxide, KOH). After several days, cleared tick larvae may be mounted in Hoyer's medium, or some equivalent aqueous mountant, as follows:
1. Remove specimens to a white porcelain crucible and wash in 3-4 changes of 70% ethanol until the lactophenol-ethanol interface disappears.
2. Using the clean glass rod of a balsam bottle, place a drop of mountant in the center of a 1" x 3" microscope slide.
3. Lift a specimen from the crucible with a fine (0000) camel's hair brush and transfer it to one of the tines of a pair of jeweler's forceps.
4. Touch the tine to the mountant and press the specimen to the bottom of the droplet, arranging it on a vertical axis with the capitulum facing the preparator. By convention, larvae are mounted ventral side down (against the slide).
5. With a clean pair of angular dissecting forceps, pick up a 12 mm, 0-thickness circular coverslip at its rim, apply the opposite edge to the rim of the droplet of mountant, and gently lower the coverslip into place. Final orientation of the specimen may be accomplished by lightly prodding the coverslip surface with a probe.

6. Place the slide in an oven set at 45ºC for about one week.

Heat-treated slides are allowed to cool to room temperature for several hours or days. A ring of Glyptal 1201 red enamel insulating paint is then used to seal the edges of the coverslip to the slide surface. A first coat may be applied using a No. 6 sable brush, but to assure an impervious seal, a second coat is applied with a somewhat smaller (No. 4 or 5) brush after the first ring has dried. At this point, the slides are ready for labeling and incorporation into the collection.

5. Prevention of Tick-borne Diseases
a. Information Gathering

Obtain as much information as possible about the status of tick-borne diseases in and around an installation. Personnel who may possess relevant information include the preventive medicine officer, occupational health nurse, community health nurse, post veterinarian, environmental science officer, wildlife biologist, entomologist, game warden, and local or state health officials and university researchers. Also, obtain copies of any operational plans, guidelines, literature, or raw data on tick-borne diseases that may have been compiled by the installation.

b. Education

It is imperative that personnel learn what diseases ticks transmit on their installation, how to recognize ticks, and how to avoid them. A variety of agencies can be contacted to obtain information or speakers for the training of medical, pest management, or other personnel. Additional educational methods include: making brochures, pamphlets and fact sheets available for in-processing personnel; publishing periodic notices in the installation newspaper or plan of the day, particularly warm months and the fall hunting season; and posting warning signs in tick-infested woods or other areas frequented by troops, hunters or hikers.

c. Personal Protective Measures (CHPPM Fact Sheet)

Ticks are small, especially as immatures, and can therefore easily go undetected. Develop the habit of conducting tick checks by routinely examining clothing and exposed parts of the body while in tick habitat. Use the buddy system to check areas that you cannot see yourself. After leaving tick habitat, carefully recheck your clothes and entire body. Ticks are frequently found on the head, neck, groin, and underarms but may situate themselves anywhere on the body, including the torso, arms, legs, and ankles. Proper tick removal is especially important. Tick bites should be monitored and the ticks themselves saved for identification if symptoms develop. Early symptoms of many diseases are either similar or mimic other conditions, so confirmation of the tick vector species is usually an important clue in the clinical picture. Although many victims of tick-borne disease do not recall a tick bite, those who do should be alert for disease symptoms, such as fever, chills, headache, fatigue, muscular and joint aches and pains, and/or rashes up to one month following the bite. If symptoms do develop, seek medical attention immediately. Some tick-borne diseases progress quickly and can be life-threatening (e.g., Rocky Mountain spotted fever), while others progress more slowly but may persist indefinitely if not promptly treated (e.g., Lyme disease).

Proper clothing will limit access of ticks to the skin, thereby helping to prevent bites. Pants should be bloused or tucked into the boots or socks, and the shirt should be tucked into the pants. This forces ticks to crawl up the outside of the pants or shirt, where they are more likely to be seen. If the uniform shirt cannot be tucked in, the next best thing is to wear an undershirt that is tucked into the shorts. This will serve as a second layer of defense. Long sleeves will help, and a hat will be useful if crouching or crawling in bushes or undergrowth. Light-colored clothing will make ticks much easier to detect. A roller-type adhesive lint remover is useful for collecting ticks from clothing.

Repellents (CHPPM Fact Sheet) are very important in preventing tick bites. The DoD Arthropod Repellent System is a philosophy that stresses the simultaneous use of both a skin and a clothing repellent – deet and permethrin, respectively – for maximum protection from arthropod attack.

Deet (N,N-diethyl-m-toluamide or N,N-diethyl-3-methylbenzamide) is the standard military skin repellent, and it should be used in conjunction with permethrin-treated clothing to provide maximum protection. The extended-duration, or long-acting, formulation (NSN 6840-01-284-3982) is a lotion that contains 33% deet and provides protection for up to 12 hours, depending on environmental conditions. It should be applied in a thin film over exposed skin surfaces, according to label directions. It is not as greasy, smelly, or destructive to plastics as earlier military formulations of deet.

Permethrin is the standard military clothing repellent, and it is the most effective repellent for use against ticks. Since permethrin is actually a contact pesticide, it incapacitates and eventually kills ticks. Permethrin is available through the military supply system in several formulations: a 6-ounce aerosol can that is used to treat a single military uniform, a 5.1-ounce bottle of concentrate that is dissolved in a 2-gallon sprayer for treatment of 8 uniforms, and an impregnation kit for treatment of a single uniform. Desert camouflage uniforms are permethrin-impregnated at the factory, and so automatically offer protection from tick attack for their combat life. Manually treated clothing should be dried prior to wearing, and permethrin should only be applied to clothing – never to skin.

Permethrin aerosol (NSN 6840-01-278-1336) contains 0.5% permethrin. The aerosol should be sprayed liberally, to the point of dampness, over the entire outside surface of the uniform (a color change in the fabric indicates wetting; it dries to its original color). When treated with ¾ of a can, the uniform will provide excellent tick protection through up to 6 launderings. It is especially important to apply permethrin to the lower pant legs, crotch, waistband, shirt sleeves, collar, front placket and lower edge of shirt.

Permethrin concentrate (NSN 6840-01-334-2666) is used in a 2-gallon sprayer to treat 8 sets of uniforms. When dry, the uniforms offer effective protection for the combat life of the uniform.

The Individual Dynamic Absorption Application Kit (IDAA kit, "baggie" method) (NSN 6840-01-345-0237) is used to impregnate a single uniform with permethrin. The kit consists of two small vials of permethrin concentrate, two plastic treatment bags, two pieces of twine, and a black permanent marker. The pants and shirt are treated separately. Each is rolled up, tied with a piece of twine, and placed in a treatment bag into which a vial of permethrin and ¾ canteen

cupful of water have been mixed. After three hours, all the liquid will have been absorbed by the clothing, and it is then hung to dry. The kit imparts effective protection from tick bites for the combat life of the uniform.

Companion animals should be closely monitored for ticks on a daily basis. Dogs are particularly vulnerable, having been shown to be several times more likely to acquire Lyme disease than their owners. A number of commercial products are available for controlling fleas and ticks on pets, but these should be used cautiously. To avoid toxic synergistic effects, contact a veterinarian for advice on using more than one type of treatment on an animal. A veterinarian may also provide prescription chemicals. Under no circumstances should pet flea and tick collars be used on humans. Dogs and cats have few or no sweat glands in their skin and therefore do not absorb chemical toxicants from collars. However, human skin readily absorbs chemicals, and people who foolishly wear pesticide-impregnated flea and tick collars risk skin damage or poisoning. Use of other commercial products (e.g., bath oils) in a manner inconsistent with their labeling may also be hazardous or may fail to provide protection equivalent to standard military repellents.

6. Tick Control (CHPPM Fact Sheet)
       a. Integrated Control

Tick control measures should be tailored to the biology and seasonality of particular tick species. Additional considerations include the type of habitat involved, density and activity of the human population, incidence of infection in the vector species, extent to which tick control is necessary, and degree of environmental modification that is permissible. Based on a knowledge of when infected stages are most active, a medical or installation authority may recommend complete avoidance of some areas at certain times. Integrated tick control comprises four functions: personal protection (see above), habitat modification, acaricide application, and deer exclusion (Fig. 1).
       b. Habitat Modification

Where acceptable, clearing of edge habitats by leaf litter removal, mechanical brush control, and mowing or burning vegetation are effective means of tick control in residential areas, bivouac sites, and certain recreational areas. Removal of low-growing vegetation and brush eliminates the structural support that ticks need to contact hosts, thereby reducing the incidence of tick attachment. Removing leaf litter and underbrush also eliminates tick habitats and reduces the density of small mammal hosts, like deer mice and meadow voles. Without leaf litter, ticks are denied suitable microhabitats that provide the necessary environmental conditions for survival, such as high relative humidity. Mowing lawns and other grassy areas to less than 6 inches (16 cm) greatly reduces the potential for human-tick contact. Of course, the environmental consequences of habitat modification must be thoroughly evaluated to avoid creating additional problems.

When environmentally acceptable, controlled burning has been shown to reduce tick abundance for six months to one year. Besides killing all active stages, burning reduces questing success by destroying the vegetation that is normally used to contact passing hosts. Success is dependent on several factors: amount of combustible brush and ground cover present, environmental and

climatic conditions at the time of burning, size of the area to be burned, tick activity, and rate of tick reintroduction due to animal utilization. There must be a critical mass of combustible material to support an effective controlled burn, and its moisture content must be low enough to allow a thorough burn. It is best to burn in the late evening, when ambient humidity is higher and winds are minimal. These conditions favor a slower burn that consumes some of the protective ground cover and permits penetration of high temperatures to the lower levels of unburned cover or leaf litter. Burning when winds are high increases the risk of wildfire and greatly reduces the consistency and effectiveness of the controlled burn. Fires in areas less than 5 acres (2 hectares) generally provide only temporary reductions in tick numbers due to the rapid reintroduction of ticks by animals passing through the burned site or the reestablishment of animal populations in the burned area. Even large controlled burns may produce only a temporary decrease in tick populations. In some instances, if the amount of vegetation is not regulated, controlled burns may actually increase the browse available for deer and other animals, thus eventually increasing the tick population above pre-burn levels. Success in habitat modification is largely dependent on removal of ground cover, especially the mulch that shelters all tick stages. Burns can be conducted in winter, when trees are dormant, to avoid damage to timber resources. All burning must be coordinated through proper installation channels.

Simple mechanical clearing of underbrush, to the extent that it allows 70-80% sunlight penetration, has been shown to be an effective means of reducing numbers of *Ixodes scapularis*. In developed areas, frequent mowing of grass will also definitely control tick populations. If mechanical clearing is accompanied by herbicide application, larval populations may be reduced by 90% and adult and nymphal populations may decline by 50% or more. Like burning, the success of this technique depends on various ecological, environmental, and control factors: amount of vegetation removed, size of the area, tick activity, extent of reinfestation due to animal utilization, and extent to which control procedures are maintained. Clearing also helps reduce the attractiveness of an area to small mammals, birds, and deer. As with controlled burns, the smaller the area cleared, the faster it is likely to be reinfested by ticks. In order to reduce tick populations to acceptable levels, vegetation control must be implemented on a regular basis. In most instances, control by mechanical means will be the preferred technique because it does not contaminate the environment with pesticides. However, this method is labor intensive and only slowly reduces tick populations. Another habitat modification technique is to thin early successional shrubs and grasses in early to mid fall, stressing the overwintering tick population and reducing survivability. This should be done late enough in the season that regrowth does not occur.

c. Acaricide Application

Use of an acaricide, or any other pesticide, must be consistent with label requirements of the Environmental Protection Agency (EPA) and the Federal Insecticide, Fungicide and Rodenticide Act of 1972, as amended. Outside the jurisdiction of the EPA, DoD Directive 4150.7 requires that pesticide application be in accordance with the accepted standards of the host country, or any host-tenant agreement between the United States and that country. In the absence of any host country standards and agreements, the application must be consistent with EPA requirements or the regulations of the respective service, whichever are more stringent. Beyond these requirements, pesticide selection and application should be in accordance with the

recommendations set forth in AFPMB Technical Guide 24, "Contingency Pest Management Pocket Guide." Chemical control can be accomplished by conventional ground application, aerial application, or both, but area chemical treatments should be a last resort in tick control operations.

Experience has shown that applications of liquid pesticides to control ticks may be impractical because of incomplete penetration of vegetation, especially when aerial dispersal is employed. However, adult *Ixodes scapularis* generally quest in the shrub and grassy layer after the autumn leaf drop and again in the spring before leaves appear. The lack of protective foliage during these periods makes adults of this species vulnerable to chemical sprays. Effective control of *I. scapularis* in all stages has been achieved in small areas using a backpack sprayer or hand-operated granule spreader, thereby also reducing environmental contamination of nontarget areas. But such applications are very labor intensive and unsuitable for larger areas. As well, small areas may require frequent retreatment because ticks may be quickly reintroduced by animal hosts. Seasonal applications of acaricides target overwintered nymphs as they become active in the spring and early summer, and possibly larvae in late summer or early fall. Large-scale applications using vehicle-mounted air-blast sprayers or aerial dispersal of liquid or granular pesticides have provided 50-90% control for 6-8 weeks. Generally, the more active ticks are, the better the control achieved with pesticides (tick activity usually stops when temperatures fall below 54ºF or 13ºC), and localized control provides only localized and temporary relief. When minimal resources are available, treating an area two weeks before it is to be used achieves maximum control for the effort expended.

The formulation (e.g., dust, granule, emulsifiable concentrate) is one of the primary considerations in selecting the type of equipment that will be used to apply an acaricide. In addition, the formulation may be a very important factor in limiting pesticide drift to nontarget areas and in determining the amount of time required before tick control is achieved. Normally, liquid formulations of pesticides provide immediate reduction in tick populations, while granular formulations require a few days before the pesticide is released from the granules into the ticks' habitat. Liquid formulations of pesticides can be applied to vegetation at various heights to kill questing ticks, whereas granular formulations only affect ticks at ground level. However, granular formulations are generally easier to apply and are less likely to contaminate nontarget areas through pesticide drift. In comparative studies, both formulations gave about the same level of control when evaluated over a period of 4-6 weeks. Several factors are associated with successful acaricidal tick control, including type of acaricide, ambient temperature, dosage, penetrability of canopy, extent of coverage, susceptibility of the tick species, and tick life stage and physiological condition.

In recent years, Agricultural Research Service entomologists at the Knipling-Bushland U.S. Livestock Insects Research Laboratory in Kerrville, Texas, have proposed an alternative to earlier acaricidal or medicated bait approaches for controlling such important vectors as *Amblyomma americanum* and *I. scapularis*. A passive topical treatment system called the "4-poster" attracts white-tailed deer to a food source and, as they feed, allows self-application of an acaricide, such as amitraz, to the head, ears, and neck to control ticks. Through grooming, acaricide is transferred to other body areas, such as the brisket, axillae, groin, and vent. The 4-poster has a central reservoir for whole kernel corn that fills two feeding/application stations at

either end of the base. These stations each have a single feeding port adjacent to two vertical acaricide-impregnated applicator rollers. A horizontal plate partly obscures each feeding port and forces deer to rub against the rollers as they feed. The rollers permit treatment of both antlered and antlerless deer and allow upward retraction of the head if deer are startled while feeding. Preliminary field tests in Texas and throughout the Northeast Corridor have indicated that, at least locally, very high percentages of tick control are possible with this device.

d. Deer Exclusion

As with the 4-poster (above), the specificity of *Ixodes scapularis* for deer provides a point of vulnerability that may be exploited to reduce tick population size. Experience has shown that reducing the number of deer will not immediately reduce the number of ticks; instead, ticks "double up" on the remaining deer and, to some extent, utilize alternate hosts. Tick densities are not reduced immediately because of the long life cycle of three-host ticks. Following a reduction in deer density, populations of nymphs and adults will take one or two years to fall. Because deer provide over 90% of adult *I. scapularis* blood meals, eliminating deer, either by exclusion or removal, is ultimately an effective tick control measure.

A variety of fences (Fig. 2) have been developed to exclude deer, ranging from single electrified wires for areas where the deer population is low, to complex, multi-wired electric and nonelectric designs, required in areas where deer pressure is great. Fence selection and design are also strongly influenced by location, terrain, vegetation and cost. A 9-foot (3 m) high, woven-wire, nonelectric fence has been used successfully to exclude deer from recreation areas. A 6-wire, vertical, high-tensile, electric anti-deer fence is effective in many situations. Voltage energizers of various capacities, which can electrify short or long runs of fence, are used to charge wires spaced so that deer cannot pass through without contacting them. The hot and ground return wire sequence can be changed with a switching device to provide the most effective shock for snow cover, tall grass, or very dry situations. Vegetation must be controlled with herbicides in a strip 12-18 inches (0.3-0.5 m) wide directly under the fence and by mowing for 5-8 feet (1.5-2.5 m) on the deer side of the fence to minimize voltage drops and to provide the deer with an approach zone. This zone allows deer to perceive the fence as a no-entry barrier that is to be avoided, and encourages a pathway around the fence rather than through it. Electric fences will also work without a mowed strip when conditions like rough terrain or dense timber make mowing impractical. Although electric fences are expensive, and require regular outlays for maintenance, vegetation control, and the costs of battery replacement and/or AC current, such fencing may be appropriate around residences and in selected recreational areas.

Appendices

Suggested Materials for Surveillance of Tick Vectors and Their Hosts

1. General Equipment

    * Insulated shipping boxes for shipping serum samples (approx. 1 cu. ft. size)

    * White cotton coveralls or white cotton pants and shirt (NOT treated with repellent)

    * White socks NOT treated with repellent

    * Boots, preferably without eyelets, for protection during mammal trapping

    * Flagging (surveyor's tape)

    * Wide (6 cm) masking tape for sealing eyelets, boot tops, shirt openings, and for removing immature ticks collected during tick walks

    * Mosquito head nets

    * Coveralls

    * Data forms (small mammal sampling form, deer sampling form, tick walk form. See Appendix J)

    * Refrigerator foam packs for shipping serum or tick samples

    * Dry ice

    * Rubber gloves (e.g., disposable surgical) for handling small mammals and deer

    * Pencils and permanent marker pens

2. Tick Walk Survey

    * High top shoes (boots), bloused pants

    * White drag cloth (1 x 1/2 m, or 1 x 1 m) mounted on dowel

3. Small Mammal Survey

    * Folding aluminum Sherman box traps, approx. 3.5 x 3.5 x 9 in. (9 x 9 x 22.5 cm) - 60 to 300 traps for trap lines plus 5 extra for replacement of damaged, lost, or stolen traps

* Cotton balls (nesting material for traps)

* Peanut butter/oatmeal bait recipe: Place approx. 4 cups of dry oatmeal (preferably the long-cooking style) in a large mixing container such as a 5-pound coffee can. Add approximately 1 tablespoon of peanut butter per cup of oatmeal and mix well, using your hand to knead the mixture. Continue kneading until the peanut butter is evenly distributed and the mixture is crumbly and does not clump together. Approximately 1 tablespoon of this mixture is used per trap.

* Felt-tipped marking pens for traps

* Cloth or leather gloves (e.g., gardening gloves) for handling traps

* Large (approx. 30-gal.) plastic bags for carrying traps/animals to lab

* 10-ml vial of 100 mg/ml or 50-ml vial of 10 mg/ml Ketamine hydrochloride injectable for small mammal anesthesia

* Tuberculin syringes with needles (1-cc syringes with approx. 26-gauge x 1/2-in. needles) for small mammal anesthesia

* 30-cm ruler

* 100-g Pesola spring scale for weighing small mammals

* Materials listed under BLOOD/SERUM COLLECTING EQUIPMENT

* Materials listed under TICK COLLECTING EQUIPMENT

* Small mammal blood collection protocols (TIM 40)

4. Deer Survey Materials

* Rubber gloves (e.g., disposable surgical) for handling small mammals and deer

* 5-cc syringes with 19 or 21-gauge x 1.5-in. needles for deer blood collection

* 5- to 10-ml glass screw-cap centrifuge tubes or 7-ml Vacutainer tubes for all blood samples

* Materials listed under BLOOD/SERUM COLLECTION EQUIPMENT

* Materials listed under TICK COLLECTION EQUIPMENT

** Deer blood collection kits for hunters, which include:
- Twirlpak bag
- Disposable polypropylene transfer pipette
- Labeled 5- to 10-ml glass, screw-cap centrifuge tube or 7-ml
Vacutainer tubes
- Vacutainer blood collection tube
- Protocol for Blood Collections from Deer, Deer Sampling Survey
Form, and Lyme Disease Fact Sheet
- Small pencil
- Disposable gloves

5. Tick Collection Equipment

* Squeeze bottle for water

* Squeeze bottle for 70% alcohol

* Cotton dressings for use with alcohol to surface-sterilize equipment, etc.

* Fine paint brush for transferring ticks

* Needle-nose forceps, Swedish or Dumont forceps for small mammal tick collection

* White enameled pan or white plastic cloth for processing small mammals

* Labeled snap-cap or screw-cap plastic vials (for tick samples) with a filter paper strip insert for moisture

* Scalpel blade and handle for aid in dislodging ticks from deer

* Medium forceps for tick collection from deer

6. Blood/Serum Collecting Equipment

* 1-cc syringes with approx. 23- to 25-gauge x 1/2-in. needles for small mammal cardiac puncture, if necessary

* 100-count micro hematocrit tubes for retro-orbital blood sampling of small mammals
* 5- to 10-ml glass or plastic, capped centrifuge tubes or 7-ml Vacutainer tubes for all blood samples

* 2-ml Wheaton or Sarstedth vials for all serum samples

* Glass Pasteur pipettes for serum transfers

* Pipette bulbs

* Plastic Twirlpak disposable bags for carcasses and medical waste

* White labels for labeling sample vials

* Disposable wood applicators, 6 x 1/2-in. (swab sticks, minus cotton) for clot removal from coagulated blood

* Scalpel blade and handle for performing venous cut-down

Protocol for Blood Collections from Deer

I. Pre-arrangements

    A. Hunters must first be notified of the need to collect blood specimens and ticks from their deer. This notification is coordinated through the installation wildlife biologist, game warden, safety officer, or other personnel responsible for the installation hunting program. These arrangements are best made several months before hunting season to allow incorporation into the hunting program.

    B. Several subject areas can be covered when the hunter is contacted.

        1. Lyme disease information, including personal protective measures, should be provided to the hunter through pamphlets or a briefing.

        2. Options for collecting blood specimens can also be communicated to the hunter, who may either obtain the blood sample from the deer himself or bring the undressed deer to the check station.

        3. Hunters should also be asked to allow a brief period of time (15 min.) at check-out for tick collection by investigators/survey personnel.

    C. If hunters will be responsible for obtaining blood samples, prepare or arrange for an adequate supply of blood collection kits. Each kit should contain a collection tube (non-heparinized glass or plastic, stoppered tube, 7-ml or greater volume), disposable plastic pipette (eyedropper), pencil, deer data record form, plastic disposable glove or other hand covering, tissue paper, and an information sheet on Lyme disease for hunters. Package the kit in a resealable plastic bag. A copy of the deer data record form should be provided.

    D. To optimize collection of specimens, ask personnel responsible for the hunting program to provide the typical time period for hunter check-in and check-out. Schedule tick collection times based on this information.

II. Blood Collection by the Hunter. This procedure should be used when a high level of hunter cooperation is expected and there are enough blood collection kits on hand to distribute to each hunter or hunting party.

    A. Distribute kits to hunters. Instruct hunters in taking a blood sample (see Section B, below) and providing the deer carcass for a 15-minute examination at the check-out point upon returning from the field.

    B. Take a cardiac blood sample from each deer immediately following the kill. Blood should be taken directly from the heart if possible. Blood can be obtained by making a small cut (1/4 inch or 6mm) in the heart with a hunting knife and using the pipette to remove the sample.

Extract as much blood as possible. If blood cannot be obtained this way, collect pooled blood from the chest cavity. As a last resort, the hunter can bring the heart with the deer to the check-out station.

        C. The hunter must fill out the top part of the deer data record sheet and mark the blood tube with his/her name so that it can be properly identified. All information provided by the hunter remains confidential.

        D. The blood sample and data sheet will be turned in at check-out to the survey personnel, who will then record the proper identification number on the tube (see section III. C. below).

        E. Collect all unused kits at check-out.

III. Blood Collection at Check-out Station.

        A. If blood collections cannot be made in the field, arrange to have all hunters bring their intact deer to the check-out station. This will likely require the survey personnel to perform or assist in field dressing of the deer.

        B. Collect blood using either the procedures described in paragraph II. A. above or a needle (1 1/2" or greater length, 19-gauge or larger bore) and syringe (5-ml or greater volume). Syringe-drawn specimens are obtained by laying the deer on its right side and inserting the needle through the left flank, piercing between the ribs about two to four inches (5-10cm) behind the left shoulder.

        C. Print the sample number on the collection tube and the survey form. This number will consist of the year, month, day, ARLOC/UIC number for the installation, and sequence number of deer processed that day. Example: a sample taken on 12 September 1997 for the 10th deer sampled that day would be 970912; 63117 is the UIC for the Navy Environmental Preventive Medicine Unit, Norfolk, and 10 is the tenth deer taken that day; therefore, the complete number would be recorded as 970912-63117-10. The ARLOC/UIC code number for the installation will be different depending on what branch of Service is doing the sampling.

        D. If possible, obtain at least 50 samples. This is the minimum number of samples needed to detect a true prevalence rate of at least 5% (at a 95% probability level).

        E. Allow the blood samples to remain at room temperature for at least two hours in order to clot. Remove each clot with a wooden applicator swab stick by spinning the clot around the stick. Dispose of the clot, and retain the remaining serum sample. Use a Pasteur pipette to draw off the serum and transfer it to a clean vial (1-ml or larger volume Wheaton or Sarstedth vial).

        F. If a centrifuge is available, spin the original samples (2000 x g, 5 minutes) after "rimming" each clot away from the tube wall with a wooden applicator. Pipette the serum to a vial, as above, and label with the sample number. Freeze the serum samples, and transport them frozen if possible. Store the collected serum at -5°F (-20°C) or colder for serological analysis.

G. Record age (fawn, yearling, adult), sex, time of day deer was shot, time processed at check-in station, hunting area or location (e.g., deer stand number), and deer registration number on the deer survey form. Note any other special events or observations on the form as well.

Protocol for Establishing Serology Evaluations for Dogs

Purpose and Significance

Efforts to mitigate the impact of Lyme disease in the northeastern United States are currently limited by our inability to track the early spread of deer ticks into new areas and, subsequently, to educate residents and physicians about the increased risk of tick bites. Dogs suffer from Lyme disease (canine borreliosis) and, because of a generally higher exposure to ticks in the environment, may acquire the disease earlier than humans in the same area. Examination of canine blood samples is an important surveillance tool for detecting the presence of the Lyme disease spirochete. Positive results of canine serology tests denote high local risk of infection in dogs, which are typically asymptomatic, and in humans. Such information can contribute to our understanding of the epidemiology of Lyme disease and its spread to new areas, and should facilitate efforts to inform the public before human involvement becomes significant.

A. Methods

Obtain at least 15-30 blood samples from different dogs, up to a maximum of 100. Serum should be submitted from animals that are most likely to have been exposed to tick vectors. Outdoor dogs, hunting dogs, and dogs known to have had tick infestations are good candidates. If there is an installation hunt club, consider briefing the club about the survey and obtaining samples from their hunting dogs. Dogs to be tested should be over six months of age and selected at random; that is, no preference should be given to dogs with symptoms suggesting canine borreliosis. Blood can be collected at the same time it is being drawn for heartworm tests. However, such samples would not be random because dogs tested for heartworm represent a subset of the canine population that receives superior veterinary care. With a syringe, collect 3-4 cc of blood, place it in a "red cap" tube (no additives), and centrifuge for 15 minutes at medium-high speed. Serum should be drawn off with a clean pipette and placed in a sterile, labeled tube. The label should include location, name, and date. The tube should be capped and frozen until sent to the laboratory. If a centrifuge is unavailable, refrigerate the whole blood until it can be shipped to the laboratory. NOTE: Uncentrifuged whole blood samples should not be held any longer than 2 days before being forwarded.

B. Include a sheet with the sample, listing the following information:

1. Identification number or name of each sample for individual test results.
2. Date the sample was collected.
3. Age and sex of the dog.
4. Street address and town where the dog lives.
5. If the dog has a history of borreliosis, describe it and give symptoms.
6. State whether the dog been vaccinated for leptospirosis.

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