

TECHNICAL INFORMATION MEMORANDUM

LYME DISEASE - VECTOR SURVEILLANCE AND CONTROL

MARCH 1990



**TECHNICAL INFORMATION MEMORANDUM NO. 26**  
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## **ACKNOWLEDGMENT**

This Technical Information Memorandum (TIM) was prepared through the efforts of the Lyme Disease Subcommittee, under the Medical Entomology Committee of the Armed Forces Pest Management Board (AFPMB), LCDR H. Rob Stevenson, MSC, USN, Subcommittee Chairman. Members of the Lyme Disease Subcommittee included MAJ A. Lynn Hoch, MSC, USA; CPT George W. Korch, MSC, USA; and Dr. Jerome Goddard. Special mention goes to MAJ Dale Hamilton, MSC, USAR, and LT Cole Church, MSC, USN, who contributed information to various sections of this TIM. CDR James Trosper of the AFPMB's Defense Pest Management Information Analysis Center played a major editorial role. Many members of the AFPMB, and agencies associated with the Board, were instrumental in reviewing and revising this TIM. Their many worthwhile comments were incorporated into the final text. The contributions of all participating individuals and organizations are greatly appreciated in the development of this document.

## **DISCLAIMER**

Mention of specific insecticides, equipment items, or other materials does not constitute an official endorsement by the U. S. Armed Services. Further, the Armed Forces Pest Management Board does not endorse specific brands of any such insecticides or other materials.

## **FOREWORD**

This Technical Information Memorandum, written in order to consolidate information and procedures on the surveillance and control of Lyme Disease vectors, is being made available to military and civilian agencies having interest in this disease. As the distribution and magnitude of Lyme Disease as a health problem become better known, updating of the information in this TIM will become necessary. With the current pace of developments with this disease, I fully expect that our Board will need to revise this TIM in a very short time. Your constructive comments are most welcome and will be given full consideration in the updating of this document.

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## I. INTRODUCTION

### A. Background

1. Lyme disease is a spirochetal infection currently recorded from North America, Eurasia, and Australia. The disease is most often referred to as Lyme disease or Lyme arthritis in the United States, and erythema migrans (EM) in Europe. The initial most frequent clinical feature is a characteristic skin eruption, EM, with the main subsequent clinical manifestations being a variety of widespread nervous disorders and arthritis. Some patients have a very mild disease while others develop severe and prolonged illness. It is rarely fatal.

2. Lyme disease was named after the Connecticut community of Lyme, where outbreaks of juvenile arthritis had occurred in 1974 and 1975.<sup>1</sup> Two housewives living in Old Lyme questioned the diagnosis, and the Rheumatology Department of Yale University Medical School conducted an epidemiological investigation. These Yale researchers subsequently described a new disease entity called Lyme arthritis. Later, Dr. Willy Burgdorfer and colleagues at the Rocky Mountain Laboratories discovered the causative agent of the disease, a spirochete which was subsequently named *Borrelia burgdorferi*.<sup>2,3</sup> These organisms were isolated from the midgut diverticula of deer ticks, *Ixodes dammini*, captured on Long Island, New York. Soon thereafter, it became apparent that EM, tickborne meningopolyneuritis, and acrodermatitis chronica atrophicans of Europe were caused by the same or closely related spirochetes, and that these clinical entities may represent various stages of the more complex entity called Lyme disease.

3. This illness is a potentially serious health threat to troops, personnel, and residents at military installations in many parts of the world. Direct effects on the mission (active Lyme disease in troops) as well as indirect effects (Lyme disease in dependents or DoD civilian personnel, and Lyme disease-related health care costs, etc.) may be lessened through aggressive public health coordination, education, surveillance, and prevention/control programs, as well as prompt diagnosis and treatment.

### B. Purpose of the Technical Information Memorandum

1. To provide a surveillance protocol to evaluate the current and potential threat of Lyme disease at military installations.

2. To provide reliable control alternatives, both chemical and non-chemical, for reducing populations of tick species responsible for transmitting Lyme disease.

C. Specific Objectives. This Technical Information Memorandum (TIM) is written to give direction and guidance in specific areas to the Pest Management Professional, such as:

1. Determining the known or suspected vector(s) of Lyme disease on a military installation.

2. Determining the prevalence of infection by the Lyme disease agent, *B. burgdorferi*, in these vectors.

3. Ascertaining the presence of other tick species at the installation, and evaluating their possible role in Lyme disease epizootiology.

4. Collecting sera from available mammal hosts of tick vectors, and determining sera infection prevalence of *B. burgdorferi*.
5. Identifying habitats at the installation which present a high risk of human exposure to infected ticks.
6. Assessing the potential spread of Lyme disease onto, or within, the installation based on the existence of suitable habitats, vector ticks and wild mammal hosts.
7. Providing assistance in Lyme disease education for installation personnel and dependents.
8. Gathering information on the epidemiology of Lyme disease at the installation in humans, their domestic pets and other animal hosts.
9. Recommending or performing appropriate prevention/control measures based on surveillance data, the environment, the mission of the installation at risk and other pertinent factors.

## **II. EPIDEMIOLOGY OF LYME DISEASE**

### **A. Background**

1. Lyme Disease is now one of the fastest growing vector-borne diseases in the United States, with cases being reported in almost every state. Major foci are located in the Northeast (NY, CT, RI, NJ, MA), the upper Midwest (WI, MN), and the West Coast (CA, OR).
2. Switzerland, Austria, and West Germany are three of the most highly endemic areas in Europe. Sporadic cases have been reported from most of Eurasia and possibly Australia.
3. Lyme disease is not yet federally reportable in the United States, but estimates of incidence range from 5,000 - 15,000 new cases per year. Several additional thousands of cases are reported in Europe annually. Because it is a "newly discovered" disease, it is likely that many cases go unreported each year.
4. Diagnosis of Lyme disease is often based largely on clinical criteria because of the variable sensitivity and specificity of current serological tests for the disease. Both indirect fluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA) tests are used to detect antibody response to Lyme disease spirochetes. There is, however, considerable cross-reactivity with other spirochetal infections such as syphilis and tick-borne relapsing fever. In addition, recent studies have shown that only 13-50% of clinical cases of Lyme disease (with the EM present) test positive for Lyme disease by IFA or ELISA. New tests are being devised that employ a membrane-EIA antibody assay format, capable of simultaneously detecting IgG and IgM classes of immunoglobulin antibodies, to indicate early and late stages of pathogenesis. Other tests are being developed which will not depend on detection of antibody but will detect Lyme disease antigen in urine. A promising and sensitive technique called polymerase chain reaction (PCR) to detect *B. burgdorferi* DNA has recently been developed.<sup>4</sup>

## B. Symptoms

1. Lyme disease may manifest itself in three general stages.<sup>5,6,7</sup> In the early stage of Lyme disease, patients experience a flu-like syndrome often accompanied by the characteristic skin eruption, EM. The EM, a slowly expanding red rash, begins to appear three to thirty days following the bite of the tick, and is usually located at the site of the bite. If the EM is atypical or absent altogether, as seen in about 30% of the patients, the symptoms mimic a viral infection. Other symptoms in stage one are commonly fatigue, fever, arthralgia (joint pain) and headache. These symptoms may disappear without treatment. Antibodies are developed in man, but these are strain-specific and do not confer lasting immunity. Re-exposure and re-infection are possible.

2. If the infection is not treated, the spirochete disseminates through the body, and pathology is seen in the brain and its surrounding membranes, other skin sites, lymph nodes or joints. Neurological abnormalities may occur in 10-20% of patients roughly four weeks after the tick bite and include a noninfectious type of meningitis (inflammation of the membranes covering the brain and spinal cord), encephalitis (inflammation of the brain), chorea (constant, rapid, jerky, involuntary movements), cerebellar ataxia (failing muscular activity associated with the cerebellum of the brain), facial paralysis, headaches and myelitis (inflammation of the spinal cord). Cardiac abnormalities and arthritic pain in major joints may also occur.

3. In the late stage or chronic phase, arthritis develops in many untreated patients (about 60% in the United States). Arthritis is usually of sudden onset, affecting one or multiple joints, and it is occasionally migratory. The knee seems to be the joint most commonly involved. Duration of the arthritis is 7-10 days, but it occasionally persists for several months. Recurrent attacks of arthritis are the rule, sometimes lasting for years and mimicking chronic rheumatoid arthritis. Before Lyme disease became a prominent consideration, the common diagnosis for a patient was juvenile rheumatoid arthritis. Other symptoms can continue for years, mimicking such disorders as multiple sclerosis, brain tumors, stroke, anorexia, Alzheimer's Disease, and schizophrenia.

4. Transmission through the placenta to the fetus has occurred in pregnant women, resulting in abortions and stillbirths, or physical and cognitive defects in full term, surviving individuals.

**C. Treatment.** Treatment commonly involves the use of antibiotics such as tetracycline, doxycycline, and penicillin. Delayed treatment is less successful because the spirochete apparently becomes sequestered in "immune-privileged" sites (around the brain) where antibiotics have little or no access. Ceftriaxone, however, has been used successfully in delayed treatment cases.<sup>8</sup>

**D. Causative Agent.** Lyme disease is caused by the spirochete, *Borrelia burgdorferi*.

**E. Reservoirs.** The Lyme disease agent circulates in nature between small rodents and ticks. In the northeastern United States, the primary reservoir is the white-footed mouse, *Peromyscus leucopus*. Deer are important in the Lyme disease cycle, at least in the United States, by providing blood meal sources for the ticks, and pet dogs may bring infected ticks into close contact with people. In general, the Lyme disease spirochete is acquired by larval ticks from small rodents and transmitted to people via the bite of the subsequent nymphal or adult stage. Birds also appear to play an important role in the epizootiology of Lyme disease. *B. burgdorferi* has been isolated from larval deer ticks after feeding to repletion on wild caught woodland songbird species, and nymphs from these molted larvae successfully transmitted spirochaetes to mammalian hosts. In addition, birds are thought responsible for short and long distance dispersal of ticks.

F. **Vectors.** The first vector to be incriminated in the United States was the tick, *I. dammini*, in the northeastern and upper midwestern states. Other known tick vectors are *I. pacificus*, in northern California and southern Oregon, *I. scapularis* in the southern states, *I. ricinus* in Central and Western Europe, and *I. persulcatus* in Japan, the Eastern USSR, and China. *I. angustus* has been incriminated as a vector in the Pacific northwest. A highly related strain of *B. burdorferi* has also recently been isolated from rabbit ticks, *I. dentatus*, in New York, Massachusetts, and Maryland. Additional tick species such as the Lone star tick, *Amblyomma americanum*, the American dog tick, *Dermacentor variabilis*, and the Brown dog tick, *Rhipicephalus sanguineus*, have been found naturally infected with the spirochetes, but based upon laboratory studies their vector potential is currently considered to be minimal. In addition, blood-sucking insects such as mosquitoes, fleas and horseflies have been found infected, so other hematophagous insects may also be involved in Lyme disease transmission. Their ability to transmit the disease has not yet been confirmed.

#### 1. *Ixodes dammini*, **Dammin's Northeastern Deer Tick**

a. **Distribution:** This tick occurs in the New England states and New York south into Virginia. There are also established populations of *I. dammini* in Wisconsin and Minnesota as well as Ontario, Canada. Recent reports (1987-88) of *I. dammini* from Michigan, Iowa, Illinois, and Indiana indicate that this species is extending its range. While primarily found in well established deciduous woodland, *I. dammini* also frequent "edge habitats", where the forests open to fields, trails or clearings. Exposure to infected tick vectors in endemic areas is common in woodland recreation areas, although investigators have also found ticks in highly endemic areas of New York, on lawns in homesites adjacent to forests and other areas frequented by humans.<sup>9,10</sup>

b. **Hosts:** Immatures feed on a wide variety of small mammals, especially rodents like the white-footed mouse, *P. leucopus*, which is an excellent reservoir of the Lyme disease spirochetes, producing spirochetemias high enough to infect feeding ticks.<sup>11</sup> Other hosts fed upon by immatures include migrating deer, fox, shrews, voles, chipmunks, sheep, horses, cattle, dogs, cats, birds, and man. Adult ticks feed primarily on deer, such as the white-tailed deer, *Odocoileus virginianus*. Mating occurs both on the host and on the ground before attachment to the host. The engorged, mated female<sup>12</sup> drops off the host to lay her eggs while the male remains on the host, mating with other females.<sup>12</sup>

c. **Seasonality:** Larvae are active from July through September, while nymphs are active from April through July. This is illustrated by the tick's two-year life cycle (Figure 1). Adults are most numerous in the late fall and early winter.<sup>13</sup>

Figure 1. Life cycle of the deer tick, *Ixodes dammini*.

d. **Remarks:** *I. dammini* nymphs bite people aggressively and this behavior is very important in the epidemiology of Lyme disease. In the northeastern United States, 30-70% of *I. dammini* nymphs or adults become infected after feeding on spirochete-infected hosts. Vertical or transovarial transmission of the spirochete and horizontal (transtadial) transmission (from larva to nymph and nymph to adult) have both been demonstrated to occur in *I. dammini* and may occur in other vectors as well. Other potential vectors, such as *A. americanum*, *D. variabilis*, *D. albipictus*, and *I. scapularis* have variable, but usually much lower, infection rates; usually less than 1%, but 3% in one study of *A. americanum*.<sup>12,14</sup>

## 2. *Ixodes pacificus*, Western Black-Legged Tick

a. **Distribution:** This species occurs along the Pacific coastal margins of British Columbia, Canada, and the United States, possibly extending into Baja California and mainland Mexico.

b. **Hosts:** *Ixodes pacificus* immatures feed on numerous species of small mammals, birds, and lizards. Adults feed primarily on Columbian black-tailed deer, *Odocoileus hemionus*.

c. **Seasonality:** Adults are primarily active from late October to early June, with immatures being active in the spring and summer.

d. **Remarks:** Adult *I. pacificus*, like *I. scapularis* and *I. dammini*, have long mouthparts, making their bites especially painful to man. Adults are most abundant in the early spring. Infection rates of *I. pacificus* with Lyme disease spirochetes are usually in the range of 1-5%. These low rates may be related to host availability for the immatures. *Ixodes pacificus* immatures feed predominantly on the western fence lizard which is refractory to *B. burgdorferi* infection.

## 3. *Ixodes scapularis*, Black-Legged Tick

a. **Distribution:** This species occurs in the southern Atlantic Coast states and throughout the South including Texas and Oklahoma. It has also been reported from the Mexican states of Jalisco and Tamaulipas.

b. **Hosts:** Immatures feed on lizards, small mammals and birds, including quail, blue jay and thrush.<sup>15,16</sup> Adults prefer deer, but will bite people. In certain sections of the southern United States adults have been reported to occur in considerable numbers on dogs, cattle, sheep and horses, as well as deer.<sup>16</sup> In Mexico, there is an additional host record from jaguar.

c. **Seasonality:** In the United States, adults are active in the fall, winter and spring, whereas immatures are active in the spring and summer.

d. **Remarks:** *Ixodes scapularis* congregates along paths, trails and roadways in various types of forested areas such as those exhibiting mature pine-hardwoods with dogwood, wild blueberry, huckleberry and sweetgum. *Ixodes scapularis* inflicts a painful bite. The majority of hard ticks acquired by persons in the south central and southeastern states in the winter months

are of this species. The very low level of infection in this species is likely due to its heavy reliance on lizards as hosts, and lizards are refractory to infection with the Lyme disease spirochete.

#### 4. *Ixodes ricinus*, European Castor Bean Tick

a. **Distribution:** This tick is thought to have habits similar to those of *I. dammini*, and is common throughout most of Europe, including the British Isles. It is also found in scattered locations in northern Africa and parts of Asia.

b. **Hosts:** Immatures have been recorded from lizards, small mammals and birds, whereas the adults feed primarily on sheep, cattle, dogs, rabbits, horses and deer. Unlike *I. dammini*, *I. ricinus* is found in the absence of deer.<sup>12</sup> *Ixodes ricinus* adults are avid parasites of humans.

c. **Seasonality:** In the temperate regions of its range (central Europe), *I. ricinus* feeding in all stages is most active in spring, April through June and autumn, August through September. Adults and nymphs may be seen in the spring three to four weeks before the appearance of the larvae.<sup>7</sup> In northern Africa, it is most active in winter.

d. **Remarks:** *Ixodes ricinus* is one of the most commonly encountered ticks in Central and Western Europe. Three years are usually required to complete the life cycle, with larvae feeding the first year, nymphs the second year, and adults the third year. Females deposit 2,000 - 3,000 eggs.

#### 5. *Ixodes persulcatus*, Taiga Tick

a. **Distribution:** This species occurs in Central and Eastern Europe, the USSR, China, and Japan.

b. **Hosts:** Larval and nymphal *I. persulcatus* feed on a wide variety of small forest mammals and birds. The adults parasitize larger wild and domestic mammals. The species readily bites humans.

c. **Seasonality:** *Ixodes persulcatus* is found on hosts in the late spring and summer months.

d. **Remarks:** *Ixodes persulcatus* apparently is more cold hardy than *I. ricinus*, thus inhabiting harsher, more northern areas. It inhabits small-leaved forests near primary coniferous forests, such as spruce-basswood combinations. This habitat is commonly referred to as taiga.

### III. TICK SURVEILLANCE MEASURES

A. **General.** There are several tick surveillance methods that can be used to determine the number and type of ticks in a given area, including tick drags, tick walks, dry ice traps and tick collections from animal hosts. Whichever method is chosen should be used consistently throughout a given evaluation.

#### B. Tick Drags and Tick Walks

##### 1. Tick Drags

a. Tick dragging collects representative samples of the hard ticks present. This technique is very manpower intensive and nets few ticks in areas of low to moderate tick densities. However, as a method for a quick pre-treatment survey, or "spot check" of an area, it is the most practical technique available. This method is particularly useful for *I. dammini* surveillance, since *I. dammini* surveillance using CO<sub>2</sub> traps requires an extensive trapping period.

b. The basic principle involves dragging a piece of cloth over or around the vegetation where ticks are waiting (questing) for a passing host. Ticks cling to the cloth. After dragging the cloth over the sampling site in the area of concern, the ticks are removed, counted and identified.

c. A tick drag can be made from a sheet of white muslin or flannel, 1 meter long by 1 meter wide, hemmed at all edges. Sewing a 1.2-meter stick or dowel into each end of the sheet serves to keep the sheet spread open as it is pulled over vegetation and small obstacles. A 3-meter cord is attached to both ends of one of the sticks to form a loop, which is used to pull the drag.

d. In areas of high vegetation, a tick flag can often be used more easily than a drag. A tick flag is made by attaching the flannel or muslin to a stick or dowel to resemble a flag. The flag is waved back and forth on the surface of the vegetation.

##### 2. Tick Walks

a. The purposeful collection of ticks while walking in a prescribed area can be an effective surveillance tool. Appropriate clothing should be worn to highlight any ticks that may be encountered. Several clothing possibilities are available. White, 100-percent cotton clothing (pants and shirt, or coveralls, and socks) which has not been treated with repellents can be used. The pants are bloused into the socks, and low cut shoes are worn to provide greater surface area for tick collection on the socks.

b. Surgical stocking net (NSN 6510-00-559-3159) has also been used by pulling it over the legs of the regular battle dress uniform (BDU). It can then be taped down around the lower part of the boots to prevent ticks from getting under the material. This method is advantageous since there are no special clothing requirements. Even permethrin-treated BDUs can be covered in this fashion. The stocking net will adequately shield the effects of the permethrin against the ticks.

### 3. Additional Considerations

#### a. Surveillance Sites

(1) When doing tick surveys, select several different locations. When also conducting CO<sub>2</sub> surveillance (see below), select survey locations near the CO<sub>2</sub> traps for comparative samples. Select sampling sites for tick walks based on a representative sample of the different habitats available on the installation. Take into account the areas of human activity across the installation. A recommended procedure is as follows: If any one habitat type accounts for more than half of the outdoor or field activities taking place on the installation, then perform 50% of the tick walks in this type of habitat, and select the remaining sites according to estimated extent of cover of each habitat type.

(2) Note that some habitats contain more ticks than others. Edge habitats, such as where forests open to fields, trails or clearings, may have the highest tick concentrations; however, don't overlook other types of habitat. Seek to maximize captures by selecting areas where tick concentrations are high for tick drags and CO<sub>2</sub> traps.

(3) Sampling can be done during different times of the day to improve the chances of collecting during peak activity periods. Early mornings may not be a good time for tick collection due to overnight dew and low temperatures which reduce tick activity. Late morning from 1100-1230 and during the afternoon between 1500-1630 may be better periods to survey. Time of day and weather conditions affect tick activity and can influence the number of ticks caught on the drag or the walker.

#### b. Measurements

(1) A standard distance of 100 meters can be used. The cloth or clothing should be examined for ticks every 20 meters or so. If at all possible, orange flags should be used to mark the beginning and end of the 100 meters. During future surveys these same areas can be examined again or new areas can be selected for survey.

(2) Carry along a collection vial, labeled with a sample number, site location, and date. If additional collection vials are necessary as a result of a large tick population, they should be labeled identically.

(3) Pick off all ticks, and place them in the collection vial. Remove adult and nymphal ticks with a pair of tweezers or forceps. Adult and nymphal ticks can be removed from the cloth with forceps. A lint roller is a preferable method to quickly remove large numbers of nymphal or larval ticks. The adhesive sheet can then be marked as to date, time, location, collector and drag distance. A piece of waxed paper placed over the adhesive surface then allows the sheet to be folded on itself and placed into a vial or a plastic bag. The ticks can be counted and generally identified while still adhering to the sheet, and they can then be removed for further processing.

(4) After all collections are made, place a small strip of lightly moistened filter paper or paper towel in each vial.

(5) Note basic weather conditions, including temperature, on a data sheet (see Appendix A for a tick survey form).

(6) Dragging and tick walks should not be done in the rain, when vegetation is wet or

during times when the air temperature is less than 54<sup>0</sup>F (13<sup>0</sup>C). These conditions normally reduce tick questing and the resultant effectiveness of these methods.

(7) Check Appendix B for the methods of surveillance which work best for the various species of ticks.

### C. Carbon dioxide trapping

1. Carbon dioxide (CO<sub>2</sub>) trapping provides the most ticks per man-hour expended. This technique relies on the ability of ticks to sense CO<sub>2</sub> and move toward the source. The sensitivity of this method varies with the species of tick. Lone Star ticks are attracted to CO<sub>2</sub> traps to a greater extent than are American dog ticks. Nonetheless, more American dog ticks can be collected by this method than by dragging, per man-hour expended. I. dammini ticks are attracted, but 12 to 24 hours of CO<sub>2</sub> trapping may be required to attract these smaller, slower-moving ticks.

#### 2. Procedures

a. Dry ice is placed in vented, insulated containers. Tape can be stapled, sticky side up, around the perimeter of the trap to capture attracted ticks. A half pound of dry ice will last about 2 hours at 80<sup>0</sup>F (27<sup>0</sup>C) using an insulated container. Traps have been designed to collect ticks over a 7-day period using a 12-kg block of dry ice.<sup>18</sup>

b. Where dry ice is not available, CO<sub>2</sub> gas cylinders or chemical-generation may be used as a CO<sub>2</sub> source for traps. To generate CO<sub>2</sub> chemically, mix 128 g. sodium bicarbonate (baking soda) with 88 g. of dry succinic acid in a 1.0-liter chamber having a 1/4-inch (7mm) hole in its side. Insert a 0.5-liter cup with a 1/32-inch (0.75mm) hole in its bottom into the chamber. Activate the CO<sub>2</sub> generator by filling the cup with water.

c. Setting more than one trap at each chosen location will provide a more reliable count of the actual population.

d. Record date, site (use Defense Mapping Agency map grid coordinates if possible), time traps were set, time ticks were counted, species and numbers of ticks present for each of the traps at each site (Appendix A). Calculate a trap index (TI) for each site to adjust results for differences in time that traps were operating at various sites. The TI is calculated by adding the total counts from all traps at one site and dividing by the number of traps (usually 3) to get the average catch for the site. This number is then divided by the time (in hours) that the traps were operating. Time in hours is calculated by dividing the number of minutes of trap time by 60 minutes. For example, 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. 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 Lone Star ticks conducted in Oklahoma showed that when all stages of ticks are counted, the number of ticks found on a trap per hour approximates the number that would be expected to attach to a human who remains in that one spot for an hour.

(NOTE: If upon returning to count ticks, you discover that all of the dry ice is gone from a trap, disregard the data from that trap and use only the data from the remaining traps to calculate the TI.)

e. Threshold TIs for the initiation of tick control can be determined by combining information from all available sources, and by evaluating the nature and frequency of human use in

a given area. In sites used infrequently, and for brief periods of time, more ticks could be tolerated than in heavily used recreational areas. It is not unreasonable to use a threshold TI of one for a heavily used picnic area.

#### **D. Host Trapping and Examination**

1. Host trapping provides the most accurate assessment of a local tick population when appropriate hosts are sampled. While nothing works as well as the natural host to evaluate tick populations, difficulty in catching some wild hosts is a limiting factor of this surveillance technique.

2. Ensure the efficiency and validity of survey measures by thorough coordination, scheduling and preparation. If possible, begin coordination at least 60 days in advance of the anticipated survey.

3. The following is a checklist of pre-survey preparations for tick collections from natural hosts.

a. Contact the installation Environmental Science or Preventive Medicine Office for preliminary coordination. Find out what other personnel should be contacted, e.g. natural resources, security, veterinarian, game warden, pest control, range control, etc.

b. Obtain maps in advance, if possible [e.g., installation training map (1:50,000) and terrain analysis map (1:50,000)].

c. Solicit recommendations for sampling areas from the personnel listed above based on the potential for contact with ticks and known problem areas. If possible, select potential sites in advance for tick walks and small mammal sampling.

d. For sampling small mammals, determine whether trapping permits are required, or whether trapping can be conducted under pre-existing permits issued to the installation. Arrange for permits, if necessary. Arrange for laboratory space for processing.

e. Coordinate deer sampling with appropriate personnel, such as the wildlife biologist or game warden. Determine the duration of the hunting season, and location and number of check-in stations.

f. Based on the above coordination, schedule survey dates and prepare a notification letter for the wildlife biologist, game warden or other designated point of contact.

g. Schedule time(s) for presentation of Lyme disease educational material to installation personnel who will be assisting you or who need information for such projects as news releases.

h. Assemble necessary supplies. See Appendix C.

4. The following is a checklist for host trapping and tick collection from small mammals.

a. Select three or more areas for study. Record these areas on installation maps or a logbook, including a narrative description, grid location, etc. Emphasize areas where human exposure to ticks occurs.

b. In locations known or strongly suspected to have vector species for Lyme disease, select two representative sites for that species, and one additional site to allow for collection of other potential vectors (e.g. two woodland sites and one grassland site, where *I. dammini* is the suspected vector).

c. In locations where the vector species is unknown, select three sites differing in vegetative composition to sample for as many tick species as possible (e.g., one woodland site, one grassland site, and one transitional area).

d. Set up two trap lines (transects "A" and "B") per site with transects arranged perpendicularly, if possible, to form a "T" or "+" configuration. Locate ten trap stations along each transect at ten-meter intervals. Place a marking flag and a folding aluminum live trap (Sherman trap) at each station.

e. Put a small wad of synthetic batting in each trap to serve as bedding material. The amount used should not impede proper function or closing of the trap.

f. Set and bait traps in the evening.

g. Check traps in the early morning to minimize capture distress and to avoid mortality of the animals. Collect traps containing animals, marking the trap site both on the trap and the map or log book. Transport in a large plastic bag back to the laboratory or other predetermined location for processing.

h. Perform trapping for two consecutive nights.

i. Animal processing for anything other than ectoparasite removal can best be done in a laboratory. Appendix D outlines a protocol for anesthetizing and collecting blood from small animals if samples are required for serological testing. Serology is an important aspect of the surveillance process. Even if the animal is uninfested, the sera will still indicate whether it was infected with *B. burgdorferi*.

j. Utilize a small mammal data form such as the example in Appendix A. For specimens submitted to Army laboratories, sample numbers should follow this sequence: year, month, day, ARLOC/UIC code, and the sequential number for small mammals processed that day. Example: sample number 890810-24004-09, indicates the ninth small mammal processed from Aberdeen Proving Grounds on 10 August 1989. The trap number should follow this sequence: site number, transect A or B, and trap location along the transect (1 through 10). Example: trap number 01A09, indicates trap number 9 along transect A at site number 1.

k. Handle all animals with rubber gloves (e.g., disposable surgical gloves).

l. Place the opening of a clear plastic bag around the door of the trap and seal mouth of bag tightly around the trap with one hand. Open the trap door by pressing open the trap door through the plastic with the other hand, shake trap forcefully to dump the animal into the bag, immediately closing end of bag with hand to prevent escape. The animal can then be properly anesthetized (see Appendix D). An aerosol form of starter fluid for cold weather starting of vehicles can be used easily in the field, since it contains ether and can be administered quickly. Ectoparasites will not be killed if this is used judiciously.

m. Place animal on a white surface, such as a white plastic cloth or white enamel pan.

n. Remove ticks over the enamel pan using needle-point forceps. Use a moistened fine-tip (000 or 0000) paint brush to assist in picking up detached ticks from the enamel pan. Store the collected ticks in a labeled plastic snap cap (pill) vial which has been humidified with a small strip of lightly moistened filter paper or paper towel. Remove as many ticks as possible from the animal, and count or estimate those unremoved. Store tick vials at room temperature. Record the number of collected ticks by species and stage, if possible. Record the total number of ticks infesting the animal, and the actual number collected (if different). For those species and stages for which you have collected more than one individual, save a representative sample (i.e., one each) in a single, labeled vial of 70-percent ethyl alcohol for confirmatory identifications.

5. The following are procedures for collecting ticks from deer. Usually, the most efficient technique for obtaining deer is to monitor check stations during the hunting season.

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

b. Use fine-tipped forceps (tweezers) to collect ticks from the head/neck region and ano-genital region. Spend 5 minutes in each of the 2 areas, recording the number of ticks collected within each 5-minute period. If more time is available, collect more ticks. Priority should be given to collection of unengorged ticks. If no ticks are seen in a given region, the remaining time may be spent examining other areas. Record these collection efforts separately (area, number of ticks, amount of time). It is useful to have a large white pan in which to place ticks while collecting from the deer. The ticks may then transferred to the vial. Use a moistened fine-tip (000 or 0000 size) artist's paint brush to pick up small ticks from the enamel pan.

c. The ticks may be difficult to remove if deeply embedded. Try to keep the tick intact during removal. An intact specimen is needed for species identification, and live specimens are required for laboratory analysis for the spirochete. It may be necessary at times to cut the skin tissue around the ticks, removing both skin and attached tick.

d. Place ticks in labeled, plastic vials which have each been humidified with a small strip of lightly moistened filter paper or paper towel. With vials containing a water-absorbent base (plaster-of-paris base), place several drops of water in the vial to lightly moisten. Do not put more than 10 fully engorged ticks in a vial, but split the sample among as many vials as are needed. Excess numbers of engorged females tend to increase mortality in the vial, and can destroy the sample.

e. Print the sample number on the label. This number will be in the same format as that described in Appendix D for specimens submitted to Army laboratories.

f. Store tick vials at room temperature. If ticks are being stored for several days prior to shipment, it may be necessary to provide additional moisture. This can be done by briefly chilling the specimens in a refrigerator, and then rewetting the filter paper strip.

g. Ship ticks as soon as possible, if Lyme disease analysis is to be performed at another location (Appendix E).

h. Appendix F is a protocol for collecting blood from deer for serological testing.

## 6. Domestic Animal Surveillance for Ticks

a. Since many domestic animals are in close contact with tick-infested environments, as well as having close human contact, the chances for Lyme disease transmission are high.<sup>19</sup> Hence, surveillance of domestic animals may be a good method for determining the presence of Lyme disease .

b. Dogs could be considered as "sentinel" animals in those areas of the world not yet identified as foci of Lyme disease infection. There are several justifications for this strategy:

(1) Dogs, as previously mentioned, are at greater risk of tick infestation and Lyme disease than are humans.

(2) Dogs have a strong, clear-cut antibody response to the spirochete in contrast to the response seen in humans and deer.

(3) They are compliant and easily sampled, particularly when sampled in conjunction with heartworm surveys, which are already in place in many areas of the world.

(4) Since dogs frequently develop an asymptomatic condition which may progress into severe lameness, civilian and military dog owners are well motivated to have their animals tested.

(5) Most military bases have veterinary support, which can serve as the coordinating point for on-base and off-base studies.

c. Appendix G is a general canine serology protocol adapted from the Medical Entomology Laboratory of New York Medical College. In addition to the serology testing, tick collections should also be accomplished. Collecting ticks from animals such as dogs, cats, or birds can sometimes be accomplished by using a lint roller. The roller should be moved against the grain of the fur, getting it as close to the skin as possible. This technique will not work for long-haired animals or for embedded ticks.

## E. Tick Identification

1. Once surveillance programs are established, identification of the ticks is necessary. This can be done using a dissecting scope, which magnifies from 60 to 90 times, and a proper taxonomic key. Identification keys to the genera of hard and soft ticks are in Appendix H. A pictorial key to the adults of hard ticks east of the Mississippi River has recently been published.<sup>20</sup>

2. Many keys are designed for a specific sex or life stage. Always check the particular key you are using to see if any differentiation is made between males or females, adults, nymphs or larvae. Adult ticks are the easiest to identify, but problems can occur if a nymphal key is being used to identify adult ticks. A quick method of differentiating between male and female ixodid adults is by examining the scutum (the shield-like structure behind the head). Males are covered totally by the scutum, whereas the female scutum does not extend the entire length of the body. In engorged ixodid ticks this difference is much more distinguishable.

## IV. LYME DISEASE PREVENTION MEASURES

### A. Information gathering for Lyme disease

1. Obtain as much information as possible concerning the status of Lyme disease at and around the installation. The following is a list of personnel who may have pertinent information:

- a. Preventive Medicine Officer
- b. Entomologist
- c. Occupational Health Nurse
- d. Post Veterinarian
- e. Environmental Science Officer
- f. Community Health Nurse
- g. Wildlife Biologist or Game Warden
- h. Local health officials
- i. State health officials
- j. University researchers

2. Obtain copies of any operational plans, guidelines, existing data and literature prepared by the installation concerning Lyme disease.

**B. Education.** Dissemination of information is paramount in preventing the continued spread of Lyme disease. The most effective and least costly protection against ticks and tick-borne diseases is educating people about ticks and the personal protective measures needed to avoid contact with ticks.

1. The agencies listed in Appendix I can be contacted for assistance in obtaining information or speakers for the training of medical, pest management or other personnel.

2. Other educational methods can be utilized as follows:

a. Posting of Lyme disease warning signs in the woods where troops, hunters or hikers will see them.

b. Making information pamphlets a part of the check-in material for in-processing personnel at installations with risk of Lyme disease (See Appendix J).

c. Publishing periodic notices in the base newspaper and Plan of the Day, particularly during spring and summer periods.

**C. Personal Protective Measures.** Because of its small size, the *I. dammini* tick can easily go undetected. Twenty to thirty percent of Lyme disease patients in one study did not recall a tick bite.<sup>9</sup> Personal protective measures are important in any area suspected of harboring ticks. Figure 2 (Section V) shows how personal protection can be used in conjunction with tick control measures.

1. Performing a thorough, visual check of one's body after leaving a tick-infested area is one of the most effective methods of preventing Lyme disease. Research indicates that an *I. dammini* tick will search for one-half to two hours on a person before beginning to feed. Additionally, it requires from 12 to 24 hours after feeding begins for the spirochete to leave the tick and enter the host. Therefore, a thorough examination at the end of the day should still allow

sufficient time to remove even a feeding tick before disease transmission would normally occur. The person's entire body should be checked. Adult ticks are more prone to be located on the head and neck regions, while nymphs and larvae are more generally seen on the lower extremities.<sup>9</sup>

a. **Proper Tick Removal is Important.** The tick should be grasped behind the mouthparts with forceps, and slowly but firmly withdrawn. A slow, steady pressure encourages the tick to withdraw its mouthparts. If the head is detached during this procedure, the wound site should not be probed with a pin. Removal of detached body parts should be performed by medical personnel. To avoid injecting tick body fluids into the bite, the tick body should not be squeezed during removal. Ticks may react to any violent trauma to their bodies by regurgitating. Thus, any method resulting in trauma to the tick may also result in injection of tick fluids and possibly disease organisms into the wound. Do not use any chemical agents, like alcohol or petroleum oils, hot match heads, or other similar methods to make the tick withdraw its mouthparts. Ticks should not be handled or smashed with fingers as disease inoculation by such action is possible. Clean the wound and apply an antiseptic.

b. Tick bites should be monitored and the ticks saved for identification. An individual who has been bitten should be alert for a rash and other symptoms which may develop up to eight weeks following the bite. If either develops, seek prompt medical attention.

2. Proper clothing will decrease access of ticks to the skin, thereby helping to prevent bites. Pants should be bloused into or tied around the boot or sock tops. Long sleeves are also helpful. Light-colored clothing will make ticks easier to detect. A roller-type adhesive lint remover is useful for removing ticks from clothing.

3. Application of the insect clothing repellent, permethrin (NSN 6840-01-278-1336), before entering tick-infested areas will greatly reduce tick infestation of personnel.<sup>21</sup> The aerosol should be sprayed liberally, to the point of dampness, over the entire outside surface of the clothing. Treated clothing should be dried prior to wearing. The battle-dress uniform (BDU), when treated with two-thirds of a 6-oz. permethrin aerosol can (until there is a visible color change that indicates wetting), will provide excellent tick protection through 5 commercial launderings. Special emphasis should be placed on application to the lower pant legs, crotch, waist band and shirt sleeves. It should be emphasized that the permethrin repellent should only be applied to clothing and never to bare skin.

4. The extended duration DEET repellent (NSN 6840-01-284-3982) has 50% less active ingredient, longer protection time, greater user comfort, greatly reduced odor, and less tendency to degrade plastic items than the old military DEET formulation. This skin repellent should be used in conjunction with permethrin-treated clothing to provide maximum protection.

5. Companion animals should be closely monitored for ticks on a daily basis. Dogs are particularly vulnerable to exposure and have been shown to be several times more likely to acquire Lyme disease than their owners.<sup>22</sup> A number of commercially available flea and tick collars, shampoos and dips will provide good control. An aerosol formulation of permethrin is also registered for flea control on pets. Veterinarians can be contacted for advice and prescription chemicals.

6. The use of flea or tick collars on humans (such as around ankles or pant legs) should not be allowed under any circumstances. These collars are labeled for use on animals, such as dogs and cats, which have few, if any, sweat glands in their skin, and thus do not absorb chemical toxicants from the collar. Human skin readily absorbs chemicals, resulting in poisoning of people who wear these pesticide-impregnated flea or tick collars.

D. **Prophylaxis.** No current vaccine or prophylactic drug is available for protection of humans against Lyme disease, however studies to develop a vaccine are underway.

## V. TICK CONTROL MEASURES

### A. Integrated Control Measures

1. The control measure(s) selected must be relevant to the biology and seasonality of the tick species. See Section II. F. of this TIM. Control measures will require tailoring to the vector's species-specific biological and seasonal characteristics. Equally important, all tick control efforts must be compatible with environmental concerns and human safety.

2. Additional considerations include the type of habitat involved, relative degree of human density and activity, the incidence of infection in the vector species, the degree to which tick control is necessary and the amount of environmental modification that is permissible.

3. Based on the knowledge of when the infected stages are most active and abundant, the medical or installation authority might recommend complete avoidance of certain areas during these limited periods.

4. The various integrated control methods to be covered can be placed into four distinct categories - habitat modification, acaricide application, deer exclusion, and personal protection. These various methods can be effectively used for the control of ticks. Figure 2 outlines which control methods are most appropriate for the areas requiring control.

Figure 2. Appropriateness of Tick Control and Personal Protective Measures.

### B. Habitat Modification

1. Brush clearing of the edge habitat by leaf litter removal, mechanical brush removal, mowing, and/or burning vegetation where acceptable, represents an effective means of tick control in residential areas, bivouac sites and selected recreational areas. Removal of low growing vegetation and brush eliminates the structural support that ticks use to come in contact with 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 used by the ticks, like mice and meadow voles. In addition, without leaf litter the ticks are denied suitable microhabitats which provide the necessary environmental conditions for survival, such as high relative humidity. Mowing lawns and grasses to less than 6 inches (16 cm) greatly reduces the potential for human-tick contact. The environmental consequences of habitat modification must be thoroughly evaluated to avoid eliminating one problem only to create another.

2. Controlled burning, where environmentally acceptable, has been shown to reduce tick abundance for six months to one year. Besides the direct killing of active and questing stages, burning also reduces questing success by destroying the vegetation that is normally used for coming in contact with passing animals or man. The degree of success for control burning is dependent on several factors: the amount of combustible brush and ground cover present, the environmental and climatic conditions at the time of burn, the size of area to be burned, tick activity, and the rate of tick reintroduction due to animal utilization. There must be a critical mass of combustible material in an area to support an effective control burn. The moisture content of the ground cover (leaf litter) and standing vegetation must be low enough to allow for a good consistent burn. It is best to burn in the late evenings with higher ambient humidity and when wind conditions are minimal. These conditions allow for slower burn which consumes some of the protective ground cover and permits the penetration of higher temperatures to the lower levels of unburned ground cover or leaf litter. Burning during conditions of moderate to strong winds increases the chances of an uncontrolled fire and greatly reduces the consistency and effectiveness of the control burn. Its success can also be affected by the size of the area burned. Depending on the ecological situation, small areas of less than 5 acres (2 hectares) normally 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 control area. Even large areas of control burns may provide only a temporary decrease in tick populations. In some instances, if the amount of vegetation in an area is not regulated, control burns may actually increase the amount of browse for deer thus eventually increasing the tick population above the pre-burn levels. The success of habitat modification is largely dependent on the successful removal of ground cover, especially the mulch which gives harborage to all stages of ticks. Burns can be conducted in the winter when trees are dormant to avoid damage to timber resources. All burning efforts must be coordinated through the proper installation channels prior to a control burn for ticks.

3. Simple mechanical clearing of underbrush, to the extent that it allows 70-80% sunlight penetration, is an effective means of reducing deer tick numbers. In developed areas, the frequent mowing of grassy areas will definitely help in the control and reduction of tick populations. If mechanical clearing is accompanied by herbicide application, a 90% reduction in larval population and better than 50% reduction of adult and nymph populations may be expected. The success of this technique, like control burning, is dependent on various ecological and environmental conditions and control procedures: the amount of vegetation removal, the size of the control area, tick activity, the amount of tick reinfestation due to animal utilization and the extent to which the control procedures are maintained. In addition, it helps to reduce the attractiveness of these habitats to small mammals, birds and deer. As with control burns, normally the smaller the area being modified the quicker the area will be reinfested with populations of ticks. In order to maintain a tick population at acceptable levels, vegetation control must be maintained on a regular basis. In most instances, vegetative control by mechanical means will be the preferred technique for tick control since it does not contaminate the environment with pesticides. However, it requires that the environment be modified and maintained on a frequent basis, and provides a slow reduction of tick populations.

4. Another habitat modification technique is to thin early successional vegetation and grasses in early to mid-fall, stressing the overwintering tick population, and reducing survivability. This should be accomplished late enough in the season so that regrowth will not provide sufficient winter cover for ticks.

### **C. Acaricide Application**

1. The 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 of the jurisdiction of the EPA, DoD Directive 4150.7 requires that pesticide application must 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 the 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 Technical Information Memorandum #24, the Contingency Pest Management Pocket Guide, available from the Armed Forces Pest Management Board.<sup>23</sup> Area chemical treatments should be a last resort in tick control operations.

2. Experience has shown that liquid pesticide application to control ticks may be impractical because of the difficulty of penetrating the vegetation, especially when aerial dispersal is used. The control of *I. dammini* is exceptional, however, because the adults quest in the shrub and grassy layer in the fall, generally after the leaves drop, and again in the spring before the leaves appear. The lack of protective foliage during these periods makes adult ticks vulnerable to chemical sprays. Effective control of deer ticks of all stages has been achieved in small areas using a backpack sprayer or hand-operated granule spreader which also reduces the amount of environmental contamination to nontarget areas. The main disadvantage in applying acaricide using these techniques is that they are very labor intensive and only relatively small areas can be treated. Small areas may require frequent retreatment since ticks may be quickly reintroduced into the area by animal hosts. Seasonal application of acaricides is aimed at the overwintered nymphs as they become active in the spring and early summer and possibly again in the late summer or early fall to control larval stages. Larger scale control using vehicle-mounted air-blast sprayers or aerial application of liquid or granular pesticides has provided 50-90% control for 6-8 weeks.

3. The type of acaricide formulation (e.g. dust, granular, emulsifiable concentrate) will be one of the primary considerations in determining the type of equipment that will be required to apply the pesticide. In addition, the type of pesticide formulation may be a very important factor in limiting pesticide drift to nontarget areas and the amount of time required before tick control is obtained. Normally, liquid formulations of pesticides provide for immediate reduction in tick populations while a granular formulation will require a few days before the pesticide is released from the granule into the ticks' habitat. Liquid formulations of pesticides can be applied to vegetation at various heights where questing ticks are located, while granular formulations only affect ticks at ground level. However, granular formulations are generally easier to apply and are less likely to contaminate nontarget areas due to pesticide drift. In comparative field studies, it has been shown that the two formulations give approximately the same level of control when evaluated over an extended period of 4-6 weeks. Several factors are associated with successful control of ticks with acaricides. These include ambient temperature, acaricide, dosage, penetration of canopy, coverage, susceptibility level of tick species, life stage and their physiological age.

4. Chemical control can employ conventional ground application, aerial application or a combination of both methods. The most commonly used pesticides for tick control are diazinon, carbaryl and chlorpyrifos. Most organophosphates and carbamates are less effective at cooler temperatures, less than 70°F (22°C). These acaricides are mentioned because they are available through the federal stock system and are labeled for tick control. Numerous other pesticides which can provide effective tick control are available through local purchase from commercial sources.

- a. Ground application of diazinon 48% EC (NSN 6840-00-782-3925) directed

against the adult deer tick effectively reduces its density in treated areas. See the EPA-approved label for directions for its proper use. It is also labelled for use indoors and outdoors around structures to control the brown dog tick.

b. Carbaryl (Sevin<sup>®</sup>) 5% dust (NSN 6840-01-033-4481) directed against adult deer ticks is effective in reducing population size when applied to the shrub layer. Carbaryl is less effective when temperatures are greater than 80<sup>o</sup>F.

c. Chlorpyrifos (Dursban<sup>®</sup>) 41% EC (NSN 6840-00-402-5411) is also effective. It is applied as a spray to roadsides, footpaths, trails, bivouac sites and other infested non-cropland areas. Low underbrush, grassy edge areas, weeds and ground surfaces should receive special attention. Treated areas should be vacated until the spray has dried. The effective dose for Lone Star tick control is 0.25 lbs/acre. This dose should also be effective for deer ticks.

d. Cyfluthrin, a nonstandard pesticide available as an emulsifiable concentrate, has been shown by various field tests to be highly effective against all stages of the Lone Star and deer ticks when applied to shrub and leaf litter by means of a backpack sprayer. Pyrethroids are more effective at cooler temperatures, but begin to lose effectiveness at temperatures above 90<sup>o</sup>F (33<sup>o</sup>C).

5. The Damminix<sup>®</sup> System (EcoHealth Inc., Boston, MA), while not in the federal supply system, offers a promising, ecologically acceptable means of controlling the deer tick. This system uses cardboard tubes filled with cotton balls impregnated with permethrin. The tubes are placed at 10-yard intervals in a grid pattern throughout and around the area to be protected (e.g. in flower beds, bushes and woodpiles). Foraging mice carry the treated cotton back to their nests to serve as bedding material. The permethrin effectively disinfests the mice of ticks on a daily basis, while the mice survive to repeat the process. Their ectoparasite load is thereby reduced, as is their potential for infection by *B. burgdorferi*. Two applications are recommended each year: in April, and again in mid-summer. The system is limited to those rodents which use cotton as a nesting material and by its relatively high cost of \$300 per acre per year. This control method was specifically designed for *I. dammini*, but may be effective against other ticks which have cotton-gathering rodents as hosts. One of the main drawbacks to this control technique is that deer tick larvae and nymphs feed readily on numerous species of wild mammals and birds, thereby limiting its effectiveness as a tick control method. It may be used in combination with other tick control methods to effectively reduce tick populations.

6. The following considerations apply specifically to Lone Star and American dog tick control using acaricides, but have general application as well. They are the result of extensive work on tick control strategies at the USDA Lone Star Tick Laboratory.<sup>24</sup>

a. The more active ticks are, the better the control achieved with pesticides. As previously stated, tick activity usually stops when temperatures decline below 54<sup>o</sup>F (13<sup>o</sup>C).

b. Localized control efforts provide only localized and temporary relief, and do not significantly reduce the tick population as a whole. Large or isolated areas must be treated to get effective long term control. A tick population may take several years to recover after a comprehensive, large-area control effort.

c. When only minimal resources are available, treating an area two weeks before it is to be used achieves the maximum control for the effort expended.

#### **D. Deer Exclusion**

1. The apparent host specificity of *I. dammini* adults for deer provides a point of vulnerability that may be exploited to reduce the tick population size. Experience has shown that decreasing the numbers of deer will not immediately reduce the relative numbers of ticks. Instead, ticks will "double up" on the remaining deer. Tick densities are not reduced immediately because of the long life cycle of three-host ticks. Nymphs and adults will require one to two years before populations are completely reduced following deer density reduction. Ultimately, eliminating deer in the habitat, either by exclusion or removal, constitutes an effective strategic control measure since deer provide over 90% of the blood meals for the adult life stage.

2. A variety of fences have been used to exclude deer, ranging from single electrified wires, for areas where the deer population is low, to complex multi-wired electric and non-electric designs, required in areas where deer pressure is high. A 9-foot high (3 meter), woven wire, nonelectric fence has been used successfully to physically exclude deer from recreation areas. Fence selection and design are strongly influenced by location, terrain, vegetation and cost.

3. A 6-wire, vertical, high-tensile, electric anti-deer fence, as illustrated in Figure 3, is an effective design appropriate for many situations.<sup>25</sup> 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 which is to be avoided, and encourages a pathway around the fence rather than through it. Electric deer fences will also work without a mowed strip when conditions such as rough terrain or dense timber make mowing impractical. Contact the U.S. Army Environmental Hygiene Agency for information on other fence designs, material suppliers and costs.

4. The limitations of an electric fence system are the initial high cost, the requirements for regular maintenance and vegetation control, and the cost of battery replacement and/or AC current. However, fencing may be appropriate in residential areas and selected recreational areas.

5. Limiting the size of deer herds roaming in game management areas or range areas will ultimately reduce the deer pressure on adjoining training and recreational areas.

Figure 3. 6-Wire, Vertical, High Tensile, Electric Anti-Deer Fence.  
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**APPENDIX A - SAMPLE FORMS FOR SURVEILLANCE OF LYME DISEASE  
VECTORS AND THEIR HOSTS**

TICK DRAG OR WALK DATA SHEET

DATE: \_\_\_\_\_ START TIME: \_\_\_\_\_ END TIME: \_\_\_\_\_

STUDY SITE: 1 2 3 4 5 6 7 8 9 10 11 12 (circle one)

TOTAL DRAG/WALK DISTANCE (m): \_\_\_\_\_

WEATHER CONDITIONS: Temperature: \_\_\_\_\_ Sunny Pt. Cldy Cloudy

GROUND CONDITIONS: Wet Moist Dry

Walk 1 Random Coordinate: _____				Walk 2 Random Coordinate: _____			
Species	Larvae	Nymphs	Adults	Species	Larvae	Nymphs	Adults
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
Total _____				Total _____			

Walk 3 Random Coordinate: _____				Walk 4 Random Coordinate: _____			
Species	Larvae	Nymphs	Adults	Species	Larvae	Nymphs	Adults
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
Total _____				Total _____			

Walk 5 Random Coordinate: _____				Walk 6 Random Coordinate: _____			
Species	Larvae	Nymphs	Adults	Species	Larvae	Nymphs	Adults
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
Total _____				Total _____			

Walk 7 Random Coordinate: _____				Walk 8 Random Coordinate: _____			
Species	Larvae	Nymphs	Adults	Species	Larvae	Nymphs	Adults
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____	_____
Total _____				Total _____			

**GRAND TOTAL**

Species	Larvae	Nymphs	Adults
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____



**APPENDIX A - SAMPLE FORMS FOR SURVEILLANCE  
OF LYME DISEASE VECTORS AND THEIR HOSTS**

DEER SAMPLING SURVEY FORM

Sample Number \_\_\_\_\_

Name of Collector (optional): \_\_\_\_\_

Collector's Address: \_\_\_\_\_ Phone \_\_\_\_\_

Date of Collection: \_\_\_\_\_

Installation/State: \_\_\_\_\_

Hunting Area: \_\_\_\_\_

Time Deer Killed: \_\_\_\_\_

Time Blood Taken: \_\_\_\_\_

Deer Check Station Location: \_\_\_\_\_

Deer Registration No.: \_\_\_\_\_

Deer Measurements: Weight: \_\_\_\_\_

Sex (Circle): Male Female

Age (Circle): Fawn Yearling Adult

Number of Points: \_\_\_\_\_

Number of Ticks Collected: \_\_\_\_\_

Additional Remarks: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_



## APPENDIX B - TICK SURVEILLANCE METHODOLOGY

<u>Method</u>	<u><i>Ixodes dammini</i></u>	<u><i>Amblyomma americanum</i></u>	<u><i>Dermacentor variabilis</i></u>
Habitat Survey	2,4,7 woods, grass, depends on mammal	2,4,7 deer bedding, wooded areas	2,4,7 edge habitat, animal trails & activity
Tick Drags	2,3,4,7 immatures spring, adults	2,3,4,7 in immatures & adults	2,3,4,7 adults along animal trails in fall
Deer Checks	2,4,5,8 late fall, winter	7 - better determined by other methods	NA
Dog Checks	2,3,4,5,6,7 immatures in spring, adults in fall/winter	NA	2,4,7 adults
CO <sub>2</sub> Traps	2,3,4,6,7,8 overnight, hit or miss, ticks slow moving	2,3,4,5,6,7 spring, summer - 2 hr. in morning	2,3,4,7,8 summer for adults, few nymphs, patchy
Tick Walks (white cotton clothing)	2,3,4,5,6,7,8 spring, summer	2,3,4,5,6,7 spring & summer & fall	2,3,4,5,6,7,8 summer
Small Mammal Check	4,5,6,8 spring/summer <i>Peromyscus</i> <i>leucopus</i>	NA	4,5,6,8 spring & summer
Tick Testing	2,8,9 Lyme Disease Babesiosis Ehrlichiosis	NA	2,8,9 RMSF, 1%-2% pos. expect 1 case per 30,000+

<u>Method</u>	<u><i>Ixodes dammini</i></u>	<u><i>Amblyomma americanum</i></u>	<u><i>Dermacentor variabilis</i></u>
Small Mammal Serology	1,2,8,9	NA	NA
Canine Serology	8,9 Lyme Disease	NA	NA
Deer Serology	8,9 Lyme Disease	NA	NA

Key to numerical codes:

- 1 - Determine disease presence in amplifying host.
- 2 - Useful in determining need for control.
- 3 - Useful in control evaluation.
- 4 - Indicator of tick presence.
- 5 - Provides information pertinent to population dynamics.
- 6 - Provides quantitative/density information.
- 7 - Technique useful in nuisance assessments.
- 8 - Component of disease threat assessment.
- 9 - Indicates pathogen presence.

## **APPENDIX C - SUGGESTED MATERIALS FOR SURVEILLANCE OF LYME DISEASE VECTORS AND THEIR HOSTS**

### **1. GENERAL EQUIPMENT**

- \* Insulated shipping boxes for shipping serum samples (approx. 1-cubic foot size)
- \* White cotton coveralls or white cotton pants and shirt (NOT treated with repellents)
- \* White socks NOT treated with repellents
- \* Boots, preferably without eyelets for protection during mammal trapping
- \* Flagging (surveyors tape)
- \* Ten-meter measuring 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
- \* Lab coat or coveralls
- \* Data forms (small mammal sampling form, deer sampling form, tick walk form. See Appendix A)
- \* Refrigerator foam packs for shipping serum or tick samples
- \* Rubber gloves (e.g., disposable surgical) for handling small mammals and deer
- \* Pencils and permanent marker pens

### **2. TICK WALK SURVEY**

- \* Low cut shoes for tick walks
- \* White flannel drag cloth (1 meter x 1/2 meter, or 1 x 1 meter) mounted on dowel

### **3. SMALL MAMMAL SURVEY**

- \* Folding aluminum Sherman traps, approx. 3.5 x 3.5 x 9 inches (9 x 9 x 22.5 cm) - 60 traps for trap lines plus 5 extra for replacement of damaged, lost, or stolen traps
- \* Synthetic polyester batting (nesting material for traps)
- \* Peanut butter/oatmeal bait recipe: Place approximately four cups of dry oatmeal (preferably the long-cooking style) in a large mixing container such as a five-pound coffee can. Add approximately one 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 one tablespoon of this mixture can be used per trap.
- \* Felt-tipped marking pens for traps
- \* Cloth or leather gloves (e.g., gardening gloves) for handling traps

- \* Large (approx. 30-gallon) plastic bags for carrying traps/animals to lab
- \* Ten-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-inch needles) for small mammal anesthesia
- \* Thirty-centimeter ruler
- \* One hundred-gram 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

#### 4. DEER SURVEY MATERIALS

- \* Rubber gloves (e.g., disposable surgical) for handling small mammals and deer
- \* Five-cc syringes with 19 or 21-gauge x 1.5-inch needles for deer blood collection
- \* Five 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 enamel 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**

- \* One-cc syringes with approx. 23 to 25-gauge x 1/2-inch needles for small mammal cardiac punctures, if necessary

- \* One hundred count micro hematocrit tubes for retro-orbital blood sampling of small mammals

- \* Five to 10-ml glass or plastic, capped centrifuge tubes or 7-ml Vacutainer tubes for all blood samples

- \* Two-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-inch (swab sticks, minus cotton) for clot removal from coagulated blood

- \* Scalpel blade and handle for performing venous cut-down



## APPENDIX D - PROTOCOL FOR CONDUCTING SMALL ANIMAL BLOOD SAMPLING

1. This is not a routine procedure. If special circumstances require serological testing of animals other than deer or dogs, early coordination with the supporting laboratory is necessary to define the extent and determine the feasibility of the support required.
2. Use the small mammal data form. The sample number should follow this sequence: year, month, day, ARLOC/UIC code, and sequential number of small mammal processed that day. Example: sample number 870810-24004-09, indicates the ninth small mammal processed from Aberdeen Proving Grounds on 10 August 1987. The trap number should follow this sequence: site number, transect A or B, and trap location along the transect (1 through 10). Example: trap number 01A09 indicates trap number 9 along transect A at site number 1.
3. Handle anesthetized or dead animals with rubber gloves (e.g., disposable surgical gloves). Use heavy cloth or leather gloves when handling live animals in traps.
4. Place the opening of a clear plastic bag around the door of the trap and seal the mouth of the bag tightly around the trap with one hand. With the other hand, open the trap door by pressing through the plastic, then forcefully shake the trap to dump the animal into the bag, simultaneously closing the end of the bag to prevent escape. Work the animal into a corner of the bag. Exercise care not to squeeze the animal so tightly as to cause suffocation. Inject the animal with anesthetic intra-muscularly into the hip through the plastic bag at the following dosage rate:
  - a. For an animal weighing less than 100 grams, use 0.015 to 0.02 ml (100 mg/ml) Ketamine HCl.
  - b. For an animal weighing greater than 100 grams, use 0.03 ml (100 mg/ml) Ketamine HCl.
  - c. Use tuberculin syringe (1-cc syringe with 26-gauge, 1/2-inch needle).
5. Keeping the animal in the bag, watch for signs of drug effect, then remove the animal when it appears immobilized. If the animal has not responded satisfactorily in five minutes, administer another 1/2 dose.
6. Place the animal on a white surface, such as a white plastic tarp or white enamel pan.
7. Obtain blood sample by a retro-orbital bleed in the following manner: Hold the animal in the palm of the left hand with thumb below the eye and index finger above the eye. Stretch the skin around the eye so that the membrane behind the rear corner of the eyeball is exposed. Avoid squeezing the animal so hard as to kill it. Using a heparinized micro-hematocrit tube, insert the non-colored end into the exposed membrane behind the corner of the eye. Twist the tube while exerting steady pressure against the orbital membrane until membrane is punctured and blood enters tube. Continue to hold tube in the retro-orbital area and allow blood to flow through the tube and into a blood collecting tube (5-ml vacutainer tube with rubber stopper is ideal). Collect at least 0.5 ml of blood or more, if possible.
8. If no blood can be obtained by the retro-orbital technique, then attempt cardiac puncture using a 1-ml tuberculin syringe with 23-gauge, 1-inch needle. Obtain as much blood as possible by either technique.
9. Take animal measurements: total length (nose to fleshy tip of tail), body length (nose to base of tail), right hind foot length (heel to tip of longest toe, not including toenail), ear length (ear notch to tip of ear), and weight to nearest tenth gram (100-gram Pesola spring scale is ideal).
10. Note sex, age (e.g., juvenile versus adult), and any other special features.
11. If necessary sacrifice the animal by cervical dislocation. If possible, place animal on a surface

where its feet cannot get a firm grip. Place pencil or other similar object across the neck at the base of the head. While firmly pressing pencil down, pull sharply on tail. As an alternative to sacrifice, the animal can be marked by ear notch and released at the point of capture following arousal from anesthesia if the animal isn't needed for proper identification or other reasons.

12. Allow blood samples to clot at room temperature for at least two hours. Remove clots with a wooden applicator swab stick by spinning the clot around the stick. Dispose of clot, but retain the remaining serum sample for analysis. As a preferred alternative to this procedure, centrifuge the clotted blood sample after "rimming" the clot away from the tube wall with the wooden applicator. Transfer the serum to a clean, labeled vial (1-ml Wheaton or Sarstedth vial). Freeze the serum samples, and transport frozen, if possible. Store at minus 5 °F (-20 °C) or colder.

13. Dispose of animal carcass and all other medical wastes (e.g., syringes) according to the guidance of the military installation medical authority.

**APPENDIX E - DEPARTMENT OF DEFENSE AND OTHER FEDERAL AGENCIES  
PROVIDING LYME DISEASE LABORATORY SUPPORT**

USAEHA  
Aberdeen Proving Ground, MD 21010-5422  
Services provided 1,2

USAPACEHEA - Sagami  
APO San Francisco 96343-0079  
Services provided 1

USAEHA - DSA North  
Fort Meade, MD 20755-5225  
Services provided 1,2

USAEHA - DSA South  
Fort McPherson, GA 30330-5000  
Services provided 1,2

U. S. Army Regional Veterinary Lab  
Fort Meade, MD 20755-5225  
Services provided 3

USAEHA - DSA West  
Fitzsimmons Army Medical Center  
Aurora, CO 80045-5001  
Services provided 1,2

10th Medical Laboratory  
APO New York 09180-3619  
Services provided 1

NEPMU-2  
Naval Station  
Norfolk, VA 23511-6288  
Services provided 1, 2(projected)

NEPMU-5  
Naval Station, Box 143  
San Diego, CA 92136-5143  
Services provided 1, 2(projected)

NEPMU-6  
Pearl Harbor, HI 96860  
Services provided 1, 2(projected)

NEPMU-7  
FPO New York 09521-4200  
Services provided 1, 2(projected)

Bacterial Zoonoses Branch  
Division of Viral Diseases  
Centers for Disease Control  
Fort Collins, CO 80522  
Services provided 1, 2, 3, 4

Department of Entomology  
Museum Support Center  
Smithsonian Institution  
Washington, DC 20560  
Services provided 1

1. Tick identifications
2. Detection of spirochetes within vectors
3. Deer and canine serologies
4. Human serologies (performed only when specimen is submitted through a state health department)



## APPENDIX F - 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. Alternatives for acquiring the blood specimens can also be provided to the hunters. Alternatives include the hunter's preference of either obtaining the blood sample from the deer himself or bringing 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 to allow tick collection by investigators/survey personnel.

C. Prepare or arrange for an adequate supply of blood collection kits for hunters if blood will be obtained by the hunter. Each kit should contain a collection tube (non-heparinized glass or plastic, stoppered tube, 7-ml or greater volume), a 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, obtain information from the personnel responsible for the hunting program concerning 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 in situations where a high level of hunter cooperation is expected, and there is a sufficient number of blood collection kits available to distribute to each hunter or hunting party.

A. Distribute kits to hunters. Instruct hunters in how to take a blood sample (see Section B, below) and to provide the deer carcass for a 15-minute examination at the check-out point upon return 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)(6mm)] in the heart with a hunting knife, and using the pipette to remove the sample. Get 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 in 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. Arrange to have all hunters bring in the intact deer to the check-out station if blood collection cannot be made in the field. This will likely require the survey personnel to perform or assist in field dressing of the deer.

B. Collect blood either using the procedures described in paragraph II. A. above, or by use of 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 of the deer, 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 be composed of the year, month, day, ARLOC/UIC number for the installation, and sequence number of deer processed that day. For example: a sample taken on 12 September 1987 for the 10th deer sampled that day would be 870912, 63117 is the UIC for the Navy Environmental Preventive Medicine Unit, Norfolk, and 10 is the tenth deer taken that day; therefore, the number would be recorded as 870912-63117-10. 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 to clot. Remove the 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 to a clean vial (1-ml or larger volume Wheaton or Sarstedth vial).

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

## **APPENDIX G - 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 the inability to track the early spread of deer ticks into a new area 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 be exposed to 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 will denote high local risk of infection to dogs, which are typically asymptomatic, and of Lyme disease infection to humans. Such information will contribute to our understanding of the epidemiology of Lyme disease and its spread to new areas, as well as facilitating efforts to inform the public before human involvement becomes significant.

### **A. Methods**

Obtain a minimum of 15-30 blood samples from different dogs, up to a maximum of 100. Canine serum should be submitted from animals that are most likely to have had exposure to tick vectors. Outdoor dogs, hunting dogs, and dogs known to have had tick infestations would be 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 randomly selected, that is, no preference should be made for dogs with symptoms suggesting canine borreliosis. Blood can be collected at the same time it is being drawn for a heartworm test. However, this would not be a random canine sample since the heartworm tested canines represent a subset of the canine population that receives better veterinary preventive care than the general canine population. With a syringe, collect 3 or 4 cc of blood, place it into a "red cap" tube (no additives), and centrifuge for 15 minutes at medium-high speed. Serum should be drawn off with a clean pipette, placed into a sterile, labeled tube. The label should include location, name, and date. The tube should be capped and frozen until sent to the [NAME OF LAB]. If a centrifuge is unavailable, refrigerate the whole blood until it can be shipped to the [NAME OF LAB]. NOTE; Uncentrifuged whole blood samples should not be kept any longer than 2 days before being forwarded.

**B. Samples should be sent to:** [PERSON / POINT OF CONTACT]  
[NAME OF LAB]  
[LOCATION OF LAB]

Please include a sheet with the sample, listing the following information:

1. An identification number or name for each sample if you would like individual test results.
2. The date the sample was collected.
3. Age and sex of the dog.
4. Street address and town where the dog lives.
5. Does the dog have a history of borreliosis? If so, describe the history and symptoms.
6. Has the dog been vaccinated for leptospirosis?

Any questions can be directed to [NAME] at [PHONE]. Your assistance and cooperation in this matter is very much appreciated.



## APPENDIX H - KEY TO THE GENERA OF IXODIDAE OF THE WORLD

### (ADULTS)

J. E. Keirans (unpublished, 1986; revised 1987)  
Acarology Summer Program  
The Ohio State University

1. Eyes present (may be difficult to see in some male *Boophilus* and *Anocentor nitens*) ..... 5  
Eyes absent..... 2
2. Festoons absent; anal groove distinct, extending anteriorly around the anus; Prostriata (cosmopolitan, common)..... *Ixodes*  
Festoons present; anal groove distinct or indistinct but never extending anteriorly around the anus; Metastriata..... 3
3. Scutum ornate or, uncommonly, inornate; palpi elongate, subcylindrical (almost always on reptiles) ..... *Aponomma*  
Scutum inornate; palpi elongate or short, conical, not subcylindrical (on birds and mammals, almost never on reptiles) ..... 4
4. Basis capituli square to rectangular dorsally; palpi short, conical, segment II at least as broad as long and usually extended laterally (cosmopolitan, common) ..... *Haemaphysalis*  
Basis capituli of male quadrangular dorsally with posterolateral margins diverging anteriorly; basis capituli of female hexagonal; palpi elongate, conical, segment II at least twice as long as broad, not extending laterally (Russia, China, Nepal, rare) ..... *Anomalohimalaya*
5. Spiracular plate with irregular ridges and partially ornamented with ivory coloration; festoons number 9 (Africa, rare) ..... *Cosmiomma*  
Spiracular plate normal, without ridges or coloration; festoons present or absent, if present, never 9 festoons ..... 6
6. Palpi much longer than basis capituli, palpal segment II much longer than broad ..... 7  
Palpi about as long as basis capituli, palpal segment II about as long as broad ..... 9
7. Scutum and palpi ornamented, palpal segment III with a dorsal and ventral flange; male with paired adanal, accessory and subanal plates (India, rare)..... *Nosomma*  
Scutum ornate or inornate, palpal segment III without both a dorsal and ventral flange; male with or without ventral plates ..... 8
8. Scutum inornate; male with adanal and usually with subanal plates; festoons irregular, partially coalesced (India, Mideast, Africa, common)..... *Hyalomma*  
Scutum usually ornate; male without anal and subanal plates; festoons regular, not coalesced (essentially pantropical, common) ..... *Amblyomma*
9. Palpi extremely short, ridged dorsally and laterally; anal groove indistinct, festoons absent (cosmopolitan, cattle ticks) ..... *Boophilus*  
Palpi not extremely short nor ridged dorsally and laterally; festoons present or absent ..... 10
10. Festoons absent; leg IV of male greatly enlarged (Africa, rare)..... *Margaropus*  
Festoons present; leg IV of male normal ..... 11

11. Festoons number 7; spiracular plate round with goblets few (ca. 8) and large (Neotropical, common on horses) ..... *Anocentor*  
 Festoons number 11; spiracular plate round or with dorsal extension, goblets numerous ..... 12
12. Basis capituli rectangular; usually ornate  
 (cosmopolitan, common) ..... *Dermacentor*  
 Basis capituli hexagonal dorsally; usually inornate ..... 13
13. Scutum usually inornate (4 ornate species); adanal and accessory plates present in the male; coxa IV of male not much larger than I-III, without two long spurs, two short pointed spurs absent on female coxa IV ..... *Rhipicephalus*  
 (*Rhipicephalus sanguineus* cosmopolitan, others primarily African)  
 Scutum inornate; adanal and accessory plates absent in the male; coxa IV of male larger than I-III, with two long spurs, two short pointed spurs present on female coxa IV (Africa, rare)  
 ..... *Rhipicentor*

**KEY TO THE GENERA OF THE ARGASIDAE OF THE WORLD**

**(ADULTS AND NYMPHS)**

J.E. Keirans (1986, unpublished)  
Acarology Summer Program  
The Ohio State University

1. With a definite sutural line separating the dorsal and ventral surfaces.....*Argas*  
Lacking a definite sutural line..... 2
2. Nymphs with integument beset with spines, hypostome well developed; adults with integument granular, hypostome vestigial .....*Otobius*  
Nymphal and adult integument lacking spines; hypostome of adult various in form but not vestigial3
3. Dorsum of adult and nymph with a smooth raised area or false scutum; palpal segment I with a large flange partially obscuring the hypostome .....*Nothoaspis*  
Dorsum of adult lacking a false scutum; palpal segment I without a flange..... 4
4. Hypostome broad at the base and scoop-like (associated with  
bats) ..... *Antricola*  
Hypostome of various forms but with denticles and never scoop-like (found on various animals including bats) ..... *Ornithodoros*



**APPENDIX I - DEPARTMENT OF DEFENSE AND OTHER FEDERAL AGENCIES  
PROVIDING LYME DISEASE TRAINING INFORMATION**

Commander  
10th Medical Laboratory  
APO New York 09180-3619  
06371-86-8113/8381  
Autovon 2223 Ext 7837

Commander  
USAEHA - DSA North  
Fort Meade, MD 20755-5225  
30330-5000  
(301) 677-5281/6502  
Autovon 923-5281/6502

Commander  
USAEHA - DSA West  
Fitzsimmons Army Medical Center  
Aurora, CO 80045-5001  
(303) 361-8090  
Autovon 943-8090

Commander  
U. S. Army Health Services Command  
Fort Sam Houston, TX 78234-6100  
(512) 221-3167/68  
Autovon 471-3167/68

Officer-in-Charge  
NEPMU-2  
Naval Station  
Norfolk, VA 23511-6288  
(804) 444-7671  
Autovon 564-7671/72

Officer-in-Charge  
NEPMU-6  
Box 112  
Pearl Harbor, HI 96860  
(808) 471-9505  
Autovon 430-0111 Ext 471-9505

Commanding Officer  
Naval Environmental Health Center  
2510 Walmer Ave  
Norfolk, VA 23513-2617  
(804) 444-4657  
Autovon 564-4657

Commander  
USAPACEHEA - Sagami  
APO San Francisco 96343-0079  
9426-51-1520 Ext 228-4835  
Autovon 228-4835/5113

Commander  
USAEHA - DSA South  
Fort McPherson, GA  
  
(404) 752-2564/3984  
Autovon 572-2564/3984

Commander  
USAEHA  
Aberdeen Proving Ground, MD  
21010-5422  
(301) 671-4131  
Autovon 584-4131

Officer-in-Charge  
NEPMU-5  
Naval Station, Box 143  
San Diego, CA 92136-5143  
(619) 556-7070  
Autovon 526-7070

Officer-in-Charge  
DVECC  
NAS, Box 42  
Jacksonville, FL 32212-0043  
(904) 772-2424  
Autovon 942-2424

Officer-in-Charge  
NEPMU-7  
FPO New York 09521-4200  
Autovon 625-4468  
39(81) 724-4468/69

Officer-in-Charge  
DVECC  
Naval Air Station - Bldg. 130  
Alameda, CA 94501-5039  
(415) 263-2806  
Autovon 993-2806

**APPENDIX I - DEPARTMENT OF DEFENSE AND OTHER FEDERAL AGENCIES  
PROVIDING LYME DISEASE TRAINING INFORMATION**

USAF School of Aerospace Medicine/EK  
Brooks AFB, TX 78235-5301  
(512) 536-3471  
Autovon 240-3471

Insects Affecting Man and Animals Lab  
USDA, ARS, BOX 14565  
Gainesville, FL 32604  
(904) 374-5900  
FTS 974-7500

Bacterial Zoonoses Branch,  
Division of Viral Diseases  
Centers for Disease Control  
Fort Collins, CO 80522  
(303) 221-6400  
FTS 330-6430

## APPENDIX J - LYME DISEASE FACT SHEET

### FACT SHEET

### LYME DISEASE

Acknowledgement: This Fact Sheet is taken, in most part, from a Public Health Fact Sheet Produced by the Massachusetts Department of Public Health.

#### WHAT IS LYME DISEASE (LD)?

Lyme Disease is an infectious disease syndrome that often begins with a characteristic rash, and which can later involve the joints, nervous system and/or heart. It is caused by a spiral-shaped bacterium called a spirochete that is transmitted to humans by the bite of an infected tick.

#### WHERE IS LD FOUND?

In 1975, an investigation of geographic clustering of children with arthritis in Lyme, Connecticut led to the description of this newly recognized disease. It is now apparent that LD occurs over wide areas of the United States. These areas correspond to the distribution of the known tick species that carry the disease. Currently, the major affected areas are the Northeast from Massachusetts to Maryland, the Midwest in Wisconsin and Minnesota, and the West in California and Oregon. Cases have been reported in other states, however, as well as in Europe, Asia and Australia.

#### HOW IS LD TRANSMITTED?

In the Northeast and Midwest, the "deer tick" or "bear tick" (known by the scientific name, *Ixodes dammini*), and in the West, the western black-legged tick (*Ixodes pacificus*), are the known tick transmitters (or vectors) of the disease. In either case, the tick is very small (i.e., much smaller than the well known dog tick, *Dermacentor variabilis*, or the Lone Star tick, *Amblyomma americanum*), and the immature stages are no larger than the period on a printed page. It is currently unclear whether other ticks may be involved in transmission of LD. The ticks cling to vegetation and are most numerous in a bushy, wooded, or grassy habitat. The tick's two year life cycle requires that the tick feed (take a blood meal) on three separate hosts. These hosts include a variety of animals, including birds, but white-footed mice and deer are preferred. The spirochete that causes LD, *Borrelia burgdorferi*, is acquired by juvenile ticks (larvae) that feed on an infected animal, usually is a mouse. The next juvenile stage of the tick (nymph), attaches to vegetation and is transferred by direct contact to the skin of a passing animal or human. The bite of the infected nymphal tick can then transmit the infectious organism to the new host. Thus, the greatest chance of becoming infected by the bite of the tick occurs while walking barelegged through brush or tall grass May through August, the period of greatest nymphal tick activity in most areas. The adult tick feeds mainly on deer but may also become attached to, and infect humans. It is important to remember that not all ticks carry Lyme Disease. Thus, a tick bite does not necessarily mean that disease will follow, and prompt removal of a tick will lessen any chance of disease transmission.

#### WHAT ARE THE SYMPTOMS?

Early - The first symptom of LD is usually a skin rash, called erythema chronicum migrans (ECM), that occurs at the site of the tick bite. The actual tick may go unrecognized. The rash, which begins 3 to 32 days after the tick bite, begins as a small red area which gradually enlarges, often with partial clearing in the center of the lesion so that it resembles a donut or bulls-eye. There may be multiple secondary lesions. The skin lesion is occasionally described as burning or itching. A small number of people with LD may not have the early skin rash, and symptoms may appear only in the later stages of the disease. Other skin signs include hives, redness of the cheeks and under the eye (malar rash), and /or swelling of the eyelids with reddening of whites of the eyes.

These skin signs may be accompanied by flu-like symptoms such as fever, headaches, stiff neck, sore and aching muscles and joints, fatigue, sore throat and swollen glands. If not treated, these symptoms may disappear on their own over a period of weeks; however, the rash may recur in about 50% of untreated people, and more serious problems may follow later. If treated with appropriate antibiotics, the skin rash goes away within days, and complication may be avoided.

**Late** - There are three major organ systems involved in later stages of the disease - the joints, the nervous system, and the heart. These can become affected weeks to months after the initial symptoms, although symptoms typically appear 4 to 6 weeks after the initial tick bite.

Symptoms in the joints occur in up to 60% of untreated persons. This is an arthritis affecting the large joints, primarily the knee, elbow and wrist, which can move joint-to-joint, and can become chronic.

Neurologic complications occur in 10-20% of infected persons. The most common symptoms include severe headache and stiff neck (aseptic meningitis), facial paralysis (or other cranial nerve palsies), and weakness and/or pain of the chest or extremities (radiculoneuritis). These symptoms can persist for weeks, often fluctuate in severity, and may respond to intravenous antibiotics.

Heart symptoms occur in 6-10% of infected persons. The electrical conduction in the heart may be affected and an inflammation of the heart muscle (myocarditis), or heart block may occur.

## **HOW IS LD DIAGNOSED?**

Diagnosis is based primarily on recognition of the typical symptom of LD such as the characteristic skin rash occurring in a person who lives in or has visited one of areas mentioned earlier. **PROMPT TREATMENT OF EARLY SYMPTOMS MAY PREVENT LATER AND MORE SERIOUS PROBLEMS.**

Atypical cases, or cases presenting with only later stage complications, are difficult to diagnose. In these persons, a blood test looking for antibody to the causative bacteria is often helpful. It should be noted that early in the disease, this blood test can be negative even though disease is present; only with later disease does the test become reliably positive.

## **WHAT IS THE TREATMENT?**

Oral antibiotic treatment is beneficial early in the illness and often prevents late complications. Tetracycline appears to be the most effective drug. In children under 7 years, penicillin is the drug of choice (tetracycline can stain the enamel of developing teeth in children). In penicillin-allergic persons, erythromycin can be substituted, although it may be less effective.

## **HOW CAN LD BE PREVENTED?**

The only known way to get LD is from a bite from an infectious tick. Knowledge of where these ticks are found, avoidance of such areas, and, if bitten, prompt removal of the tick, are the primary preventive measures. Persons living in areas where ticks are prevalent, particularly if the known tick vector species is present, should be aware of the following preventive measures:

Don't walk barelegged in tall grass, scrubby areas or woods where the ticks may be found.

If you do walk in such areas, wear a long-sleeved shirt, long pants, and high socks (with pants tucked tightly into the socks). Light colors will help the recognition of tick on clothing.

Conduct daily "tick checks". The ticks are most often found on the thigh, flank, arms, underarms, and legs, and are very small. Look for new "freckles".

To remove a tick, use tweezers to firmly grip the tick's mouthparts as close to the skin as possible, and pull it straight outward. If using fingers, place a protective covering between your

fingers and the tick, and wash your hand afterward. Apply an antiseptic to the bitten areas.

After removing, destroy the tick by immersing it in alcohol. Save the tick in the event that symptoms arise and identification of the tick becomes necessary.

Be aware of the symptoms of Lyme Disease. IF YOU HAVE BEEN IN AN AREA WHERE THE TICK IS FOUND AND YOU DEVELOP SUCH SYMPTOMS, PARTICULARLY THE SKIN RASH AND/OR 'FLU' SYMPTOMS DURING THE PERIOD FROM MAY THROUGHOUT EARLY FALL, YOU SHOULD PROMPTLY SEE A PHYSICIAN FOR EVALUATION AND TREATMENT.



## APPENDIX K - REFERENCES\*

1. Steere, A.C., Malawista, S.E., Hardin, J.A., Ruddy, S., Askenase, P.W. and Andiman, W.A. 1977. Erythema chronicum migrans and Lyme arthritis: the enlarging clinical spectrum. *Ann. Intern. Med.* 86: 685-698.
2. Burgdorfer, W., Barbour, A.D., Hayes, S.F., Benach, J.L., Grunwaldt, E. and Davis, J.P. 1982. Lyme Disease - a tick-borne spirochetosis? *Science* 216: 1317-1319.
3. Johnson, R.C. et. al. 1984. *Borrelia burgdorferi* sp.nov.: etiologic agent of Lyme disease. *Int. J. Syst. Bacteriol.* 34: 496-497.
4. Magnarelli L.A. 1989. Quality of Lyme disease tests. *JAMA* 262: 3464-3465.
5. Fox, J.L. 1989. Interest in Lyme disease grows. *ASM News* 55: 65-66.
6. Hamilton D.R. 1989. Lyme disease: the hidden pandemic. *Postgraduate Medicine* 85: 303-314.
7. Barbour, A.G. 1988. Laboratory aspects of Lyme borreliosis. *Clin. Microbiol. Rev.* 1: 399-414.
8. Treatment of Lyme Disease. 1989. *Med. Lett. Drugs. Ther.* 31: 57-59.
9. Falco, R.C. and Fish, D.J. 1988. Ticks parasitizing humans in a Lyme disease endemic area of southern New York state. *Amer. J. Epidem.* 128: 146-1152.
10. Falco, R.C. and Fish, D.J. 1989. Potential for exposure to tick bites in recreational parks in a Lyme disease endemic area. *Amer. J. Public Hlth.* 79: 12-15.
11. Levine, J.F., Wilson, M.L. and Spielman, A. 1985. A mouse reservoir of the Lyme disease spirochete. *Am. J. Trop. Med. Hyg.* 34: 355-360.
12. Matuschka, F.R., Spielman, A. 1986. The emergence of Lyme disease in a changing environment in North America and Central Europe. *Exper. and App. Acarology* 2: 337-353.
13. Spielman, A., Wilson, M.L., Levine, J.F. and Piesman, J. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu. Rev. Entomol.* 30: 439-460.
14. Magnarelli, L.A., Anderson, J.F., Apperson, C.S., Fish, D.J., Johnson, R.C. and Chappell, W.A. 1986. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York State and North Carolina. *J. Wildlife Dis.* 22: 178-188.
15. Bequaert, J.C. 1945. The ticks, or Ixodoidea, of the northeastern U.S. and eastern Canada. *Entomologica America* 25: 144-147.
16. Hooker, W.A., Bishop, F.C. and Wood, H.P. 1912. The life history and bionomics of some North American ticks. *USDA Bull.* #106.

17. Walter, G. and Liebisch, A. 1980. Untersuchungen zur Biologie und Verbreitung von Zecken (*Ixodoidea*, *Ixodidae*) in Norddeutschland. III. *Ixodes ricinus* (L.) Z. Angew. Zool. 67: 449-476.
18. Gray, J.S. 1985. A carbon dioxide trap for prolonged sampling of *Ixodes ricinus* L. populations. Exper. and Applied Acarology 1: 35-44.
19. Bukowski, J.A. 1988. Lyme Disease: A tick-borne threat to people and pets. Veterinary Medicine, April, pp 346-358.
20. Keirans, J.E. and Litwak T.R. 1989. Pictorial key to the hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi river. J. Med. Entomol. 26: 435-448.
21. Schreck, C.E., Snoddy, E.L., and Spielman, A. 1986. Pressurized sprays of permethrin or DEET on military clothing for personnel protection against *Ixodes dammini* (Acari: Ixodidae). J. Med. Entomol. 23: 396-399.
22. Eng, T.R., Wilson, M.L., Spielman, A. and Lastavica. 1988. Greater risks of *Borrelia burgdorferi* infection in dogs than in people. J. Infec. Dis. 158: 1410-1411.
23. Anonymous. 1988. Contingency Pest Management Pocket Guide, 3rd Ed., Technical Information Memorandum #24, Armed Forces Pest Management Board, Washington, D.C., 69pp.
24. Barnard, D.R., Mount, G.A., Koch, H.G., Haile, D.G. and Garris, G.I. 1988. Management of the Lone Star tick in recreation areas. U.S.D.A. Handbook 682, 33pp.
25. Prepared by the Agricultural Extension Committee on Deer Damage and Control: Baugher, T.A., Carcaterra, S.M., Davidson, W.R., Grafton, W.N., McConnell, T.R., Selders, A.W., Williams, C.E., and Workman, D.J. 1985. 6-Wire Vertical, High-Tensile, Electric Anti-deer Fence. Publication #814, West Virginia University, Cooperative Extension Service, Morgantown, W. Va.

\* LYMCITE is a comprehensive database of the most important citations relevant to Lyme disease from 1902 to the present. It is available free upon written request on letterhead from:

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