Tickborne Infectious Diseases

Diagnosis and Management

edited by
Burke A. Cunha

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Winthrop-University Hospital, Mineola, and
State University of New York School of Medicine
Stony Brook, New York

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Foreword

The identification of emerging infectious diseases is a new and growing field. There are many newly described agents that are transmitted by tick bite, including those that cause Lyme disease, rickettsioses, ehrlichioses, and several viruses. Ticks play a major role as the vector, and sometimes reservoir, of disease agents. The infectious agent usually comes to interact with human beings accidentally because the prevalence of tick-transmitted diseases is dependent on multiple causal factors. This explains why the geographical distribution of tickborne diseases remains limited, as ticks are specifically adapted to particular flora and fauna. The purpose of *Tickborne Infectious Diseases: Diagnosis and Management* is to condense in a single book different approaches and paradigms of tickborne infectious diseases. Three chapters are devoted to background information, including the natural history of ticks, the diagnostic procedures of tickborne diseases, and the new tick-transmitted diseases. Then four chapters are devoted to Lyme disease, four to viral diseases, two to rickettsial diseases, and one to parasitic tickborne disease.

This book gives an overview of all the traditional and new findings on tickborne infectious diseases by authorities on all aspects of the subject, and should be useful to clinicians interested in understanding this rapidly moving field.

Didier Raoult
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Preface

Tickborne infectious diseases are of worldwide importance. There is an increase in recognition as well as in the incidence of infections derived from ticks. Some infectious diseases acquired from ticks are common, e.g., Lyme disease, and several can be life-threatening, e.g., Rocky Mountain spotted fever (RMSF). Lyme disease is most frequently encountered with much diagnostic and therapeutic confusion. For this reason, Lyme disease is covered in this book in more detail than other tickborne infections.

Tickborne infectious diseases are among the most interesting and potentially devastating infections of mankind and may be viral, bacterial, or rickettsial. The tickborne rickettsioses include some of the most interesting infectious diseases, which fortunately are treatable, e.g., RMSF, ehrlichiosis, typhus, and Q fever. The tickborne encephalitides are distributed worldwide and include such illnesses as Powassan encephalitis and Russian spring-summer encephalitis. Some of the important hemorrhagic fevers are tickborne: included in this group are Crimean-Congo hemorrhagic fever, Kyasanur Forest disease, and Omsk hemorrhagic fever. Lastly, other tickborne infectious diseases include relapsing fever, tick paralysis, tularemia, and Colorado tick fever.

Tickborne infections constitute a broad spectrum of complicated and dangerous diseases that vary in their vectors and clinical manifestations and are easily confused with a wide variety of other infectious and noninfectious diseases. *Tickborne Infectious Diseases: Diagnosis and Management* includes the differential diagnosis of many illnesses, and it is the only single-source text on the diagnosis and management of tickborne infectious diseases.

The need for a separate book on this subject arose from the increase in the

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number of tickborne infections that have affected the human population worldwide. Some of the information contained in this book is available in many other sources, but no single-source publication has previously put all of this information together for the use of clinicians dealing with tickborne infectious diseases. In addition to the classic zoonoses, comprehensive coverage of babesiosis and ehrlichiosis is included here. Tickborne encephalitis and tickborne hemorrhagic fevers, which are a constant threat to the human population, are also reviewed.

Each contributor was selected for his expertise in an area of tickborne transmitted infections. They come from many academic disciplines and range from basic scientists to clinical academicians. This single-source book should provide a handy reference to all clinicians dealing with tickborne infectious diseases.

Burke A. Cunha

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1

The Natural History of Ticks: A Human Health Perspective

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Harvard School of Public Health, Boston, Massachusetts

The rapid proliferation of ticks in the evolving landscapes of eastern North America and Europe commands increasing public attention. Many wooded sites that once nurtured the carefree traveler are now regarded as a threat to our health and peace of mind. Publicity concerning the recent emergence of Lyme disease, in particular, causes people to demand protection from ticks and fear the forest edge. This trend dates back only to the early 1980s. This chapter, therefore, is designed to acquaint medical practitioners with the natural history of vector ticks and provide them with a basis for understanding the epidemiology of tickborne infections.

STRUCTURE

A tick is a large, hematophagous mite with a dorsoventrally flattened, sack-like body. Its eight legs retract by muscular action but depend on hydrostatic pressure to extend. Its mouthparts, concealed by hinged palps, consist of a pair of articulated, retractable chelicerae that serve to penetrate the skin of a vertebrate host and of a multitoothed hypostome that is drawn into the resulting wound, thereby anchoring the feeding tick in place. Certain ticks have a pair of simple but remarkably effective eyes embedded in their dorsum, and all have an array of olfactory setae, including specialized structures arranged in pits near the apices of the anterior pair of legs. Within the body cavity, the tube-like gut, reproductive tract, and a ganglionic mass hang suspended in the hemolymph. Respiratory function

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is provided by an ectodermally derived system of ramifying tubules that open ventrally in a prominent pair of ornately perforated plates.

Ticks fall into two general taxonomic categories that differ radically in form, pattern of development, behavior, and disease relationships. The body of the soft, or argasid, ticks is leathery, whereas that of the hard, or ixodid, ticks remains rigid until they begin to gorge with blood. The feeding apparatus of soft ticks lies ventrally, concealed from above by the carapace. The feeding apparatus of hard ticks projects prominently from the anterior margin of the body. Soft ticks develop through numerous nymphal molts. Hard ticks, in contrast, molt only twice; the egg stage gives rise to a six-legged larva, which develops into an eight-legged nymph which in turn molts to produce a similar but larger adult. Adult soft ticks feed and oviposit repeatedly, but hard ticks do so only once. With the exception of certain ornithophilic species, soft ticks generally are restricted to arid parts of the world. The distribution of hard ticks is more cosmopolitan. Soft ticks feed briefly while their hosts sleep; hard ticks remain attached for days. Any tick that attaches persistently to a person will be a hard tick. Soft ticks mainly transmit the endemic relapsing fever spirochetes, including *Borrelia duttoni* and *Bo. hermsii*, but the microbial repertoire of hard ticks is vast and varied. The discussion that follows, therefore, will be restricted to hard ticks, and the word ''tick'' applied solely to members of this group of ectoparasitic arthropods.

Four kinds of ticks, belonging to four genera, are most frequently encountered by medical practitioners in the northern temperate parts of the world. Those in the *Ixodes ricinus* complex of species, including the taiga ticks and wood ticks of Eurasia and deer ticks of North America, attack people as larvae, nymphs, or adults. They attach firmly. Before they begin to engorge, these ticks are a lustrous black; the posterior dorsum of the female is red (Fig. 1). The much larger American dog tick, *Dermacentor variabilis*, attaches lightly to people or dogs and only in the adult stage. The dorsum of these ticks is dark brown ornamented with white. The American lone star tick, *Amblyomma americanum*, attacks people in all three of its trophic stages. It attaches firmly. This oval-bodied tick is black with a central white spot, its ''lone star.'' Brown dog ticks, *Rhipicephalus sanguineus*, frequently attack people in southern Europe but almost never in the Americas. These ticks sometimes ''paint light brown'' the lower walls of kennels and of dog owners' homes.

FEEDING

Vertebrate blood is a tick's sole source of nutriment. Growth progresses stepwise, punctuated by a series of molts and terminating with mating and the formation and deposition of eggs. Each developmental or reproductive event requires previous contact with a host and the imbibition of blood. Vast quantities of blood are ingested during the several days that a tick retains host contact; its mass may

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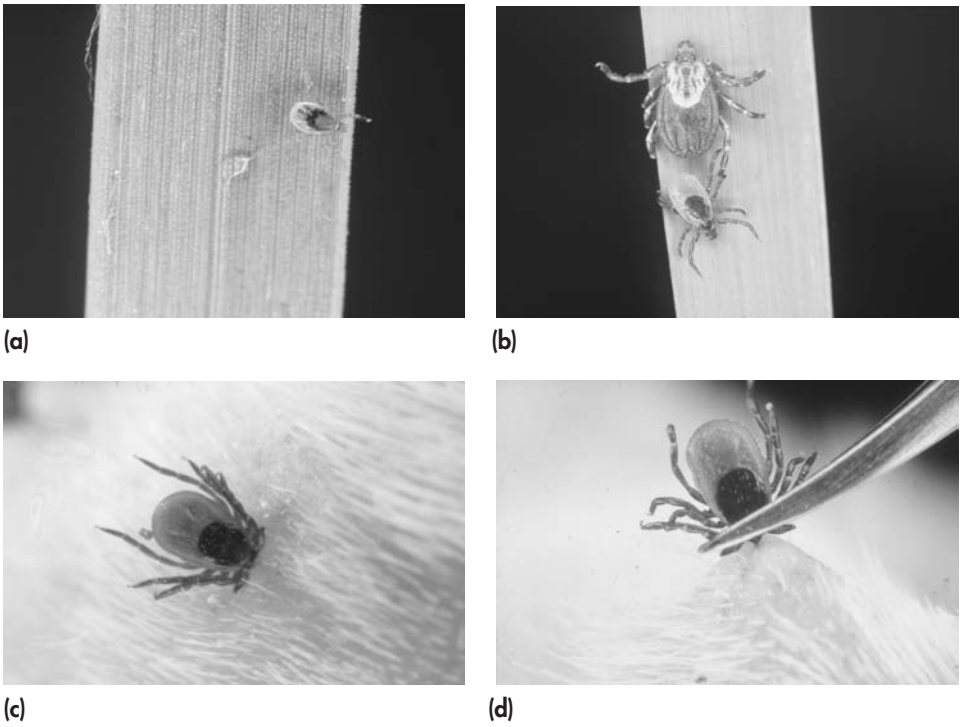

FIGURE 1 Deer ticks (*Ixodes dammini*) transmit the Lyme disease spirochete (*Borrelia burgdorferi*) in the northeastern and north central United States. The same ticks also transmit the agents of human babesiosis (*Babesia microti*) and human granulocytic ehrlichiosis (*Ehrlichia microti*). As adults they parasitize deer, but subadult deer ticks acquire pathogens as they feed on infected mice. (a) The nymphal deer tick (shown on a blade of grass) is only about the size of a poppy seed, so its attachment to a person often goes undetected. Nymphs generally feed on mice, but this developmental stage is also that which most often transmits the agent of Lyme disease to humans. As nymphs rest on the margins of low vegetation between early May and mid-July, they may be brushed against by a passing mouse or a person's feet and may attach and feed for a few days. (b) For comparison, an adult dog tick (*Dermacentor variabilis*) is shown above the smaller adult female deer tick. The latter is the size of an apple seed, and its posterior portion is reddish-orange in color. Adult-stage deer ticks quest for hosts during winter months, resting on grass or brush at the height of a person's knees. (c) Adult deer ticks insert their mouthparts deep into the skin of their hosts and may feed for a week or more. (d) As soon as they are discovered, nymphal or adult ticks should be gripped with forceps near the point of attachment and gently pulled loose. Stepping on ticks may not kill them. Taping them instead to a piece of paper ensures that they will not seek out another host. Such a specimen card, labeled with the date and the part of the body to which the tick was attached, may prove useful to doctors should symptoms of a tickborne illness appear. (Courtesy of Dr. Franz-Rainer Matuschka, Charité, Humboldt-Universität zu Berlin.)

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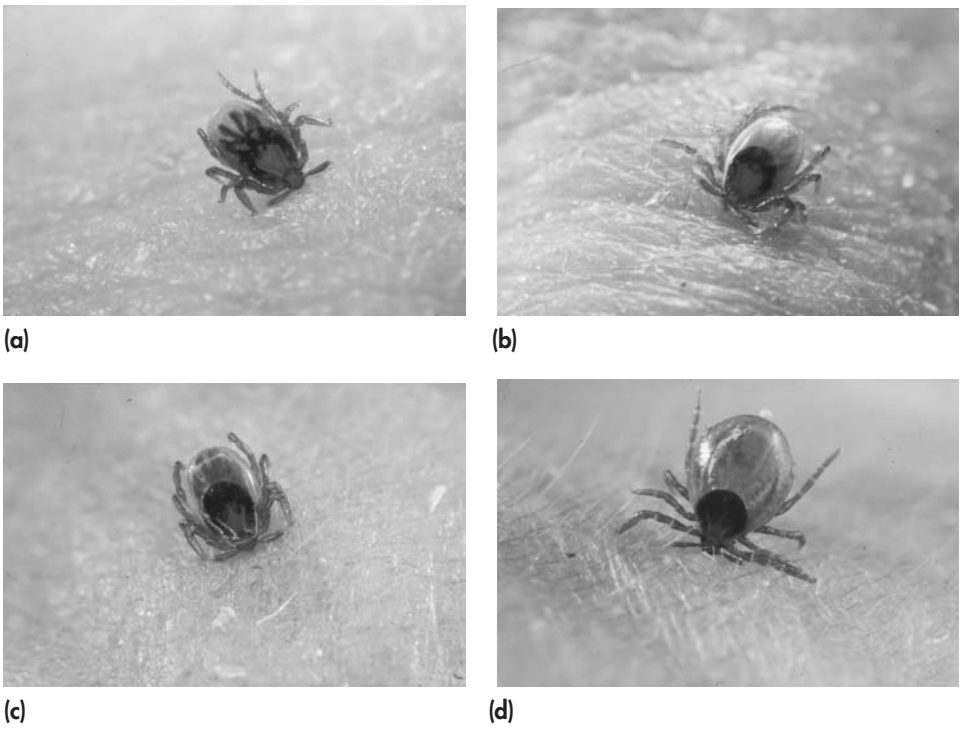

FIGURE 2 The appearance of a nymphal *Ixodes dammini* tick after (a) 12 hours, (b) 1 day, (c) 2 days, and (d) 4 days of attachment. (Courtesy of Dr. Franz-Rainer Matuschka.)

increase several hundredfold with the most rapid expansion occurring just before detachment, during the ''big sip'' (Fig. 2). A series of hormonally mediated changes occurs in the body of the feeding tick during the first day or so of host contact; the rigid body wall becomes plastic and unfolds accordionlike to accommodate the mass of blood being ingested. Massive diuresis follows, with the salivary glands functioning as kidneys to return water to the host. Like an aphid feeding on a plant, the tick ''urinates'' copiously in this manner, thereby protecting the host from dehydration. The tissues of the replete tick ultimately stretch envelopelike around the compacted mass of ingested blood.

Ticks digest their blood meals intracellularly. Enzyme sufficient to digest, as a unit, such a large quantity of blood could not derive from the tick's relatively minute tissue mass. The gut wall, however, includes cells that progressively engulf bits of the congealed mass of blood. Digestion proceeds inwardly and may take weeks.

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The formation and nature of the feeding cavity that a tick creates within the skin of its hosts is poorly understood. This venue of interaction between vector ticks, any microbes they may carry, and the animal destined to serve as host for these pathogens remains a partial mystery. It is known that hemostasis within the cavity is prevented by the secretion of an elaborately integrated salivary pharmacopeia. Platelet activation, the coagulation cascade, and pain are blocked by specific enzymatic action. Anti-inflammatory products of the salivary glands quiet specific components of the host's local immune response so that blood flows readily through the feeding cavity and into the attached tick. No proteolytic enzymes capable of forming such a cavity, however, have been identified. Ill-adapted ticks that attach to the ''wrong'' kind of host tend to be rejected, particularly after the host has had repeated contact with similar ticks.

HYDRATION

In addition to blood, ticks ingest water, but they do so from the atmosphere. Ticks dehydrate when perched on vegetation and their legs retract because of loss of hydrostatic pressure, which causes them to fall to the relatively moist ground. To drink, they secrete a hygroscopic salt onto their mouthparts, reingesting the mass after a period of hydration. Water is removed and the salt is repeatedly reprocessed. Immersion in free water may waste this hygroscopic salt. A tick's ability to drink from the air improves with increasing temperature, and certain kinds of ticks are more capable of rehydrating in cooler air than others. A cold, dry winter tends to destroy ticks, particularly in the absence of snowcover.

QUESTING BEHAVIOR

Many ticks quest passively, lying in wait for a host to brush against them. They mount on vegetation to a height commensurate with the stature of their preferred hosts. Adults in the *I. ricinus* complex of species, including the main vectors of the agent of Lyme disease, ascend a meter or so on brushy vegetation to about the height of a deer's body; subadults tend to cling to fallen leaves where a mouse is likely to encounter them. These eyeless ticks do not move laterally to any great extent. Instead, they position themselves on the undersides of twigs or leaves with their forelegs protruding as they wait in ambush for contact with a passing animal. Although the questing process appears to be entirely passive, a degree of host specificity in retaining contact is evident. In North America, for example, deer ticks tend to discontinue their feeding contact with cats, detaching prematurely. Subadults are found feeding more often on mice than on voles. Such selectivity by ticks varies with age: Nymphal deer ticks are far more host specific than are larvae, and although 3.5 times as many larval as nymphal deer ticks feed on American white-footed mice, this ratio approaches 20:1 on European

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yellow-necked mice. *I. dammini*, the deer tick that transmits the Lyme disease spirochete in the northeastern United States, readily attaches to people (and to deer) in all three of its trophic stages. In contrast, the closely related but morphologically distinct *I. scapularis*, which is found in the extreme southeastern United States, only attaches to people in its adult stage, and subadults are far more likely to feed on lizards than on mice. Lone star ticks, *A. americanum*, also feed mainly on deer and do so during each developmental stage. Although these annoying pests frequently feed on people, they seldom, if ever, attack rodents. They might serve, therefore, as vectors of deer-associated but not mouse-associated pathogens. Numerous factors contribute to host specificity among ticks.

Although American *D. variabilis* dog ticks, the main vector of the rickettsial agent of Rocky Mountain spotted fever (RMSF), mainly quest passively, they move great distances in response to a source of carbon dioxide, crawling a kilometer or more toward windborne fumes emanating from automobiles. To these ticks, an automobile probably smells like a dog. The leeward sides of parking lots or highway margins, therefore, provide an important setting in which these potentially dangerous ticks attain contact with people and dogs. Traps, baited with dry ice, facilitate efforts to monitor the density of these ticks. Functional eyes may aid them in their travels. Dog ticks in the subadult stages feed mainly on voles, seldom on mice, and never on people or dogs.

Certain ticks are startlingly aggressive. The Old World *Hyalomma* vectors of Crimean-Congo hemorrhagic fever, for example, raise their bodies on their extraordinarily long legs and run toward dark, moving objects. These sharp-eyed, ''cursorial'' ticks generally parasitize ungulate hosts.

Other ticks, in contrast, seldom disperse. The *Ixodes* ticks that feed on American groundhogs and European hedgehogs, for example, are strictly nidicolous. They quest and attach underground in the host's nest, becoming replete over a period of days as the host moves about to forage and detaching in the nest toward the end of the host's period of rest.

REPRODUCTION

Few ticks reproduce parthenogenetically. They generally must mate before their bodies become plastic, permitting them to feed to repletion. Perprandial mating (during feeding) is the general rule. *D. variabilis* dog ticks, for example, mate after a preliminary period of feeding, the male being stimulated and guided to the feeding female by a pheromone she emits. The male then appresses his venter to that of the female, and extrudes from his genital pore a delicate spermatophore which he places within the genital opening of the female via his mouthparts. The endospermatophore everts, thereby delivering sperm into the female's oviduct. After the female engorges to repletion, sperm remain viable in the reproductive

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tract through the several months required for vitellogenesis, finally penetrating the mature eggs just before deposition.

Only *Ixodes* ticks are capable of mating before they attain host contact, and certain of these never mate while feeding. For example, the New World mouse tick, *I. muris*, is strictly nidicolous; adult males never leave the nests of their rodent hosts and never ingest blood. After mating in the nest, adult females engorge on their hosts and detach in the same site. *I. dammini* deer ticks can mate both pre- and perprandially, with the female accepting spermatophores from more than one male. Male deer ticks only feed transiently, never becoming gorged with blood.

Vitellogenesis commences after the bloated female tick detaches and falls from the host. She digests her meal of blood while sheltered in a relatively humid site, converting it into an enormous egg mass. Such masses can contain tens of thousands of eggs. Each egg is coated with a viscous fluid secreted by a pair of erectile organs that evert from the anteriodorsal margin of the body. The shriveled hulk that remains of the female's body cannot return to its virginal form; death soon follows.

DEVELOPMENT

A unique ability to endure long periods of food deprivation complements a tick's exclusively parasitic mode of life. Certain desert-dwelling soft ticks, for example, survive a decade or more in an apparent state of ''suspended animation'' while awaiting the return of a suitable host. They lie motionless in relatively protected sites, and the pathogens they transmit similarly suspend development in order to spare the energy reserves of these vectors. Such dormant ticks reanimate rapidly once they sense the presence of a suitable host.

Ticks generally detach from their hosts after gorging on blood. The final, particularly rapid phase of engorgement, the ''big sip,'' commences at a particular time of day such that the time of detachment is constant but varies with host species. Those that separate from their host after each feeding are designated as three-host ticks. Certain ticks, however, retain contact with their hosts after they have become replete; they molt there and begin to feed once again on the same host animal. The Texas cattle fever tick, *Boophilus microplus*, illustrates this one-host developmental cycle. Other ticks feed in a corresponding two-host pattern.

The seasonal cycle of certain ticks is regulated closely, probably signaled by daylength. That of *I. dammini* deer ticks of eastern North America, for example, is closely integrated with the cycle of seasons. Their larvae hatch early in the month of August and begin to quest for hosts soon thereafter. Questing activity ceases about 2 months later, as autumn approaches. Nymphs that develop from these fed larvae, as well as those unfed larvae that survive the winter, begin to quest once again with the onset of warm weather in May. Resulting adults

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begin to quest in October and continue to do so throughout the winter, as the weather permits. The eggs they deposit generally hatch 2 years after those of the previous generation. This precisely punctuated 2-year cycle may extend to 3 years when hosts are scarce, and occasionally is compressed into a single year in the presence of a surfeit of hosts. Interestingly, other ticks often lack such a structured seasonal pattern of activity. All three trophic stages of the sibling species of *I. ricinus* wood ticks of Europe, for example, are active simultaneously throughout the warm months of the year. In North America, all trophic stages of *D. variabilis* dog ticks and *A. americanum* lone star ticks feed during May and June. *D. albipictus* winter ticks, which are one-host ticks, begin to feed on deer in the fall, as do adult deer ticks. The seasonal cycles of ticks are complex, defying generality.

PATHOGEN RELATIONSHIPS

Ticks transport numerous pathogens back and forth between their vertebrate hosts. Indeed, Theobald Smith's seminal 1893 work on Texas cattle fever, caused by *Babesia bigemina*, was the first to demonstrate that pathogens could be transmitted between vertebrate hosts via the bites of hematophagous arthropods. Although another tickborne infection, RMSF, which impeded settlement of the Bitter Root Valley of western Montana, initially was attributed to another such ''pyroplasm,'' its rickettsial etiology became established soon after the turn of the 20th century. The list of tickborne pathogens that has accumulated subsequently is long and varied and includes viruses, rickettsiae, diverse bacteria, protozoa, and nematodes.

Until recently, *D. variabilis* dog ticks were perceived as the main vector ticks in North America. They transmit *Rickettsia rickettsii*, the agent of RMSF, a potentially fatal disease. However, in the Rocky Mountain region, where RMSF first was recognized as a threat to human health, this pathogen cycles between *D. andersoni* vector ticks and marmot reservoir hosts. The heavy health burden imposed there at the turn of the century soon diminished as the expansion of agriculture forced the range of marmots to ever-higher altitudes. RMSF later surfaced in coastal parts of North America, where the pathogen cycles mainly between *D. variabilis* dog ticks and voles. Vector ticks also may inherit this microbe. The rising risk of human RMSF results from the popularity of dog ownership, the abandonment of farmland, and a consequently broadened distribution of dog ticks. The distribution of this disease is irregular because pathogenic *R. rickettsii* predominate in certain locations while the related but nonpathogenic *R. montana* are frequent in the same cycle elsewhere. In dormant ticks, RMSF pathogens reside in an inactive state within various tissues, including the salivary glands. The ability to replicate and to infect vertebrate hosts is restored within a day after infected ticks attach to a host and begin to feed. Because RMSF is

so readily diagnosed, and is now so effectively treated, public concern over dog ticks has waned.

The emergence of Lyme disease has elevated public alarm over *Ixodes* ticks to a level never induced by dog ticks. The notorious spirochetal infection, caused by *Bo. burgdorferi* s.l., is transmitted by members of the *I. ricinus* complex of deer and wood ticks. Although Lyme disease originally was noted in Europe, the first clustered cases of human disease were documented in 1975 in North America. The public health impact of *I. ricinus* ticks is exacerbated by an array of cotransmitted infections, including: human babesiosis, caused by *Babesia microti* in North America and *Ba. divergens* in Europe; human granulocytic ehrlichiosis (HGE), caused by *Ehrlichia microti* in North America and *E. phagocytophila* in Europe; and tickborne encephalitis (TBE) in Eurasia, caused by a flavivirus. A related virus may cause human disease in North America. The white-footed mouse, *Peromyscus leucopus*, serves as the main reservoir of these infections in the New World, and various *Apodemus* mice and dormice do so in the Old World. Other rodents, and even birds, also may contribute to the force of transmission. Although certain of these pathogens occasionally vertically pass between tick generations, transmission mainly is horizontally maintained by the subadult stages of these ticks; as larvae, the ticks acquire pathogens from the same varieties of vertebrate hosts they later feed on in the nymphal stage, releasing the pathogens into the host as they engorge. The transmission cycle of TBE virus remains uncertain. Deer ticks are known to transmit certain pathogens among ungulates that generally do not infect immunocompetent people; these include *Ba. odocoilei* of deer and *Ba. divergens* of cattle. Deer tick–associated diseases are increasing because these vector ticks mainly infest the brushy margins of forest, a habitat that is expanding.

Within vector ticks, pathogens survive prolonged interfeeding periods in various ways. The Lyme disease spirochete lies immobile against the microvillar wall of the deer tick's gut where it is coated by an outer surface protein, designated OspA, which is evident only until the infected tick begins to ingest blood. The spirochete then ''changes its coat'' and begins to migrate through the gut wall into the salivary glands of the tick. Transmission commences after about 2 days of attachment. Once delivered to the host, spirochetes remain within the feeding lesion for at least 2 days after detachment of the tick. Babesial piroplasms, in contrast, survive in the tick's salivary glands in an inactive sporoblast stage of development. Sporogony commences soon after the infected tick begins to feed and is completed within 2 days. *Ehrlichia* rickettsiae appear to mature slightly more rapidly. Viable TBE viruses, however, begin to pass into the vertebrate host as soon as the infected tick starts to feed. For most pathogens, we enjoy a prolonged grace period in which a feeding tick can be removed with little risk of acquiring infection.

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ECOLOGY

The capacity of a tick to perpetuate a microbial infection depends on several essential properties:

1. The vector tick must be a competent host for the pathogen. That is, it must be capable of ingesting the pathogen, supporting its subsequent multiplication and development, and delivering an infectious inoculum to a suitably prepared site.
2. The vector tick must focus its bites on suitably competent reservoir hosts. If one of the two relevant feeding events were to be delivered to an unsuitable host, the effect of the other would be negated.
3. The vector tick must attach to a sufficiently sessile host that it will not be transported out of the original transmission site to a site in which the tick population will not thrive.
4. In the absence of inherited infection, at least two developmental stages of the vector tick must feed on a reservoir host.
5. The vector tick population must be sufficiently dense to insure that another such tick will parasitize the reservoir host while it survives and remains infectious.

Together, these biological requirements characterize the vector capacity of ticks.

The capacity of a vertebrate animal to perpetuate a tickborne microbe depends on a complementary battery of essential properties:

1. The reservoir animal must be competent as a biological host to the pathogen. That is, if vulnerable to infection, the animal must be capable both of supporting microbial multiplication and development and of presenting such infectious pathogens to another feeding tick.
2. The reservoir animal must permit vector ticks to feed abundantly, and immunity must not be generated against the tick.
3. The reservoir animal must not be so vagile that the feeding tick is carried away from suitable transmission sites.
4. In the absence of inherited infection, reservoir animals must support the feeding of at least two developmental stages of the vector tick.
5. The population of reservoir animals must not be so dense relative to that of vector ticks that each reservoir is unlikely to be parasitized again while it remains infectious.

These biological requirements of pathogen transmission for reservoir hosts complement those of vector ticks.

Tick density is keyed in large part to the density of the hosts on which the adult stage usually feeds. Successful feeding by subadult ticks merely permits development, whereas that by adults permits enormous multiplication events. *D.*

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variabilis dog ticks become abundant only where dogs are numerous, and *I. dammini* deer ticks become abundant where many deer are present. Although adults of both kinds of ticks may feed on various alternative animals, the presence of a particular definitive host is crucial. Although their rate of proliferation varies with host availability, no other mechanism that limits the carrying capacity of the landscape for ticks has yet been identified. Nor do we know whether tick density is regulated by some feedback mechanism or by catastrophic events. Risk of human infection by tickborne microbes depends on a concatenation of environmental factors, influencing the ecology of pathogens as well as vector and reservoir hosts.

PREVENTION

Tickborne pathogens are vulnerable to a diverse array of interventions. These include immunization, case management, personal protection, and an array of antivector measures.

Vaccines have long been freely available for protection against TBE and RMSF. They appear to be effective and to save lives when appropriately used. Various factors, however, differentiate the people to whom these vaccines should be administered. TBE vaccine should be administered generally to potentially exposed people because the sequelae of the disease may be severe, transmission occurs without a grace period following attachment of infected ticks, treatment merely involves management of symptoms, and the prevalence of infected ticks may be intense. Fewer people are candidates for RMSF vaccination. Although RMSF can be fatal, the disease is readily treated and infected ticks are generally scarce. Anti-TBE vaccination, therefore, should be prescribed for all people visiting particular forested sites in central Europe and Asia, but anti-RMSF vaccination should be reserved for travelers to remote sites who may not have access to medical care. People vaccinated against TBE frequently falsely assume that they are also protected against Lyme disease, a misperception that increases risk. Antitick precautions remain necessary.

The decision to administer the newly approved Lyme disease vaccine rests on several complex issues. As in the case of TBE, risk of contact with a spirochete-infected tick may be intense in certain circumscribed sites. Lyme disease, however, is far more readily diagnosed than is TBE and treatment is simple and effective. An appropriate decision is rendered more difficult by the incomplete efficacy of this vaccine. A three-dose regimen provides only 78% protection and a two-dose regimen 50%. Because the venue of action of this OspA-based antispirochetal vaccine lies within the gut of the tick, no anamnestic response follows contact with an infected tick. Therefore, frequent revaccination, probably an annual boost, seems essential. The continued need for protection against ticks is rendered even more acute by the various coinfecting pathogens to which a vacci-

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nated person remains susceptible. People whose work or recreational activities bring them into sites where transmission is intense should be advised to receive vaccine protection while continuing to protect themselves against ticks. Lyme disease vaccination might not be warranted for the general public, or even for residents of sites in which cases are associated in space and time.

Personal protection measures against infection by tickborne pathogens are straightforward. Long pants should be worn with the cuffs tucked into the wearer's stockings. Light-colored fabrics render ticks more visible, facilitating their removal. Pyrethroid repellents (such as permethrin) applied to clothing add a large measure of protection. A nightly ''tick check'' of one's body is recommended. Ticks, including the poppy seed–sized nymphal deer tick or the apple seed–sized adult, should be promptly removed by gentle traction. A dead attached tick or a fragment remaining in the skin will cause no harm, and squeezing will inject no pathogens. A history of exposure suggesting that a tick could not have been attached for more than 2 days offers reassurance that the grace period for transmission of most pathogens has not expired. In any event, the site of attachment should be monitored during the next 2 weeks to detect the expanding, annular rash that is pathognomonic of Lyme disease. People should avoid exposure to sites likely to be infested with ticks: brush in the case of deer ticks, and meadows or roadsides in the case of dog ticks.

Various environmental antitick interventions can be applied. Most effective is the local destruction of deer. Virtual annihilation, however, is required. Because risk of exposure to deer ticks increases nearly a hundredfold when a person steps from a lawn into the ecotone, wide trails may reduce the risk to people walking through brush. Although brush clearing by burning or mowing effectively reduces long-term risk, a transient increase in risk of human infection may occur during the following season because mowing displaces hosts for subadult ticks, perhaps causing more nymphs to feed on people. Acaricidal sprays massively delivered to a tick-infested location will reduce risk, but an environmental or toxicological price may be paid. Host-targeted acaricides are less intrusive. Tubes containing permethrin-impregnated cotton are distributed across a site. Mice that build their nests from this fiber thereby become coated with acaricide and are rendered ectoparasite free. Because of the tick's 2-year lifecycle, protection is delayed and may be incomplete. (The first such formulation, Damminix, was developed and patented in the author's laboratory. He and his colleagues retain a financial interest in the product.) Various experimental treatments are being evaluated in which acaricides are either applied to or fed to deer attracted to a feeding site. However, the vast quantities of bait needed to attract these animals promotes their abundance and causes them to concentrate in the vicinity of the bait stations. The efficacy of such deer-targeted acaricidal systems remains to be determined.

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CONCLUSIONS

The array of tickborne infections currently emerging in temperate parts of the world increasingly threatens human health. Reforestation in North America and in Europe exacerbates risk. Because tickborne infections are known to be associated with certain landscapes and because protective measures are available, property owners to an ever greater degree may find that they are required to warn and protect visitors. Together, public health interests and the force of common law may compel protective measures.

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Microbiology and Laboratory Diagnosis of Tickborne Diseases

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INTRODUCTION

One of the most difficult tasks for clinicians who treat patients with potential tick-transmitted infections is an inability to obtain reliable and objective laboratory confirmation of clinical impressions. Because tick-transmitted diseases occur at relatively low rates of prevalence and because culture and isolation of the causative agents has been very difficult, laboratorians have been significantly challenged in their attempts to provide diagnostics that are sensitive and specific, timely, and useful to the medical laboratory under routine working conditions. Perhaps the most significant pitfall has been an inability to compare new diagnostic tests using a ''gold standard'' reference that has never existed for most tickborne infections. Thus, the purpose of this chapter is to highlight existing laboratory diagnostics for important and relatively prevalent tickborne infections—Lyme disease, Rocky Mountain spotted fever, the ehrlichioses, and babesiosis—that may assist the clinician in appropriate diagnostic test selection and interpretation of results for tests that may yield suboptimal information in certain situations (Table 1).

LYME DISEASE: *Borrelia burgdorferi* INFECTION

Lyme disease caused by *Borrelia burgdorferi* is a multisystem infection transmitted by *Ixodes scapularis* ticks [1]. Because of its protean manifestations, Lyme

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TABLE 1 Useful Diagnostic Tools for Lyme Disease, Rocky Mountain Spotted Fever (RMSF), Human Monocytic Ehrlichiosis (HME), Human Granulocytic Ehrlichiosis (HGE), and Human Babesiosis

			Laboratory diagnostic tools				
Disease	Causative agent	Phase of illness	Serology*	Microscopy	Culture	PCR	Other*
Lyme disease	*Borrelia burgdorferi* sensu lato	early localized and early disseminated	EIA, WB paired sera, borreliacidal assays, CSF index	skin biopsy silver stain	skin biopsy ± blood	skin biopsy, synovial fluid, CSF	±urine antigen, lymphoproliferation
		late	EIA, WB	?	?	?CSF, synovial fluid	
RMSF	*Rickettsia rickettsii*	active (acute)		skin biopsy, IF, IPx	± blood	± blood	
		convalescent	IFA, paired sera				
HME	*Ehrlichia chaffeensis*	active (acute)	frequently nonreactive	± peripheral blood film examination	± blood	blood	
		convalescent	IFA paired sera				
HGE	*Ehrlichia phagocytophila* group	active (acute)	frequently nonreactive	peripheral blood film examination	± blood	blood	
		convalescent	IFA paired sera				
Babesiosis	*Babesia microti*, WA-1, *Babesia divergens*, MO-1	active (acute)	frequently nonreactive	peripheral blood film examination	± blood animal inoculation	blood	?QBC
		persistent		peripheral blood film examination	± blood animal inoculation	blood	

* EIA, enzyme immunoassay; WB, Western immunoblot; IFA, indirect fluorescent antibody; IF, immunofluorescence; IPx, immunoperoxidase; QBC, quantitative buffy coat.

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borreliosis may mimic other conditions of infectious and noninfectious origin. Characteristically, a skin lesion is observed 10–14 days after the tick bite. The lesion, denominated Erythema migrans (EM), has erythematous and expanding borders that leave a central clear zone. During this stage of the illness patients may present with EM without accompanying symptoms (early localized) or with signs and symptoms attributed to dissemination such as fever, myalgias, arthralgias, headache, or secondary annular skin lesions (early disseminated) [2,3]. Neurological or cardiac manifestations may appear weeks to months after the initial untreated infection and include meningitis, cranial nerve palsies, or heart blocks. Late manifestations of the infection include arthritis, most frequently in the United States, and chronic skin infection (acrodermatitis chronica atrophicans), most prevalent in Europe.

Borrelia burgdorferi is a spirochete, helically shaped, motile, and measuring 20 to 30 μm in length by 0.2 to 0.3 μm width [4] (Fig. 1). Borreliae contain an outer cell membrane that covers the protoplasmic cylinder complex. Unlike other bacteria, the flagella are encased in the periplasmic space between the outer membrane and the protoplasmic cylinder. Unique to Borreliae is the presence of linear plasmids that encode outer surface proteins (Osps) [5]. Of these, OspC and OspA show differential expression between the invertebrate vector and the mammal host, phenomena that have been useful in diagnostic serologic studies and in vaccine development [6,7]. Genetic studies have found that *Borrelia burgdorferi* causing Lyme disease are a genetically heterogeneous group of organisms such that they have been classified in different genospecies. The broad group that contains the genospecies is referred to as *B. burgdorferi* sensu lato. The genospecies are *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*, and they are associated with different clinical manifestations of Lyme disease [8–10].

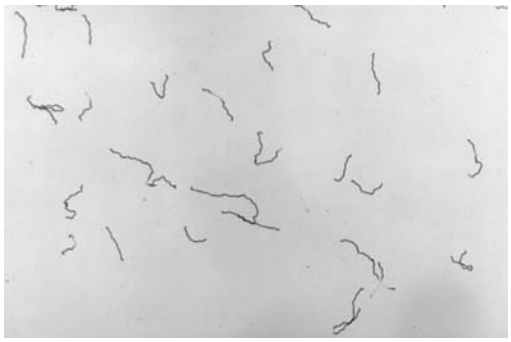

FIGURE 1 ***Borrelia burgdorferi*** **grown in culture and stained with silver stain, under 1,000× magnification. (Courtesy of Dr. G. Wormser.)**

B. burgdorferi sensu stricto predominantly produces EM and arthritis, while *B. afzelii* produces chronic skin lesions and *B. garinii* produces neurological manifestations. While *B. burgdorferi* sensu stricto causes infections in North America, Europe, and Asia, *B. afzelii* and *B. garinii* are mostly restricted to Europe and Asia.

The hallmark of Lyme borreliosis is the characteristic skin lesion EM. Problems in diagnosis arise when EM is not characteristic or absent, which is believed to occur in over 10% of patients [11]. Clinical dilemmas may also occur when patients present when the skin lesion has disappeared and with late manifestations mimicking other disorders. Because of the variable clinical presentations and the lack of reliable confirmatory laboratory methods, chronic and late manifestations attributed to Lyme disease, other than arthritis, are controversial and unsolved issues to date.

Methods for Direct Detection of *B. burgdorferi*

Microscopy

Several studies have reported on the microscopic visualization of spirochetes in different tissues and fluids by using silver stains or fluorescent techniques including acridine orange and immunofluorescence [12–16]. *B. burgdorferi* has most frequently been observed in skin biopsies obtained from the advancing edge of the lesion [13]. The sensitivity of microscopy in EM lesions is variable in different series, but by using a modified silver stain (Bosma-Steiner), deKoning reported a sensitivity of 100% [14]. With the use of microscopic methods, spirochetes have also been seen in other clinical materials such as synovial tissue, cerebrospinal fluid, as well as in infected ticks and different tissues from infected animals.

Molecular Methods

Polymerase chain reaction (PCR) has been the most frequently used method to detect nucleic acid sequences of *B. burgdorferi* in clinical materials as well as in field studies [16–25]. Primer sets used have included those directed at amplifying *ospA*, the flagellin gene, as well as chromosomal sequences. Similar to microscopy, PCR has the highest sensitivity from skin specimens where the diagnosis is already established for the most part on clinical grounds. Melchers et al. reported positive skin PCRs in three of four patients with EM and in four of five with ACA [17]. In a larger series, Schwartz et al. amplified *B. burgdorferi* sequences in 20 of 35 (57%) untreated patients with EM and from two additional cases of nine treated patients [20]. PCR has also been successful in detecting *B. burgdorferi* in CSF from patients with neuroborreliosis [21,22], and in the urine [18,19] and serum from patients with EM [23]. Keller et al. found that CSF PCR

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was positive in 45 of 55 patients (81.8%) with clinical evidence of neuroborreliosis [21]. In this study, CSF PCR was found to have higher sensitivity than intrathecal production of specific antibodies. Luft et al. also found CSF PCR frequently positive in patients with early disseminated Lyme disease, even in patients without CNS symptoms [22]. They concluded that *B. burgdorferi* invade the CNS early in the course of infection.

Of particular importance has been the finding of amplifiable sequences in synovial fluid from patients with Lyme arthritis. Nocton et al. tested 90 synovial fluids collected from Lyme arthritis patients during a 17-year period, and 69 obtained from controls using three sets of *ospA* primers and one chromosomal primer set [24]. They found PCR positivity in 75 of 88 (85%) specimens (two contained PCR inhibitors) and in none of the controls. Those untreated or partially treated patients were more likely to have a positive PCR (96%) compared with those treated for prolonged periods of time (37%). Interestingly, the primer sets with the highest sensitivity were those directed at amplifying *ospA*. This finding is attributed to the shedding of membrane vesicles containing *ospA* segments or to the multiple copies of *ospA*. The investigators concluded that the higher frequency of *ospA* sequences in the synovial fluid of untreated patients as compared with treated patients implies the presence of viable organisms.

Antigen Methods

Studies of experimental and natural animal infection have shown that *B. burgdorferi* infects the bladder and antigens are excreted in the urine [26,27]. Antigens in immune complexes have also been reported in the CSF of patients with neurological symptoms attributed to Lyme disease [28]. A Lyme urine antigen test has been developed to test patients with Lyme borreliosis [29]. However, limited information exists on the clinical utility of this test. The original evaluation showed that 30% of patients with clinical diagnosis of EM had *B. burgdorferi* antigens in urine.

Culture

The first successful in vitro cultivation was achieved in 1982 by Burgdorfer et al. [4]. The spirochete was isolated from the midguts of adult *I. dammini* (*I. scapularis*) ticks collected in Shelter Island, New York after inoculation in modified Kelly's medium [4]. This isolate was cloned by limiting dilution and one of the clones was named B31. To this date, B31 is the most frequently used antigen source in commercial antibody assays. *B. burgdorferi* has complex growth requirements. Kelly's medium, which contains salts, glucose, pyruvate, gelatin, sodium bicarbonate, and n-acetyl glucosamine, was modified by Stoenner who added Yeastolate and CMRL 1066, which may add growth factors. Further modifications (BSKII medium) deleted the glutamine from the CMRL 1066 medium among other changes [30]. The pH of the medium is adjusted to 7.6 and 6%

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TABLE 2 Barbour Stoenner and Kelly II (BSKII) Medium Formulation*

After detergent cleaning, glassware is rinsed in glass-distilled water and autoclaved
100 mL of 10X concentrate of CMRL 1066 without glutamine (GIBCO laboratories) are added to 900 mL of glass-distilled water
To the above add 5g Neopeptone (Difco)
50 g bovine serum albumin, Fraction V (Miles labs)
2 g Yeastolate (Difco)
6 g HEPES (Sigma Chemical Co)
5 g glucose
0.7 g sodium citrate
0.8 g sodium pyruvate
0.4 g N-acetyl glucosamine (Sigma)
2.2 g sodium bicarbonate
Adjust pH of medium at 20–25°C to 7.6 with 1 N NaOH
Add 200 mL of 7% gelatin (Difco) dissolved in boiling water
Sterilize by filtration (0.2 μm nitrocellulose filter) and store at 4°C
Before use add unheated rabbit serum (''trace hemolyzed,'' Pel-Freez biologicals) to a final concentration of 6%
Dispense to glass or polystyrene tubes or bottles. Fill containers to about 90% capacity and cap tightly.
Incubate at 34–37°C

* From Ref. 30.

rabbit serum is added (Table 2). Other modifications have omitted the rabbit serum from the BSKII medium [13]. Once the specimens are inoculated, the tubes are tightly capped to prevent loss of carbon dioxide and incubated at temperatures between 30°C and 37°C for several weeks. The organisms are microaerophilic, and therefore they may grow better at the bottom of the tube. The generation time is about 11 to 12 hours. Growth is detected by removing aliquots of the culture and examining them with dark-field microscopy or fluorescent microscopy using acridine orange or immunostaining.

Cultivation of *B. burgdorferi* from clinical specimens has been most successful from skin specimens collected from the advancing edge of the EM. In one report, *B. burgdorferi* was cultured from skin in 21 of 35 (60%) untreated patients with EM [20]. Berger et al. reported higher isolation from the edge of the lesion (86%) than from the normal perilesional area (57%) in 21 patients with EM [31]. Culture has been less successful from other clinical materials such as blood, CSF, and synovial fluid [1,32–34] where bacteriological confirmation is more desirable. Wormser et al. have pointed out that the yield of blood cultures could be improved by increasing the volume of blood cultured [35]. In that study, the authors found that 9 mL of sera were superior to 9 mL of whole heparinized

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blood in recovering *B. burgdorferi* from blood in patients with early Lyme disease. When 18 mL of serum were cultured, the yield of blood cultures in patients with early disease reached 26.9%. This study showed that patients presenting with multiple EM lesions had more frequently positive blood cultures than those with solitary lesions, suggesting that the former group was more likely to have a higher grade of spirochetemia.

Methods to Detect Host Response to *B. burgdorferi* Infection

Cellular Response

T-cells obtained from peripheral blood or body fluids of patients with Lyme disease have a specific response to *B. burgdorferi* antigens [36,37]. A T-cell stimulation index to *B. burgdorferi* antigens has been used as a diagnostic test in patients with Lyme borreliosis [38]. It has been reported as useful to support the diagnosis in patients who do not develop antibodies after *B. burgdorferi* infection. In this assay peripheral mononuclear cells are first separated by Ficoll-Hypaque density gradient purification. Aliquots containing 10^6 cells/mL are incubated with *B. burgdorferi* antigens in microtiter wells. Controls without antigen are also included. ^{3}H-thymidine is added 18 hours before collection at 7 days of incubation. Stimulation index is calculated by dividing the radioactivity (dpm) of the stimulated wells/radioactivity (dpm) of unstimulated wells. Tests detecting the cell-mediated immunity in *B. burgdorferi* infections are not frequently used as diagnostic tools, and because of their cumbersome nature they are currently being offered by few centers.

Antibody Detection Methods

Matrix-based Assays. Several formats have been used to detect antibodies. Earlier methods included indirect immunofluorescent assay (IFA) and its adaptation on membranes (FIAX). These methods have been for the most part replaced by enzyme immunoassays, which are easier to perform and more amenable for automation. Currently, there are several commercially available enzyme-linked immunosorbent assays (ELISAs) that use whole cell sonicates of B31 as antigen to detect IgG and IgM antibodies to *B. burgdorferi*. Western immunoblot to detect antibodies to different antigens of the organism have also been used and complement the results obtained with ELISA [39–41]. Whereas in the ELISA format antigen mixtures are coated onto polystyrene microwells, in Western immunoblots antigens are first separated by molecular size by electrophoresis and then transferred to membranes. Antibodies bound to the microwells or to the membranes are detected by an enzyme-labeled antihuman immunoglobulin conjugate. After the addition of the substrate to the enzyme conjugate, antibodies bound to the antigens are detected by the development of color. Such color devel-

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opment is measured spectrophotometrically in the case of ELISA or by the appearance of colored bands in the immunoblot. Commonly, ELISA formats detect a mixture of IgM and IgG antibodies whereas immunoblots are formatted to detect them separately.

Several antigens of *B. burgdorferi* elicit antibody response. The most immunodominant during early disease are the flagellar antigen (41-kDa) and the OspC of molecular mass of about 21 to 25 kDa [42–46]. Antigen preparations used in the first generations of immunoassays most likely lacked OspC, because they probably used *B. burgdorferi* highly passed in culture. It is now known that this antigen shows decreased expression in highly passaged cultures. Schwan et al. reported on the different expression of borrelial antigens under different culture conditions including different temperatures [47,48]. This variable expression is also found during transmission of *B. burgdorferi* from the vector to the host explaining the antibody response observed in early disease. OspA is expressed in the midgut of unfed infected ticks, but when these ticks get a blood meal from an uninfected mammal the spirochetes switch the expression to OspC. This switch is probably regulated by changes in temperature (24–37°C) or to factors present in blood. Therefore, when transmission of the agent ensues, the spirochetes are expressing OspC rather than OspA which would explain the higher frequency of antibodies to OspC in early disease. Wilske et al. first described an immunodominant antigen of molecular mass of 22 to 25 kDa (OspC) in European patients with early Lyme disease [49]. Several other studies have confirmed this finding including our own experience [43,50–52]. In a study of 100 patients with clinically defined EM, OspC was the single most frequent immunodominant antigen observed in IgM immunoblots [41]. Similar findings were observed in a study of 46 patients with culture-confirmed EM [50].

Other significant antigens of *B. burgdorferi* during early disease include a

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FIGURE 2 (a) Comparison of Lyme Index values (LIV) by ELISA on sera from patients with culture-confirmed EM with localized or disseminated disease. The graphs show the mean ± standard deviation of the LIV of specimens collected at different intervals from patients with EM of <7 days duration (top) or >7 days duration (bottom). The dashed line indicates the cutoff for ELISA positivity. Note that patients with early disseminated disease have higher LIV than those with localized disease and they remain positive for a longer time. (From Ref. 50.) (b) IgM immunoblots on sequential sera collected from a patient with early disseminated culture-confirmed EM. Lane 1 shows a positive control, lanes 2—8 patient's sera collected at baseline (lane 2), 8 days (lane 3), 20 days (lane 4), 30 days (lane 5), 3 months (lane 6), 6 months (lane 7), and 1 year (lane 8) postbaseline. Note the high number of immunoreactive bands and their persistence on immunoblots performed on posttreatment sera. (From Ref. 50.)

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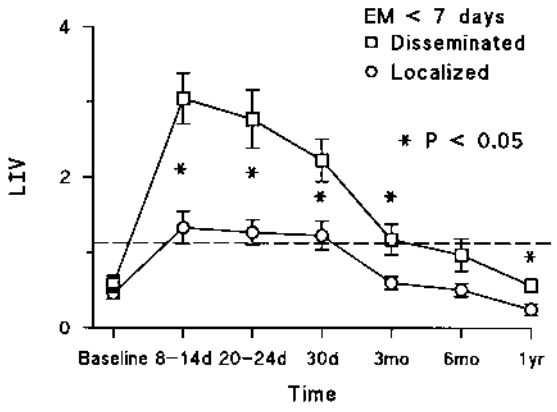
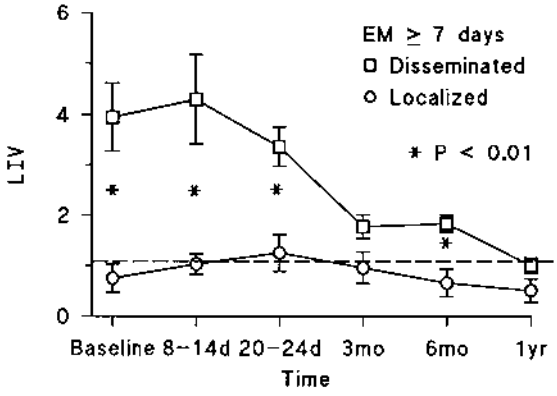
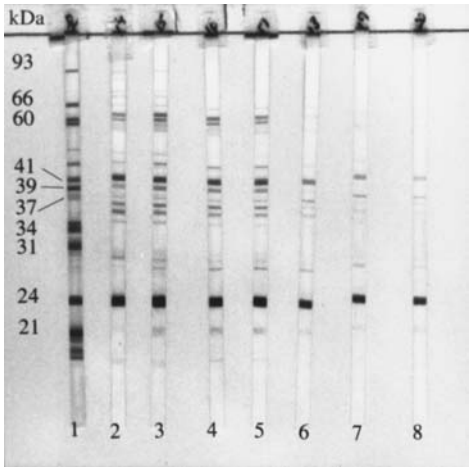




(a)



(b)

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39 kDa (BmpA), 37 kDa, 60 kDa, and 66 kDa. In our experience, IgM reactivity to 37 kDa appears more frequently than to 39 kDa as has been found in other series, which perhaps reflects differences in antigen preparations. Most published experiences using immunoblot to detect *B. burgdorferi* antibodies have used strains others than B31, the most frequently used strain in commercial kits. In fact, the guidelines established after the second national conference on serologic diagnosis of Lyme disease in 1994 adopted the IgG criteria of Dressler et al. using G39/40 strain [43] and the IgM criteria of Engstrom et al. using strain 297 [51]. Reactivity to the 39 kDa antigen is particularly frequent in individuals with longer duration of illness and especially in IgG blots.

Untreated patients with Lyme disease of more than 1 week duration or those presenting with signs of dissemination develop antibodies to a larger number of antigens and higher titers than those presenting with early localized disease (Fig. 2) [50]. Patients developing high titers will also remain positive for a longer time. The sensitivity of serology in early Lyme disease depends on the duration of illness and/or the presence of signs and symptoms of dissemination [50]. We found that 33% and 43% of patients with culture-confirmed EM had a positive ELISA and IgM immunoblot, respectively, during the acute phase. During convalescence about 90% of these patients developed antibodies as determined by ELISA and immunoblot regardless of treatment, especially if convalescent specimens are collected within 2 to 4 weeks of illness [50]. In later stages of Lyme disease patients are most frequently seropositive, and because of the lack of better confirmatory tests chronic seronegative Lyme disease is difficult to substantiate. Several of the highly immunogenic antigens of *B. burgdorferi* share cross-reactive epitopes with other spirochetes and other bacteria limiting serology particularly when assays using antigen mixtures are used [53–54]. Heat-shock proteins and flagellar antigens are among the most cross-reactive antigens [53].

Some of the problems attributed to serologic tests have been the result of many factors including (1) the use of nonstandardized tests, perhaps with different antigen preparations and variable sensitivities in detecting antibodies, in particular IgM; (2) the use of serologic tests in populations with low pretest probability for Lyme disease where positive results are most likely false positives; (3) the use of tests containing borrelial antigen mixtures, such as ELISA, using whole cell sonicates that may bind cross-reactive antibodies; and (4) the attempt to use the serologic tests for diagnostic purposes rather than using them to detect previous exposure to the agent. Current recommendations for serology of Lyme borreliosis are the use of a two-step approach: an initial sensitive enzyme immunoassay or IFA followed by Western immunoblot [55]. The guidelines for immunoblot interpretation are the IgM criteria of Engstrom et al. [51] and the IgG criteria of Dressler et al. [43]. For an IgM immunoblot to be considered positive it must show two of three IgM bands (41, 39 kDa or OspC). A positive IgG immunoblot must have five of 10 bands: 93, 66, 58, 45, 41, 39, 30, 28, OspC, and 18 kDa.

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The IgM criterion is used during the first 4 weeks of disease onset while the IgG is useful in all stages.

Most recently, the American College of Physicians published guidelines for laboratory evaluation of Lyme disease in which it stated that the pretest probability of Lyme disease must be established before the use of serologic testing [56,57]. The two-tier protocol for Lyme antibodies should be used when the pretest probability is 0.20 to 0.80. When tests are used in cases with a pretest probability of less than 0.20, tests will most likely yield false positive results.

Limitations of Current Serologic Methods. Patients who have developed a high titer of antibodies, as determined by high ELISA values and a high number of bands by immunoblots, remain seropositive for long periods of time after treatment. Therefore, antibody testing cannot be used to follow the effect of treatment [50,58]. Because immunoblots are most frequently read by visual examination, they may suffer from subjective interpretation. Patients who have received OspA vaccine preparations may test positive by initial tests, and thus would require immunoblot testing [59]. Because natural infection with *B. burgdorferi* does not usually elicit anti-OspA antibodies, screening immunoassays using a mutant strain lacking this antigen would be more desirable.

Perhaps the performance of serologic assays could be improved by using quantitative tests that contain purified immunodominant antigens in their format such that cross-reactivity and subjective interpretation are diminished.

Antibody Testing in Cerebrospinal Fluid. Neurological dissemination of *B. burgdorferi* infection can be detected by the intrathecal production of specific antibodies. Such tests have not yet been standardized and several methods have been used, including capture assays, CSF/serum indices, and immunoblot [60–62]. At the Westchester Medical Center, we measure the antibodies in cerebrospinal fluid (CSF) by comparing them to those in serum, and results are expressed as a CSF/serum ratio. Total immunoglobulins are first measured in both specimens and then serum is diluted to match the CSF concentrations. Diluted serum and neat CSF are then tested in parallel by ELISA and separate IgG and IgM immunoblots. A positive intrathecal production is indicated by CSF/serum ratios greater than 1.3 and increased intensity and number of bands in CSF as compared with serum [63]. CSF antibody testing in our experience is of low yield using the presently available methods and testing should be restricted to patients with *B. burgdorferi* antibodies in serum. Other investigators have suggested that detection of *B. burgdorferi* DNA sequences by PCR in CSF has greater sensitivity than antibody methods [21,22]. Lower yield in CSF antibody testing in American patients with neuroborreliosis as compared with German patients was also reported by Steere et al. [61]. Such differences could be attributed to the different strains infecting both populations.

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Functional Antibody Assays. Several investigators have promoted the use of assays that detect borreliacidal or immobilizing antibodies in Lyme borreliosis [64–67]. Such antibodies are, in general, more specific than those detected by matrix-based assays and also more strain specific [65]. Antibodies responsible for the borrelicidal activity of serum are probably directed to outer surface proteins of the organism [65,68]. Culture aliquots of *B. burgdorferi* are incubated with dilutions of serum and growth or motility of borreliae detected after incubation for 24 to 72 hours by color change, microscopy, or flow cytometry. Although highly specific, functional antibody assays are more time consuming than matrix-based assays and require the use of viable organisms. Furthermore, they cannot be performed while the patients are receiving antimicrobial treatment and they have similar sensitivity to nonfunctional antibody assays. Current laboratory modalities used to confirm the diagnosis of Lyme borreliosis lack the desired sensitivity and specificity. Better understanding of the pathogenesis of Lyme borreliosis and improvement of antigen and molecular methods will most likely lead to better diagnostic modalities in the future.

ROCKY MOUNTAIN SPOTTED FEVER—*Rickettsia rickettsii* INFECTION

Rocky Mountain spotted fever (RMSF) is an acute febrile illness, often accompanied by diffuse maculopapular to petechial rash, and in severe cases complicated by increased vascular permeability leading to such complications as multi-organ failure, respiratory failure, and severe meningoencephalitis [69]. RMSF is ordinarily an acute illness that either resolves owing to appropriate therapy or immunity, or results in death in 2 to 7% of patients [69,70]. Because the incubation period has a median of 6 to 7 days (in the range of 2 to 30 days), diagnostic tests may be divided into those useful during the active phase of infection, such as skin biopsy with immunohistologic demonstration of rickettsial antigens or PCR, and those useful for confirmation of a clinical diagnosis, particularly serology [69,71].

Methods to Detect *R. rickettsii* During Active Disease

Culture

R. rickettsii is an obligate intracellular bacterium, with a gram negative–type cell wall, that can only be cultivated within living eukaryotic cells. Not yet widely applied for RMSF diagnosis, spotted fever group rickettsiae have been successfully cultivated in a variety of primary cell cultures and cell lines. A method for rapid cultivation of *R. conorii*, the agent of Mediterranean spotted fever, has been developed using shell vials with immunofluorescent confirmation in cul-

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tures with cytopathic effect [72]. However, rickettsial culture is still generally perceived to be dangerous and very few laboratories outside of research and public health facilities will attempt such cultivation. Culture is usually performed by inoculation of leukocyte-enriched fractions of fresh heparinized blood, but isolations from blood collected in other anticoagulants or in clotted samples have also been achieved. The sensitivity of culture for *R. rickettsii* has not been determined.

Demonstration of Spotted Fever Group Antigens in Skin Biopsy or Tissue Samples

The most sensitive method for diagnostic confirmation of RMSF is the demonstration of *R. rickettsii* antigens in vasculitic lesions of skin biopsies [73–76]. This method requires that a typical skin lesion is available for biopsy, a situation that occurs in approximately 85 to 90% of patients with RMSF [77]. A 3- to 4-mm punch biopsy is obtained such that the suspected petechial lesion is centrally located. The biopsy may be examined immediately by preparation of frozen tissue sections, or may be transported temporarily to the laboratory by immersion in Michel's solution [71]. Formalin-fixed, paraffin-embedded tissue sections provide a superb alternative, particularly in situations where appropriate processing and assay performance are not significantly delayed [75,76]. In the immunohistologic methods, spotted fever group antigens are detected in endothelial cells within foci of vasculitis or perivascular inflammatory infiltrates. This is accomplished by the use of fluorescently labelled polyclonal *R. rickettsii* antibodies (direct fluorescent antibody [DFA]) or by the use of an indirect method that uses polyclonal antibodies to *R. rickettsii* or monoclonal antibodies to *R. rickettsii* lipopolysaccharide antigens in an enzyme-linked, light microscopic method such as immunoperoxidase. In the DFA, rickettsiae are identified by the typical bacillary morphology and apple-green fluorescence when fluorescein isothiocyanate (FITC) is used as the fluorochrome. The additional advantage of the immunoenzymatic approach is the ability to colocalize rickettsial antigens to sites of vasculitis by virtue of hematoxylin counterstain (Fig. 3). Both methods have been evaluated and achieve approximately 70% sensitivity for patients with skin lesions during the active phase of disease [73,75,76]; thus, the inability to detect rickettsial antigen should not dissuade the clinician from a diagnosis of RMSF when other clinical and historical factors are consistent. Demonstration of rickettsial antigen is adversely affected by inappropriate selection of biopsy site, inadequate examination of the tissue biopsy (several levels are recommended), and by 24 hours or more of antecedent doxycycline, tetracycline, or chloramphenicol therapy. The same polyclonal antibody may also be used to detect rickettsiae in tissues obtained at postmortem or in biopsies obtained from patients with suspected rickettsialpox (*R. akari*), imported *R. conorii*, or other spotted fever group infections [78]. The spotted fever group antibody is not useful for detection of

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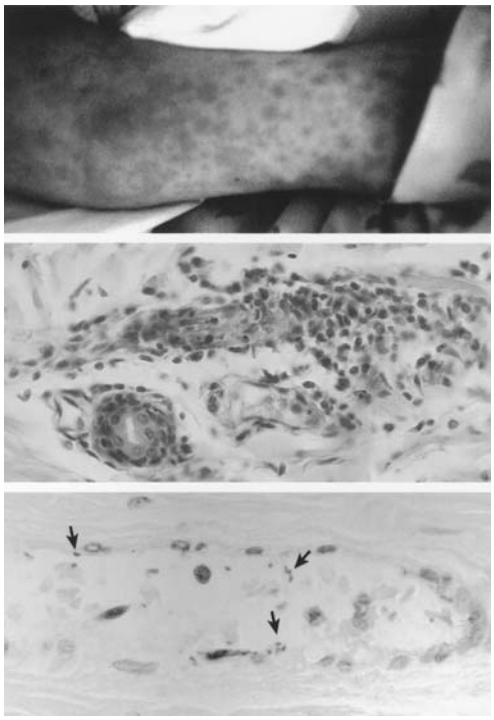

FIGURE 3 Skin biopsy for diagnosis of RMSF during the active phase of illness. Petechial skin lesions (top panel) should be biopsied and examined by hematoxylin and eosin staining for the presence of perivascular lymphohistiocytic infiltrates or lymphohistiocytic vasculitis (middle panel, magnification × 400) and by an immunofluorescent or immunoenzymatic method (bottom panel, magnification × 480) to detect the presence of spotted fever group rickettsial bacteria (arrows) and antigens in the vasculitic focus.

typhus group (*R. typhi*, *R. prowazekii*) or scrub typhus group (*Orientia tsutsugamushi*) infections.

Polymerase Chain Reaction (PCR)

Amplification of nucleic acids in specimens from patients with RMSF has been reported and appears to offer no greater sensitivity or timeliness than does skin biopsy with immunohistologic demonstration of rickettsial antigen [79]. Targets for amplification have included segments of the 16S ribosomal RNA gene, a 17-kDa lipoprotein *Rickettsia* genus gene, *gltA* (citrate synthase gene), and the major immunodominant 190 kDa rickettsial outer membrane protein A (*rompA*) gene

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[79–83]. The major shortcoming of PCR has been the attempt to amplify such rickettsial target nucleic acids in blood, where previous work has shown that the median quantity present circulating in blood at the time of active illness is $10^{1.2}TCID_{50}$ per mL, or about 10 rickettsiae [84]. One alternative not yet applied that holds promise for sensitive and rapid diagnosis is PCR performed on skin biopsy, where rickettsiae are known to be present in large quantities.

Immunomagnetic Separation and Identification of Circulating Rickettsia-Infected Endothelial Cells

Not yet applied to the diagnosis of RMSF, but of some promise because of proven diagnostic efficacy in Mediterranean spotted fever (MSF), is a method that separates circulating endothelial cells from blood of acutely infected patients by magnetically labeled endothelial cell monoclonal antibodies [85]. The cells obtained from blood are then examined by immunofluorescence for the presence of rickettsiae. For MSF, this method correctly identified nine of 12 patients during their acute illness.

Methods for Diagnosis of RMSF in Convalescence

Serodiagnosis

The current standard for diagnosis of RMSF still rests on the demonstration of a four-fold increase in antibody titer between acute and convalescent sera, with a minimum titer of 64 [69,71]. The epidemiologic case definition for RMSF established by the CDC provides that a confirmed case will have such a seroconversion in the context of a consistent clinical illness, and also allows for a probable case when a patient with consistent clinical features has a single high titer ($\geq$64) of *R. rickettsii* antibodies [86]. The latter situation must be interpreted with caution as high rates of seropositive persons with no history of RMSF have been identified in certain high-risk geographic regions [87,88]. The use of an IgM assay may provide additional assurance of a recent infection because most patients do not have IgM antibodies present several months after RMSF [89,90]. However, it must be noted that in experimental situations with known *R. rickettsii* infection, as few as 8% and up to 90% of patients develop IgM responses depending on the serologic method used [90]. Also, it is critical to understand that the majority of patients with active RMSF during the first 3 to 10 days of illness (the most critical interval for instituting antirickettsial therapy) will not have any *R. rickettsii* antibodies present by any serologic test; thus, delaying therapy while waiting for serologic results or not treating in suspected cases because of the lack of *R. rickettsii* antibodies could lead to severe complications or fatal infection [91].

Several methods have been used for serologic confirmation of RMSF. The

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most widely used method is the indirect fluorescent antibody or microimmunofluorescence assay (IFA) [90,92–94]. The sensitivity and specificity of this assay are 97% and 99%, respectively [94], and in the context of a compatible clinical illness, history, and exposure, the positive and negative predictive values can be expected to be very high. An equally sensitive and specific serologic assay is ELISA that uses purified rickettsial antigens [90]. Both IFA and ELISA have the advantage that separate IgG and IgM results may be obtained. Neither is generally available in hospital laboratories or in situations where primary care physicians are likely to require such diagnostic tools. Thus, several easy and rapid methods have been developed including latex agglutination and solid phase immunoassays on dipsticks [95,96]. Both have been shown to have high rates of sensitivity and specificity and are amenable to routine hospital laboratories. Other methods such as complement fixation, hemagglutination, and microagglutination are rarely used. The archaic Weil-Felix tests that use the cross-reactive *Proteus* spp. antigens, OX-2, OX-19, and OX-K, have been shown to be poorly sensitive and specific and cannot be advocated [97]. False positive results have occurred in pregnancy, endocarditis, syphilis, Epstein-Barr virus infections, and autoimmune disease, among other situations. Cross-reactions with typhus group rickettsiae and positive serologic responses with ehrlichial infections occur in a small, but significant, proportion of cases; thus, concurrent analysis of typhus group (*R. typhi*) and *Ehrlichia* spp. antibodies with titration will be helpful to definitively establish the etiologic diagnosis [93–95].

HUMAN MONOCYTIC EHRLICHIOSIS—*Ehrlichia chaffeensis* INFECTION

Human monocytic ehrlichiosis (HME) is so named because of the strong propensity that the causative agent, *Ehrlichia chaffeensis*, has for mononuclear phagocytic cells, including monocytes in peripheral blood [98]. The name is not meant to imply that only monocytes can be infected, because many cases in which the predominantly infected cell is not a mononuclear phagocyte have been clearly demonstrated. As for RMSF, HME is an acute febrile illness that results in a disease of limited duration in the vast majority of cases [98,99]. Based on experimental studies in animals and epidemiologic/laboratory studies in infected humans [99–102], *E. chaffeensis* establishes infection in the blood and tissues very early after tick bite (within several days) and when symptomatic, manifestations are usually detected approximately 7 to 10 days after tick bite. Ehrlichemia in humans is usually limited to a week or less in humans; however, dogs and white-tailed deer that are part of the ecologic reservoir may establish long-term, persistent, subclinical infections [101,102]. Thus, as for RMSF, diagnostic tests include those for identification of the agent in the active phase of illness (blood smear

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examination, PCR, culture, possibly serology) and those for identification of exposure during convalescence (serologic methods).

Methods for Identification of Active HME

Culture

As for other rickettsiae, members of the *Ehrlichia* genus are obligate intracellular bacteria that can only be cultivated in living eukaryotic cells. The culture of *E. chaffeensis* initially was presumed to be very difficult, requiring specialized cell culture systems, careful collection and preparation of blood for inoculation, and long intervals of examination before isolation [103,104]. However, recent investigations have shown that *E. chaffeensis* may be recovered from the blood of infected individuals in the majority of cases if blood samples are collected appropriately (during active disease and before doxycycline or tetracycline therapy) and inoculated onto cell culture in a timely fashion (within 18 hours of collection) [105,106]. When these requirements are met, isolation usually may be achieved within approximately 7 days. The single best cell culture system requires inoculation of leukocyte-enriched fractions of heparin- or other anticoagulated blood onto monolayers of the DH82 canine histiocyte cell line. Cultures are then examined every 2 days by removing nonadherent cells and examining by Romanowsky staining (e.g., Giemsa, Wright stain, Diff-Quik) for the presence of typical intracytoplasmic inclusions that appear stippled and dark blue. Confirmation is achieved by immunofluorescence or immunocytochemistry using specific *E. chaffeensis* polyclonal antibodies or by PCR.

Peripheral Blood Smear Examination

The initial cases of HME were first identified based on the observation of intracytoplasmic clusters of small coccoid and pleomorphic bacteria called morulae (Fig. 4a), in circulating leukocytes, and mostly monocytes [107]. Since that time, the majority of infected individuals have been identified by serologic means, and attempts to retrospectively examine blood films for the presence of morulae have been largely unrewarding [99]. Although no specific data exists, it is estimated from the existing literature that less than 10% of all patients with *E. chaffeensis* infection have been prospectively identified by this method. Standaert et al. have recently confirmed the low sensitivity of prospective evaluation of blood smears, but showed that careful retrospective evaluation (approximately 2 hour review) of infected individuals will often identify infected cells [106]. This further suggests that the time-consuming process of blood smear evaluation may continue to offer some hope for early diagnosis of HME. However, blood smear evaluation must be conducted by an experienced microscopist familiar with the variety of potentially confounding structures observed in blood leukocytes. No apparent

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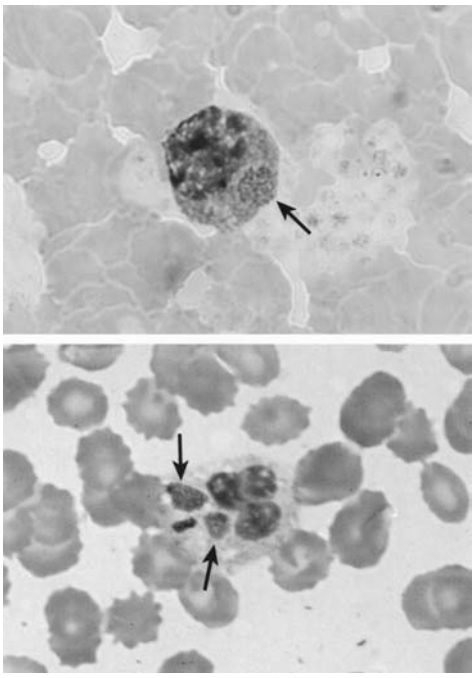

FIGURE 4 Examination of Romanowsky-stained (Giemsa, Wright stain) peripheral blood films may reveal the presence of intracytoplasmic bacterial inclusions (arrows) in (a) monocytes (magnification × 400) in human monocytic ehrlichiosis (HME, *E. chaffeensis* infection) and in (b) neutrophils or band neutrophils (magnification × 400) in human granulocytic ehrlichiosis (HGE, *E. phagocytophila* group spp.).

advantage is offered by examination of bone marrow or by conducting immunofluorescence or immunocytochemical staining directly on blood films [108], and morulae are only rarely identified in CSF mononuclear cells [109]. Examination of buffy coat smears may assist in rapid review of a larger number of leukocytes than could be examined on a thin blood film.

Polymerase Chain Reaction (PCR)

Although *E. chaffeensis* infection appears to have a component in which fixed tissue macrophages are infected [110,111], the organism is disseminated hematogenously in cells intrinsic to the peripheral blood. Thus, detection of ehrlichemia by PCR would be expected to provide a sensitive and specific alternative

for diagnosis during active infection [112]. Several previous investigations have confirmed this impression by amplification of 16S ribosomal RNA gene sequences that are specific for *E. chaffeensis* from blood [113,114]. However, as amplicon contamination remains a serious impediment to accurate diagnosis, particularly when using nested PCR, great care must be exercised when interpreting these results to assure that the clinical findings, other specific laboratory findings, and the PCR results concur and preclude misdiagnosis.

Other potential targets for PCR amplification include the newly recognized family of genes related to the p28 outer membrane protein gene (*omp*1), the 120 kDa antigen gene, the *nadA* gene, and the conserved *groESL* operon [115–119]. However, because diversity of the *E. chaffeensis*-specific protein genes seems to exist, current strategies continue to favor the use of the HE1 and HE3 primers that target a small region of the 16S rRNA gene. Prospective evaluations of this system indicate a sensitivity of approximately 85 to 90% [114]; however, other experiences with this system have been more disappointing (unpublished data). Unfortunately, few clinical laboratories currently offer PCR diagnostic testing for *E. chaffeensis* infection. The vast majority of *E. chaffeensis* infections appear to be effectively cured by antibiotic therapy or resolve spontaneously without therapy, and convincing evidence for persistent infection is strikingly sparse [99,110]; thus, follow-up PCR tests for refractory illness or recrudescent disease are unlikely to be of value. Likewise, a positive PCR test in the context of a prolonged illness and absence of any *E. chaffeensis* serologic reaction must be viewed with great skepticism.

The most appropriate sample for PCR analysis is EDTA-anticoagulated blood obtained during the active phase of disease and before antibiotic therapy. Although high levels of ehrlichemia may take several days to revert to PCR-negative, the vast majority of individuals will have a negative PCR within 24 hours of doxycycline therapy [120]. Samples should be submitted for analysis promptly, but may be stored at 4°C for several days or frozen at −20°C for shipment or if testing cannot be performed immediately.

Immunohistologic Demonstration of E. chaffeensis in Tissue Samples

Because of the tropism for mononuclear phagocyte organs (spleen, liver, lymph node, bone marrow), immunohistology for *E. chaffeensis* has only a limited role in prospective diagnosis [108,111]. A previous evaluation of bone marrow biopsies obtained for diagnostic evaluation of leukopenia, thrombocytopenia, or pancytopenia associated with *E. chaffeensis* infection revealed a diagnostic sensitivity of only 33% [108]. However, this method may be particularly useful for evaluating seronegative fatalities in immunocompromised persons likely to have fulminant infection [121,122].

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Methods for Confirmation of HME in Convalescence

Serology

After the identification of an *E. canis*-like infection in man that was based on the observation of *E. canis*-like structures in peripheral blood leukocytes and serologic reactions to *E. canis* antigens, sera from patients with potential HME were tested with *E. canis* antigens [107,123]. In 1990, *E. chaffeensis* was first isolated from a military recruit with HME and the resulting isolate was used as an antigen with increased sensitivity for serodiagnosis of HME [103]. Since then, the majority of all cases diagnosed as HME has been achieved by the demonstration of a serologic reaction to the Arkansas strain of *E. chaffeensis* by IFA. This antigen appears to be appropriately sensitive and specific given that convalescent sera of patients from whom *E. chaffeensis* has been isolated contain antibodies reactive with the Arkansas strain [103–106,124]. However, to date, no critical evaluation of the sensitivity or specificity of any of the various *E. chaffeensis* isolates for serodiagnosis of HME has been made. It has been shown that *E. chaffeensis* is antigenically diverse by virtue of different reactions of isolates with monoclonal antibodies [124]. Moreover, the 120 kDa immunodominant antigen is characterized by tandemly repeated subunits that differ among isolates [117]. Such variation has not yet been shown to result in antigenic variability for diagnostic purposes, but raises concern about the most appropriate substrate for serologic tests. Yu et al. have demonstrated the utility of a recombinant 120-kDa antigen of *E. chaffeensis* for serodiagnosis of HME using a dot blot method [125]. Moreover, a multigene family encodes at least 16 distinct but similar proteins related to the immunodominant p28 of *E. chaffeensis*, suggesting that a vast array of antigens may be expressed in any given isolate and further raising concern about the use of a single *E. chaffeensis* strain or isolate for serodiagnosis [115]. Recently, Reddy has shown that only one of the p28 family genes is transcriptionally active in vitro, a finding that lends credence to the use of a single isolate for IFA serology [116].

Regardless, IFA using *E. chaffeensis* Arkansas strain is rapidly becoming the standard tool for serodiagnosis. As for RMSF, the CDC has established case definitions that incorporate serodiagnostic studies. These case definitions are designed for epidemiological purposes, but may have some utility for laboratory confirmation of HME as well. A definite case would be characterized by a consistent clinical illness and laboratory evidence of *E. chaffeensis* infection, including a four-fold rise in *E. chaffeensis* antibody to a titer of at least 64. A probable case is defined by a clinically consistent illness in a patient with a single *E. chaffeensis* titer of at least 128. Previous studies have shown that seroconversions to *E. chaffeensis* occurred in persons exposed to ticks despite the absence of any clinical illness [126,127], suggesting several alternatives: (1) asymptomatic or subclinical infection, (2) an anamnestic response in the absence of clinical dis-

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ease, or (3) immune stimulation by a nonpathogenic organism that shares significant antigens with *E. chaffeensis*. Because the mechanism by which asymptomatic seroconversions occur is not understood, it is recommended that paired acute and convalescent sera obtained approximately 30 days apart be tested in order to maximize the positive and negative predictive values of the *E. chaffeensis* IFA [123]. Moreover, serologic tests for both *E. chaffeensis* and *E. phagocytophila* group should be obtained because the differentiation of infection by these agents may be impossible on clinical grounds [98,111]. IgM IFA can be performed, but no clinical evaluation of its utility has been performed. Interestingly, approximately 25% of infected patients are seropositive at the time of clinical presentation.

False positive results may be seen with autoimmune disorders, Epstein-Barr virus infections, Q fever, and potentially with other zoonotic and vectorborne bacterial pathogens, including *Borrelia burgdorferi*, *Rickettsia rickettsii*, *Rickettsia typhi*, *Brucella* spp., and *Legionella* spp. [123,128, unpublished data]. Strong cross-reactions with *E. phagocytophila* group (HGE agent) antigens occur in about 12% of samples [129]. Differentiation of serologic reactions can be achieved in most instances because the homologous serologic reaction will usually be of higher titer [130]. Infrequently, the homologous and heterologous titers will be similar; under these circumstances a definitive serodiagnosis may occasionally be achieved by assessing Western blots for a p28-29 band in *E. chaffeensis* and a p44 band in HGE agent antigens [128].

HUMAN GRANULOCYTIC EHRLICHIOSIS—*Ehrlichia phagocytophila* GROUP INFECTIONS

Human granulocytic ehrlichiosis (HGE) earns its name because of the frequent presence of the causative agent in neutrophils and band neutrophils in the peripheral blood of infected humans and animals [131–133]. As for HME, the name is not meant to imply that only granulocytes are infected, because it is well demonstrated that nonmyeloid lineage cells may occasionally contain morulae as well [111]. The exact taxonomic position of the infectious agent has not been assigned; however, recent data strongly suggests that it is conspecific with the veterinary pathogens *E. phagocytophila* and *E. equi* [119,133–138]. Thus, these names may be used interchangeably. HGE appears to be an infection that infrequently results in clinical illness, but when illness does occur it may be severe or even fatal [111,139,140]. Current evidence based on experimental studies in animals and epidemiologic/laboratory studies in infected humans suggests that the HGE agent establishes infection in the blood and tissues early after tick bite (within several hours to days), and infected animals are ehrlichemic well before onset of clinical signs [133]. In humans, clinical manifestations are usually detected approxi-

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mately 7 to 10 days after tick bite [139,140]. Most individuals probably develop self-limited infections; thus, ehrlichemia is probably limited to less than 2 weeks in humans and also in many animal species [132,133,139]. However, some wild rodents and white-tailed deer may function as reservoirs and establish long-term persistent infections in the absence of any clinical signs [98]. Diagnostic tests include those for identification of the agent in the active phase of illness (blood smear examination, PCR, culture, possibly serology) and those for confirmation of infection in convalescence (serologic methods).

Methods for Identification of Active HGE

Culture

Cultivation of the HGE agent was first achieved by coculture of human HL60 promyelocytic leukemia cells with anticoagulated blood from humans with suspected HGE [141]. Since that time, a large number (>20) of isolates of the HGE agent and *E. equi* have been made from infected humans, dogs, and horses. Under ordinary circumstances, the HGE agent may be identified growing within cultured cells within as little as 3 or as long as 21 days [141]. For optimal results EDTA-anticoagulated blood calculated to contain approximately 10^5 leukocytes are cocultivated with a similar quantity of HL-60 cells. Cultures are examined every 2 days by Romanowsky staining for the presence of typical intracytoplasmic inclusions that appear stippled and dark blue. Confirmation is achieved by immunofluorescence or immunocytochemistry using specific *E. phagocytophila* group polyclonal antibodies or by PCR.

Peripheral Blood Smear Examination

Although most patients are now identified by serologic means, 20 to 80% of patients may have morulae present in carefully examined peripheral blood smears; thus, blood smear evaluation is a simple and sometimes sensitive tool to establish a presumptive diagnosis of HGE. In contrast to the situation for *E. chaffeensis*, when identified, morulae may be observed in between 0.5% and up to 40% of all peripheral blood neutrophils and band neutrophils (Fig. 4b) [131, 139,140]. The HGE agent is rarely identified in other cell types; however, the presence of morulae predominantly in granulocytes is not an indication of HGE, as fulminant *E. chaffeensis* infections may result in a disproportionately high number of infected neutrophils as well [107]. As for HME, blood smear evaluation must be conducted by an experienced microscopist and examination of buffy coat smears may allow more rapid review.

Polymerase Chain Reaction (PCR)

It is apparent through series of experimentally infected animals that ehrlichemia is a key component of HGE [98,133]. Although spleen, bone marrow, liver, and

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lung may have variable quantities of ehrlichiae present during infection, ehrlichemia is detected as a self-limited process after tick bite for up to 2 weeks [133]. Thereafter, detection of ehrlichiae by culture or PCR in blood or tissue becomes increasingly difficult. Given the frequent presence of ehrlichiae in the blood at times that coincide with active clinical manifestations, PCR directed at detection of *E. phagocytophila* group nucleic acids in blood should be a highly sensitive diagnostic method. In fact, a previous prospective evaluation of PCR that targets a specific region of the *E. phagocytophila* 16S rRNA gene showed a sensitivity of 85% and specificity of 100% as compared with serologic analyses [142]. Follow-up PCR analyses of 11 patients who were PCR positive during the active phase of HGE showed that PCR was negative in all cases tested 3 days after doxycycline therapy (unpublished data). As for other rickettsial infections of humans, persistence seems to be a rare event, although at least two persons have suffered either second infections or recrudescence months to years later [98,139].

Potential targets for PCR amplification include the 16S rRNA gene, the *groESL* operon, a region of *epank1* that encodes a 150–160 kDa protein with ankyrin repeat motifs, and a newly recognized family of genes related to the p44 outer membrane protein gene and other outer membrane protein genes found broadly in the *Ehrlichia*, *Anaplasma*, *Cowdria*, and *Wolbachia* genera [119,135,141,143–147]. There are at least five different PCR systems de scribed for HGE that could potentially provide useful diagnostic information. However, the only prospective evaluation of PCR for diagnosis used the ge9f and ge10r primers that amplify a 919 bp region of the 16S rRNA gene [142], and use of other primers should be carefully evaluated for specificity in each laboratory that elects to conduct PCR testing. Few clinical laboratories currently offer PCR diagnostic testing for HGE, but the test is increasingly available through academic medical centers, reference laboratories, and public health laboratories.

As for HME, the most appropriate sample for HGE PCR analysis is EDTA-anticoagulated blood obtained during the active phase of disease and before antibiotic therapy. It is important to recall that although the vast majority of individuals will have a negative PCR within 24 hours of doxycycline therapy, therapy should not be withheld because HGE may be severe. Samples should be submitted as for *E. chaffeensis* PCR analysis, which was previously described.

Methods for Confirmation of HGE in Convalescence

Serology

Human infection with *E. phagocytophila* group ehrlichiae was first confirmed serologically by using *E. equi* antigen in equine neutrophils in an IFA method [131]. Since that time, the HGE agent has been successfully cultivated in vitro,

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and is available for the preparation of more standardized and uniform serologic reagents [141]. The initial problems with *E. equi* antigens prepared in equine neutrophils included lack of reproducibility among different preparations and from different infected horses. Recent evaluations have demonstrated the reproducibility and utility of in vitro–cultivated *E. phagocytophila* group ehrlichiae as serodiagnostic antigens [138,148–150]. Although antigenic diversity has been shown to exist among isolates derived from different geographic regions, when using infected HL-60 cells, sensitivity and specificity does not differ when a panel of sera from patients with PCR or culture-proven HGE are tested. Sensitivity approaches 93 to 97%, and specificity is between 93 and 100% for most antigens when sera from patients with HME are removed from the analysis. As anticipated, antibodies to *E. chaffeensis* cross-react and lead to false positive results in about 12% of patients [129,150]; simple titration of the sera using both *E. phagocytophila* group and *E. chaffeensis* antigens is usually sufficient to resolve discrepancies. On occasion, Western blots may need to be performed to ascertain the presence of antibodies to the p44 major surface protein of the *E. phagocytophila* group that are uniformly present in patients convalescent from HGE [128,148,151]. Western blots may also be used for initial serologic testing owing to increased specificity [152]; however, the Western blot will not generate a titer so that a seroconversion may be difficult to detect in patients with antibodies at presentation.

It is currently recommended that acute and convalescent sera obtained about 30 days apart should suffice for demonstration of seroconversion; however, between 18 and 45% of patients with HGE will have antibodies detected at onset of clinical manifestations [139,140]. Moreover, in endemic regions seroepidemiologic studies indicate that as much as 15% of the population may already be seropositive. Because of the clinical similarity between the HGE and HME, it is recommended that patients be tested for antibodies to both *E. phagocytophila* and *E. chaffeensis*. The sensitivity of IgM analysis is probably similar to that of IgG or polyvalent analyses for diagnosis during the first 40 days after infection [148,150,151]. Thereafter, IgM antibodies to *E. phagocytophila* group ehrlichiae are not detected, making this a useful tool to discern pre-existing antibody from recent infection.

HGE IFA false positive reactions may result from autoimmune disorders, Epstein-Barr virus infections, and Q fever; of particular concern are concurrent serologic reactions to *Babesia microti* or *B. burgdorferi* that would in many cases be considered diagnostic for Lyme disease despite lack of other objective findings for *B. burgdorferi* infection [153]. Clearly, some of these reactions result from simultaneous infection by *E. phagocytophila* group ehrlichiae and *B. burgdorferi* and/or *Babesia microti* [148,154,155]. However, other alternatives that have not yet been fully investigated include serologic crossreactions, low level antigenic stimulation that does not result in infection, or induction of an anamnestic sero-

logic response after the bite of coinfected ticks. That mice experimentally infected with the HGE agent do not develop antibodies reactive with recombinant *B. burgdorferi* antigens more often than uninfected controls seems to make the first alternative unlikely [156].

Several recombinant proteins, including members of the p44 major outer surface protein-1 (MSP1) complex, EPANK1, have been cloned and evaluated as potential serodiagnostic reagents with great promise [143–147]. The specificity of such reagents may be useful to diminish false positive reactions that are problematic.

HUMAN BABESIOSIS: *Babesia microti*, WA-1, AND *Babesia divergens* INFECTIONS

Babesia spp. are piroplasms, pear-shaped intraerythrocytic protozoan parasites that are genetically related to *Plasmodium* spp., and are tickborne [157–160]. Among many known species of *Babesia*, at least three species are known to cause human infection, including *B. microti, B. divergens*, and new piroplasms identified in patients from Washington state and California that are genetically close relatives of *B. gibsoni* and *Theileria* spp., as well as a piroplasm related to *B. divergens* in Missouri [157,159,161,162]. After tick bite, the piroplasm is inoculated and infects intravascular erythrocytes. The developing merozoites may on occasion form ''Maltese crosses,'' but lack detectable exoerythrocytic schizont phases. Infection is very severe in splenectomized individuals, whether caused by *B. divergens* in Europe or *B. microti* in North America [157,158]. Infection of normosplenic persons in North America may also result in significant disease, and is often discovered in the context of a coinfection with *B. burgdorferi* or *E. phagocytophila* group organisms. *Babesia* spp. have a proven capacity for chronic bloodstream infection that may last for weeks to months, even in the absence of specific clinical manifestations [157,162].

Methods for Identification of Active Babesiosis

Culture

Babesia spp. have typically resisted attempts at in vitro cultivation. Recently, cultivation of a few species has been achieved in continuous erythrocyte culture, including *Babesia* spp. from white-tailed deer (*Odocoileus virginianus*), desert bighorn sheep (*Ovis canadensis nelsoni*), mule deer (*Odocoileus hemionus*), *B. divergens*-infected humans, and a human patient after passage in hamsters [163–166]. Short-term culture of *B. microti*, *B. divergens*, and *B. rodhaini* has been achieved in erythrocyte cultures [167]. However, these methods have not been readily adapted as diagnostic methods for any *Babesia* spp.

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An alternate method for isolation of the causative agent and diagnosis of babesiosis is the intraperitoneal inoculation of small laboratory mammals, particularly hamsters (*B. microti* and WA-1-like piroplasms) and gerbils (*B. divergens*), with blood from the potentially infected patient [158,159,168]. These methods serve predominantly as mechanisms for parasite amplification such that identification may be achieved by more routine methods. Animal inoculation methods may be useful under some circumstances, but are not sufficiently timely to establish an etiologic diagnosis and may require 3 to 6 days for *B. divergens* or up to 6 weeks for *B. microti*. Infection is confirmed by examination of peripheral blood of these animals for the presence of typical intraerythrocytic forms.

Peripheral Blood Smear Examination

Romanowsky-stained (Giemsa, Wright stain) blood smears may be examined for erythrocyte infection and will assist in establishing a diagnosis of babesiosis, but automated blood and erythrocyte analysis alone is not capable of identifying infected cells [157–159]. *Babesia* spp. parasites may be recognized in peripheral blood smears with careful examination or when parasitemia achieves high levels. *Babesia microti* is identified by examination of thin blood films where the parasites are recognized as small, round to oval rings ranging from 1 to 3 μm in diameter, and having pale blue cytoplasm and one or two small red nuclei (Fig. 5). With maturation, the parasite appears more amoeboid, and infected cells that

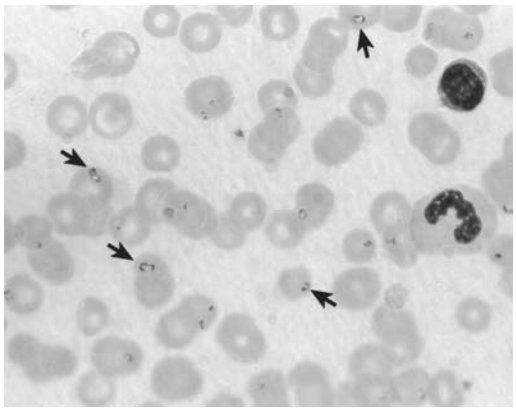

FIGURE 5 Active babesiosis may be diagnosed by examination of Romanowsky-stained peripheral blood films by the detection of intraerythrocytic piroplasms, ring-forms (arrows), amoeboid forms, and, infrequently, tetrads called ''Maltese crosses'' (*B. microti* in human blood, original magnification × 400). (Blood film courtesy Dr. Peter S. Dixon, Essex, CT.)

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contain multiple parasites may be observed. Rarely a diagnostically significant finding is the formation of tetrads of merozoites. Parasitemia in actively infected individuals usually ranges from 1 and 20% in the spleen intact, and up to 85% in asplenic patients. Pigment deposition is absent and may assist in differentiating *B. microti* infection from *Plasmodium* spp. infections. In the few cases of WA-1 babesiosis that have been identified, the morphology of the parasites was similar to that of *B. microti. Babesia divergens* has a similar morphologic appearance but possesses greater morphologic diversity, including the frequent presence of amoeboid, piriform, annular, filamentous, and tetrad forms. Morphologic identification requires an experienced microscopist, as artifacts such as Howell-Jolly bodies or Pappenheimer bodies may confound interpretation [159,169].

A modified morphologic method called the Quantitative Buffy Coat system (QBC; Becton-Dickinson, Sparks, MD) has been retrospectively applied for diagnosis of babesiosis with sensitivity equivalent to that of blood smear examination [170]. In this method, differential centrifugation of blood separates cellular components and acridine orange is used to nonspecifically stain the babesial parasites. The method is rapid (5 min) and may be useful at times when parasitemia is low.

Polymerase Chain Reaction (PCR)

PCR appears to offer a highly sensitive alternative to blood smear examination for diagnosis during active disease [166,171–173]. This is especially appropriate for patients with low levels of parasitemia, as the analytical sensitivity has been shown to be as little as three merozoites per reaction when using primers based on specific sequences in the *Babesia* small subunit rRNA gene. In a prospective evaluation of the relative utility of PCR, blood smear, and hamster inoculation for diagnosis in 19 cases of acute babesiosis (*B. microti* infection) in the northeastern United States, PCR was as sensitive as blood smear examination and more sensitive than hamster inoculation [172]. However, no differences in positive or negative predictive value were observed. PCR may also be particularly useful for detection of persistent infection, which may last up to 27 months (median 82 days) in untreated individuals and up to 13 months (median 16 days) in treated individuals [162]. PCR may also be used for detection of recrudescence that may occur even in patients who have been treated with combination quinine and clindamycin [162].

Methods for Confirmation of *Babesia* spp. Infections in Convalescence

Serology

Because *B. microti* and the WA1-like *Babesia* spp. may establish persistent infection in humans, serologic tools may be very useful for diagnosis [162,174–178].

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However, acute infection by *B. divergens* in Europe may be rapidly fatal and *B. microti* in asplenic patients in the United States may be severe, and because antibodies are not present in serum before 7 to 10 days after onset of illness serologic tests may not be useful [159]. Thus, it is prudent to establish a clinical diagnosis and to use serologic tests for retrospective confirmation after initiation of antibabesial therapy.

Serologic diagnosis of babesiosis is confounded by crossreactivity among *Babesia* spp., *Theileria* spp., and *Plasmodium* spp. and by the high seroprevalence among populations in at-risk regions [157,174–176]. Fortunately, cross-reactions among other species and genera are a problem mostly in low dilutions of sera, and the evaluation of seroconversions or IgM tests may assist in differentiating pre-existing antibodies from active infection [176–178]. Specific antigens must be used to detect infections by the WA1-like babesial pathogens that have been identified in the Pacific Northwest because of the lack of serologic crossreactivity with *B. microti* [166]. The most frequently used serologic assay for diagnosis of human infection by *B. microti* is the IFA test that uses erythrocytes from infected hamsters [176,177]. A comparison analysis, using positive cutoff titers ranging from 32 to 80, has indicated that the test is reproducible among laboratories (84–85%), relatively sensitive (88–92%), and specific (90–100%) [177]. Similarly, an IgM IFA test for *B. microti* was applied for diagnosis of patients with clinical manifestations suggestive of acute babesiosis and revealed a relatively high sensitivity (91%) and specificity (99%) when a serum titer of 32 was used as positive cutoff [178]. *Babesia* antibody appears to peak within 3 months of initial infection and drops to near baseline within 9 months in those no longer parasitemic [162]. In persistently infected individuals, antibody titers may remain elevated for 1 year or more.

False positive results have been reported in RMSF, Colorado tick fever, malaria, as well as in conditions that are associated with autoantibody production [176,177]. A high percentage of individuals with serologic evidence of babesiosis will also have antibodies to *B. burgdorferi* or the HGE agent, probably owing to the shared tick vector and not as a result of serologic crossreactions [179,180].

Seroprevalence studies indicate a high rate of subclinical infection must occur, as studies in endemic areas have revealed that 3.3 to 21% of the general population has serologic evidence of infection by *B. microti* in the northeast United States [175,181]. However, comparison studies in nonendemic regions also indicate high seropositive rates among the population. Similar studies conducted on populations potentially exposed to the WA1-type *Babesia* spp. also revealed a seropositive rate between 3.5 and 16% [181]. Thus, the judicious use of serologic tests in the correct clinical context potentially coupled with PCR and blood smear examination will optimize the positive and negative predictive values of these tests.

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Lyme Neuroborreliosis

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INTRODUCTION

Lyme disease, particularly as it affects the nervous system, has been the source of tremendous confusion and concern among both patients and physicians. Physicians practicing in Lyme-endemic areas constantly find themselves torn in trying to understand how best to help patients with troubling but ill-defined syndromes, who are convinced that they have Lyme disease which they know will finally resolve if only they could get a few more months of antibiotics. This concept is supported by advertisements in the media alluding to how this infection can so easily be confused with ''lifestyle symptoms,'' by web sites promulgating information that is less than scientifically rigorous, and by misinterpretation of legitimate descriptions of areas of scientific doubt that are often extrapolated to indicate that this disease is totally incomprehensible. At the same time, the heavy emphasis on these ill-defined phenomena has led to such widespread skepticism and misperceptions that patients who present with the classic findings of neuroborreliosis (nervous system Lyme disease) often go undiagnosed for extended periods of time.

Much of this misunderstanding can be reduced to one of four myths: (1) neuroborreliosis is a newly described illness that is, to date, little understood because it has been studied for such a brief period of time; (2) once the diagnosis has been considered, the ability of the laboratory to confirm it is so tenuous as to be totally unhelpful; (3) neuroborreliosis causes primarily ill-defined neuropsychiatric symptoms, including depression, and therefore everyone with such symptoms may well have Lyme disease; (4) treatment is highly ineffective, with the

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organism sequestering itself only to relapse in the future, and therefore extraordinarily long courses of antimicrobials should be used to effect a cure.

Like most myths, each derives from a small kernel of truth and grows to encompass far more than is rational. The power of these myths is such that Lyme disease is being considered in the differential diagnosis of individuals who epidemiologically could not possibly have this disease, which leads to unnecessary testing, misinterpretation of test results, and unneeded treatment.

None of the aforementioned should be construed to minimize the importance of this disease. It is the most common vectorborne disease in the United States and does cause real difficulty for infected patients. However, appreciating this should not lead to irrational exuberance in invoking this diagnosis where it does not fit.

THE FOUR MYTHS

History and Geography

The term ''Lyme arthritis'' was coined in 1975 to describe an outbreak of what appeared to be juvenile rheumatoid arthritis in Lyme and Old Lyme, Connecticut [1]. As it became clear that this was in fact a multisystem infectious disease, the term was changed to ''Lyme disease'' [2]. It was soon recognized that some of the clinical manifestations were quite unusual—in particular, the virtually unique evolving, enlarging rash, occurring at the site of bites by hard-shelled *ixodes* ticks. Recognition that this same rash, termed erythema chronicum migrans, had been described in the Scandinavian literature early in the century [3], and known since then to be frequently associated with neurologic symptoms [4], led to the recognition that this disorder was closely related to one well known in Europe as Garin-Bujadoux-Bannwarth syndrome [4,5]. With the identification of the causative tickborne spirochete in the United States in 1983 [6,7], and in Europe in 1984 [8], it became clear that these were closely related if not identical infectious diseases, caused by *Borrelia*. Although the name *Borrelia burgdorferi* was initially applied to the causative spirochetes in both Europe and North America, subsequent molecular biological studies have led to a subclassification [9,10]. The broad group is now known as *Borrelia burgdorferi sensu lato*, the North American subspecies as *Borrelia burgdorferi sensu stricto*, and the two predominant European strains as *Borrelia afzelii*—responsible for most chronic dermatologic phenomena—and *Borrelia garinii*—responsible for most neurologic phenomena.

Interestingly, in Europe the most widely recognized extracutaneous manifestations are neurologic, and are identical to the neurologic consequences recognized in the United States in the late 1970s. The classic triad—lymphocytic men-

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ingitis, painful radiculitis, and cranial neuropathies—was well described in the French literature in 1922, and about 20 years later in extraordinary detail in the German literature. Not only have these syndromes been widely recognized in Europe for years, but it has been known since the 1950s that they respond to penicillin or other antibiotics, and that they generally are self limited with or without treatment, with only rare long-term sequelae. As a result of this long European experience, it can safely be stated that while the full range of neurologic disorders actually caused by this infection have yet to be defined, and while there may well be room for improvement in accurate diagnosis, clinical nosology, and treatment, much more is actually known about this illness than is commonly acknowledged.

Lyme disease represents a zoonosis in which humans become infected inadvertently. Once injected into a host, spirochetes tend to migrate centrifugally from the site of inoculation, leading to the slowly expanding erythematous skin lesion. Systemic dissemination is quite common with this infection, usually leading to generalized symptoms such as fever, myalgias, arthralgias, headaches, and malaise. In contrast, the rash itself is usually asymptomatic. It is typically quite striking that such a large lesion is not painful, pruritic, or otherwise obvious to the patient, who may be completely unaware of it if it occurs in an area that is not readily visible. Although estimates vary, somewhere between 50% and 80% of patients infected with *B. burgdorferi* will have an erythema migrans [11].

The spirochetes disseminate early and widely, with several specific tropisms. Invasion of the central nervous system has been demonstrated to occur rapidly both in animal models and in patients [12–14]. Other frequently affected sites include the myocardium, joints, and liver. Although this initial infection is often described as flu-like, it is important to remember that this refers to the systemic symptoms and not to other organ-specific elements of the flu. Specifically, patients do not typically develop upper respiratory or gastrointestinal symptoms.

In this phase of acute bacterial dissemination patients may be asymptomatic, may have a nonspecific febrile syndrome, or may develop certain highly characteristic types of organ-specific manifestations. Cardiac conduction abnormalities occur in 5–10% of patients, and complete heart block in an otherwise healthy young adult in an endemic area should bring Lyme disease to mind. Other patients develop arthralgias at this stage or, less commonly, frank arthritis.

Probably the most common organ-specific types of involvement at this phase of the illness involve the nervous system, occurring in 10–15% of infected patients [15]. Described in more detail below, these typically consist of a lymphocytic meningitis, cranial neuropathies, or painful radiculitis. It is important to note that the latter can exactly mimic a mechanical radiculopathy; patients have been treated conservatively for ‘‘disc herniations’’ (usually with normal or non-

specific MRIs) only to have their symptoms ultimately explained by this infection.

Diagnosis

Before the identification of *B. burgdorferi* as the causative organism, diagnosis rested on recognition of classic clinical syndromes, such as erythema migrans, Lyme arthritis, or bilateral facial paralysis with lymphocytic meningitis. With the recognition that this was, in fact, a bacterial infection, diagnosis should have become greatly simplified. Unfortunately, this has been only partly true. Several factors contribute to this. The responsible organisms are fastidious, requiring specialized culture media known as BSK II that is not routinely stocked by most microbiology laboratories. Second, the organism is slow growing, with an in vitro doubling time of about 24 hours, requiring that cultures be maintained for far longer than most other bacterial cultures before growth or lack thereof can be described with confidence. Third, the number of organisms present in blood, CSF, or other readily obtainable clinical material is probably quite small. Although cultures of erythema migrans lesions may be positive in 70% or more of cases (a lesion so pathognomonic that culture results are totally unnecessary), results from infected spinal fluid are typically positive no more than 10% of the time [16]. This has led to a proliferation of other techniques, some of which are highly reproducible and some of which are not.

To date, the only widely used and reasonably standardized diagnostic technology remains demonstration of immunoreactivity against the responsible organism, ie, serology. Serologic testing has several inherent limitations, regardless of the organism in question. Because it takes several weeks for the antibody response to mature to the point where produced antibody is detectable against the broad background of less-specific immunoreactivity, patients are typically seronegative in the first few weeks of infection. Consequently, treatment of early disease such as erythema migrans should not be delayed for, or conditional on, the results of serologic testing. Second, because the antibody response is targeted at molecular epitopes and not ''brand name organism,'' there is cross-reactivity. In the case of Lyme disease, specific cross-reactivity is prominent with other borrelial infections (eg, relapsing fevers, which fortunately are not common in most Lyme endemic areas) and treponemal infections such as syphilis and *T. denticola* (an organism responsible for much gum disease). Nonspecific cross-reactivity occurs in circumstances in which there is prominent B cell stimulation resulting in polyclonal expansion of the B cell response, such as in subacute bacterial endocarditis [17].

As with other serologic techniques, patients who develop a measurable antibody response to an organism often will remain seropositive for an extended period of time after the antigen exposure is over. This principle, which is funda-

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mental to other serodiagnosis as well as to immunization, is often forgotten. However, because of it, serologic testing cannot be used as a measure of effective treatment. In addition, in endemic areas it can result in a great deal of confusion because previously infected patients can develop any other conceivable medical problem; in such circumstances a positive serologic result is at best misleading.

Other limitations of Lyme serologic testing are unique to this infection. There is, as yet, no agreement on the best antigen mix to use in diagnostic testing. Even among major reference laboratories, approaches range from using whole spirochete sonicate at one end of the spectrum to use of isolated fractions of a single flagellar antigen at the other. Criteria for positive and negative similarly vary, even among the most reliable labs. It should therefore come as no surprise that results vary, particularly among patients with low but detectable amounts of antibody. Although recent efforts at standardization by the CDC and other organizations have led to considerable improvement, disparities still occur.

One major improvement in standardization has come about with the adoption of a two-step diagnostic procedure, using Western blots to confirm borderline or positive results [18,19]. In Western blots, immunoreactivity is measured not as a quantitative sum of all antibody reactivity against all sampled antigens but as a qualitative assessment of the specific proteins of the organism against which patients have antibodies. Because this test is not quantitative, it should not be used in an effort to detect immunoreactivity in patients whose quantitative serologic testing (ELISAs) is negative, ie, it is usually inappropriate to use it to increase sensitivity. The role of this technique is to improve specificity, ie, to determine if a borderline or positive result is a true or false positive.

Using data derived from a large panel of clinically well characterized patients, consensus criteria have been defined for positive and negative Western blots (Table 1). When seropositive patients have two of the three defined IgM bands, or five of the 10 IgG bands, their seropositivity can be considered specific. This does not clarify if the positivity is due to an active or remote infection, nor does it necessarily differentiate between Lyme disease and antigenically similar organisms such as *T. pallidum*. However, it does help eliminate many false positives caused by nonspecific cross-reactivity.

Although this is obviously a major improvement, one important limitation must be remembered. In the studies that defined the Western blot criteria, although specificity was essentially 100%, sensitivity was not. Only 83% of patients with long-standing infection met IgG criteria, while only 32% of patients with acute illness met IgM criteria [18]. Consequently, in patients with clinically appropriate disorders, positive ELISAs, but negative Western blots, the diagnosis should not be excluded.

Despite these limitations, serologic testing can be extremely helpful if used in the appropriate context. In patients with clinically appropriate syndromes, with a real possibility of exposure to *B. burgdorferi*, the test has excellent positive

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Table 1 Western Blot Criteria

IgM (2/3 required)	IgG (5/10 required)
23 kDa	18 kDa
39 kDa	23 kDa
41 kDa	28 kDa
	30 kDa
	39 kDa
	41 kDa
	45 kDa
	58 kDa
	66 kDa

From Ref. 18.

and negative predictive value, ie, positive or negative results are very helpful [20–22]. This can be best appreciated with a few simple calculations. In most laboratories, results are defined as positive if the measured value falls three standard deviations above the mean of measured samples. Statistically this means that 99.7% of normal samples will be negative, while .3% (3/1,000) will be positive simply by statistical variation, ie, false positives. In Lyme-endemic areas, typically 10% to 15% of the population has been exposed. Therefore, for every 1,000 samples processed, between 100 and 150 will be true positives, while three will be false—a very good positive predictive value. Similarly, false negatives are known to occur. Although the exact frequency is indeterminate, it is highly unlikely to exceed 10% of samples. Therefore, assuming 10% of the population in an endemic area has been exposed, no more than 1% of samples (10% of 10%) will be false negatives. Because the total number of negatives will be 90 true negatives and one false negative, the negative predictive value of the test will again be excellent, ie, in an individual with a negative result the overwhelming likelihood is that this reflects absence of exposure.

In nonendemic areas where the background incidence of disease is much less, the negative predictive value is even more powerful. If there is one exposed individual in a population of 10,000, and 10% of exposed individuals have false negative results, for every 100,000 samples there will be one false negative and 99,990 true negatives. However, in nonendemic areas false positives become much more of a problem. Again if there is one exposed individual in a population of 10,000, for every 10,000 samples there will be one true positive and 30 false positives (0.3%), making a positive result very difficult to interpret.

Serologic testing has also been applied to the diagnosis of central nervous

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system (CNS) Lyme disease [23–25]. As in other infections, the presence of organisms stimulates local production of specific antibody within the CNS, something that can be detected by comparing cerebrospinal fluid (CSF) to peripheral blood immunoreactivity. From a technical perspective, this must be performed correcting for any disruption of the blood brain barrier or nonspecific immune stimulation within the CNS. If the CSF contains increased amounts of total immunoglobulin for either of these two reasons, and standard methodology is used to measure CSF specific immunoreactivity, all specific immunoreactivities will appear to be increased because of this nonspecific increase in antibody content. However, if the laboratory corrects for any increase in overall immunoglobulin concentration (either by measuring and adjusting for it, or by performing capture assays), it becomes straightforward to detect intrathecal production of specific antibody. The other reason to compare CSF to serum antibody concentrations is that if the peripheral blood contains significant amounts of specific antibody (particularly IgG), this will be reflected in the CSF by passive diffusion. Only if the laboratory takes this specific reactivity into account and demonstrates proportionately greater antibody concentration in the CSF can this be interpreted as evidence of local production of specific antibody.

Unfortunately, like peripheral blood antibody production this method cannot be used to follow disease evolution or resolution. Laboratory evidence of intrathecal antibody excess has been demonstrated as long as 10 years after successful treatment [26], presumably as both peripheral and CSF antibody concentrations drop in parallel.

Several nonserologic techniques have also been tried but been met with limited utility. Polymerase chain reaction, which can in theory detect the genomic material of a single organism in a sample has typically been positive in only 50 to 70% of samples that should be positive [27–29]. Antigen detection methods have been inconsistent. Promising results from some laboratories have yet to be confirmed by others. In sum, this is an area with considerable room for improvement.

Neuroborreliosis

The nervous system is probably the most frequent—and certainly the most potentially problematic—extracutaneous site of involvement in this infection (Table 2). About 10 to 15% of infected patients develop obvious neurologic involvement quite early in the illness. If untreated, an even larger proportion will later develop more subtle forms of neurologic involvement [30–32]. Use of PCR assays of patients' CSF suggest frequent early seeding of the central nervous system (CNS) [12,14]. Animal studies similarly indicate frequent, early CNS infection [12,33]. However, what has remained undetermined is the frequency with which this early

TABLE 2 Neurologic Manifestations

Acute	Chronic
Lymphocytic meningitis	Mononeuropathy multiplex
Radiculoneuritis	Encephalomyelitis
Mononeuropathy multiplex	Encephalopathy
Cranial neuropathy	
Encephalomyelitis	

seeding evolves into true CNS disease. Unfortunately, concern about these issues has contributed tremendously to the anxiety and misinformation about this disease.

Nervous system involvement can be categorized in several different ways. Anatomically, disorders can be divided into those affecting the peripheral nervous system or the central nervous system. Pathophysiologically, central nervous system disorders can be separated into inflammatory and noninflammatory. Inflammation can involve the meninges, or the parenchyma of the brain or spinal cord. In some patients, CNS function may be altered in the absence of discernible CNS inflammation.

Disease severity varies widely. Some patients develop severe neurologic problems. These typically occur relatively early in infection and have a fairly acute onset. One or 2 months after exposure a patient might develop acute radicular pain and weakness [2]. In other individuals, symptoms may develop later, be less severe, and usually have a less dramatic onset. Such a patient might gradually notice the onset of paresthesias and numbness in one or more limbs long after the acute exposure [34]. In general, the more severe and rapidly developing symptoms tend to occur earlier in disease while more insidious syndromes tend to be seen much later, suggesting a biological difference between these groups of disorders. Whether these differences are caused by the virulence of the infecting strain, inoculum size, co-infections, differences in the host's immune response, previous immune exposures, or other mechanisms remains unknown.

Acute Syndromes

The typical acute syndromes were well described many years ago by Garin and Bujadoux in Europe [4], and more recently by Reik [2] and Pachner [15] in the United States. Within 1 to 3 months of infection, about 10% of untreated patients will develop an acute lymphocytic meningitis. Like other forms of ''aseptic'' meningitis, patients typically have headache, photophobia, meningismus, fever, malaise, and other nonspecific symptoms. Interestingly, some patients with a significant CSF pleocytosis may be virtually headache free, while others with only

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a minimal pleocytosis may be highly symptomatic. Cerebrospinal fluid typically contains up to several hundred lymphocytes. Protein is typically mildly elevated (up to 100 to 200 mg%) while the glucose is normal to minimally decreased. About 95% have intrathecal production of anti—*B. burgdorferi* antibody [25] and some are PCR positive [27]. This disorder presumably reflects spirochetal invasion of the central nervous system, although *B. burgdorferi* can only be cultured from CSF in about 10% of such patients. While antibiotics are obviously helpful, this syndrome, like many of those in this disease, is typically self-limited. Untreated, some of these patients may subsequently go on to develop other evidence of chronic CNS infection.

Second, patients may develop a radiculoneuropathy [2,4,15], an acute disorder often clinically indistinguishable from a mechanical radiculopathy, affecting a limb or the trunk. Pain can be fleeting, reminiscent of tabetic ''lightning pains,'' or may be more persistent. Neurologic deficits, including segmental weakness and reflex loss, occur commonly. In fact, motor deficits often predominate. This syndrome may occur more frequently in the limb that was the site of the tick bite. It is important to emphasize that although some patients may develop this disorder together with Lyme meningitis, in others it can occur in isolation, without fever, headache, or erythema migrans. In endemic areas, if a patient develops typical radicular symptoms without a history of a precipitating injury or an appropriate cause demonstrable on imaging studies, Lyme radiculopathy should be strongly considered.

In some patients, the radicular symptoms may involve several roots resembling a plexopathy not unlike a ''brachial neuritis'' or a lumbosacral plexopathy (such as occurs as a diabetic amyotrophy). Others may develop a more typical mononeuropathy multiplex with acute onset of damage to one or several individual nerves. In all instances, patients can recover dramatically with antimicrobial therapy.

A small number of individuals has been described with a clinical syndrome resembling the Guillain-Barre syndrome [35]. These individuals develop rapidly progressive weakness and areflexia. Unlike typical patients with GBS, most have a significant CSF pleocytosis. Neurophysiologic findings have usually not provided clear evidence of conduction block or severe slowing of conduction, as would be expected in a severe demyelinating polyneuropathy such as Guillain-Barre syndrome.

The third component of the ''classic triad'' of acute neuroborreliosis is a cranial neuropathy. Most frequently this involves the facial nerve, causing facial paralysis. In endemic areas Lyme disease is a frequent cause of facial palsy; as in Bell's palsy recovery occurs in the vast majority of affected individuals. Bilateral facial nerve involvement can occur. Lyme disease is one of the few disorders that cause bilateral facial palsy, other considerations including sarcoidosis, Guillain-Barre syndrome, HIV infection, and basilar meningitis of other causes.

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Other cranial nerves can be affected as well [36]. Trigeminal involvement can lead to facial numbness or pain. Trigeminal neuralgia in a young adult in a Lyme-endemic area should bring to mind both Lyme disease and multiple sclerosis. The eighth nerve may be involved, resulting in hearing loss, tinnitus, or vertigo. The nerves to the extraocular muscles (III, IV, and VI) can similarly be involved, causing diplopia and corresponding specific eye movement abnormalities.

Involvement of other cranial nerves occurs less frequently. European series have described patients with optic neuritis [37]. Reports of this in North America have been more anecdotal. This probably can occur but is quite uncommon. Occasional patients with dysphagia and hoarseness have also been described, implicating the lower cranial nerves, but such disorders have been distinctly uncommon [38].

As in patients with radiculoneuropathies, most but not all patients with cranial neuropathies have CSF abnormalities. In all three disorders, elevations in CSF protein, white blood cell count, and intrathecal production of specific antibody occur commonly. Although measured intrathecal antibody production does not necessarily fall rapidly following therapy and consequently cannot be used to assess treatment response [26], reassessment of the CSF pleocytosis or protein elevation can be helpful, with most patients demonstrating slow improvements in these values over time after successful treatment.

These three phenomena—lymphocytic meningitis, radiculoneuropathy, and cranial neuropathy—can occur singly or in any combination. Although it was long thought that the latter two disorders were caused by damage to the nerve roots as they crossed through the inflamed subarachnoid space, detailed neurophysiologic and pathologic studies have demonstrated that these actually are disseminated inflammatory processes, involving peripheral or cranial nerves and the meninges as separate elements [30,39]. In fact, either may be involved without the other. These forms of nerve damage all appear to be manifestations of a mononeuropathy multiplex, ie, a diffuse inflammatory process affecting nerves in a patchy fashion perhaps caused by a vasculopathy. The precise mechanism remains unknown; pathologic studies have failed to demonstrate spirochetes, immune complexes, complement, or other clues to the pathophysiology. However, this syndrome responds well to antimicrobial therapy [34], indicating a significant role for active infection in its pathogenesis.

The other dramatic but fortunately uncommon neurologic disorder is a form of encephalomyelitis [31,37], an inflammation within the brain or spinal cord affecting white matter more than gray, presenting clinically with acute or chronic signs of white matter damage, eg, spasticity, sensory changes, ataxia, or eventually even a subcortical dementia. A myelopathy is particularly common, with gait and sphincter difficulty. Extensively described in both the European and North American literature, this disorder probably occurs in about 0.1% of un-

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treated, infected individuals and can respond to antimicrobial therapy, although clinical residua caused by residual white matter damage are not uncommon. Because the white matter appears to be involved preferentially, this can be confused with either the first episode of multiple sclerosis or, when severe, with an acute disseminated encephalomyelitis. However, in virtually all cases caused by Lyme disease, intrathecal production of specific antibody can be demonstrated [31]. Other helpful findings include the observation that the CSF tends to have a more vigorous pleocytosis in neuroborreliosis than in MS, and in patients with Lyme disease evoked potentials rarely demonstrate clinically inapparent lesions. Finally, when untreated this illness is typically monophasic and does not follow the typical relapsing remitting course of MS.

The course of this disorder may be acute, subacute, or chronic. In most instances, appropriate antimicrobial therapy will arrest the process and lead to significant recovery. In some individuals in whom significant structural damage has occurred, residual neurologic deficits will remain. The pathophysiology of this disorder remains obscure. Very limited pathologic data are available [40] and to date this process has not been reported in any animal model [41]. *B. burgdorferi* has been demonstrated to bind to oligodendroglia in vitro, perhaps accounting for the predilection of this syndrome for white matter [42]. Such binding might either directly trigger local myelin destruction or stimulate a localized immune response which in turn could cause myelin or other damage. The observation that many of these patients improve with antibiotics again indicates that persistence of the organism is essential for the continued pathophysiologic process.

Chronic Syndromes

In addition to these classic syndromes, several other forms of nervous system involvement have been identified more recently. The peripheral nervous system can be involved quite frequently, with abnormalities being demonstrable on neurophysiologic testing in at least 25% of patients with chronic disease [30]. This is again a mononeuropathy multiplex as is the acute radiculoneuropathy, but symptoms tend to be more indolent, developing more slowly. Pain is less common and patients are more likely to develop subtle distal sensorimotor symptoms such as paresthesias, weakness, or numbness, in more of a stocking glove pattern. In Europe, where a peculiar chronic cutaneous manifestation known as acrodermatitis chronica atrophicans has been recognized frequently, axonal nerve damage is frequently identified in the affected limb [43]. Notably, in the rhesus macaque model of Lyme disease, the only animal model to date that consistently develops nervous system involvement, essentially all infected monkeys develop a mononeuropathy multiplex [44].

The most problematic neurologic disorder associated with Lyme disease has been the confusional state referred to as Lyme encephalopathy [45–47]. This

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disorder was initially described in patients with long-standing untreated infection, most of whom had prominent systemic, extraneurologic symptoms. Typical patients had chronic relapsing oligoarthritis and malaise, and had mental status changes similar to those seen in patients with other chronic inflammatory diseases. All described difficulties with routine intellectual activities and memory, abnormalities that were demonstrable either with a minimental status exam or with formal neuropsychologic testing. Some who appeared otherwise normal neurologically had abnormal brain MRI scans and/or abnormal CSF including demonstrable intrathecal production of specific antibody.

It is likely that at least two mechanisms are responsible for these cognitive problems. In some patients, MRI scans and CSF are abnormal with a mild pleocytosis or elevation of CSF protein, relatively small areas of increased signal on MRIs, and nonfocal neurologic exams. About half of these patients have evidence of intrathecal production of specific antibody [48,49] and it is likely that most have a mild form of encephalomyelitis. Others have normal MRIs and CSF and otherwise normal neurologic exams. It is likely these individuals have a ''toxic-metabolic'' encephalopathy, similar to that seen in patients with other chronic infections or inflammatory disorders. There is some evidence to suggest this could be caused by the production of soluble neuroimmunomodulators in the periphery that could then diffuse into the CNS and affect CNS function [50]. This observation awaits confirmation by others.

Unfortunately, this description has been extrapolated to suggest that any patient with chronic subjective impairment of cognition, memory, or mood may have Lyme disease and should be treated with antibiotics. It is important to stress that the initially described patients all had clear, objective evidence of disease in addition to the mental status changes and all clearly improved with fairly brief courses of antimicrobial therapy.

Controversies

Finally, several areas of considerable controversy continue. First, there has been considerable debate regarding the link between Lyme disease and psychiatric disease. Several case reports have described patients who clearly had Lyme disease and clearly had concurrent psychiatric problems. Proving causality in such isolated instances is difficult. Other series have described patients with psychiatric symptoms in whom a diagnosis of Lyme disease has been made. In most of these, the role of *B. burgdorferi* in the pathophysiology of these patients' difficulties has, at best, been questionable. In the only systematic studies that have been performed to address this issue, it appears that depression and other psychiatric problems are no more common in patients with Lyme disease than in those with other chronic illnesses [48,51,52].

Second, confusion continues regarding a link between Lyme disease and multiple sclerosis (MS). Several possible areas of overlap need to be addressed.

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Lyme encephalomyelitis could be confused with a first attack of MS although, as previously detailed, appropriate laboratory investigations usually permit differentiation between the two. Second, individual attacks of MS are often precipitated by intercurrent infections, probably by a nonspecific mechanism involving production of γ-interferon in the CNS. *B. burgdorferi* infection could certainly be one of the large number of such triggering infections, although there is nothing specific about this mechanism.

Third, there has been considerable discussion about Lyme disease causing a form of motor neuron disease [53]. Because Lyme disease can cause both a myelopathy and a motor polyradiculopathy, neuroborreliosis certainly should be considered in the differential diagnosis of such patients, but again, it should usually be possible to differentiate between the two.

Finally, by analogy to neurosyphilis, patients with strokes attributed to a presumed Lyme-associated vasculitis have been reported [54–56]. Unlike in neurosyphilis, such cases have been quite infrequent and most individual reports have been unconvincing. If this does occur, it appears to be an extremely uncommon consequence of this infection.

Treatment

Despite the presence of several well-controlled and compelling treatment trials, there continues to be considerable controversy concerning optimal therapy for this infection, particularly in patients with nervous system symptomatology. The bacteria itself is quite sensitive to commonly available antibiotics, provided they can reach it wherever it might be sequestered in the body. There is increasing evidence that oral antimicrobials are effective in the majority of patients, and studies have even demonstrated substantial efficacy of oral doxycycline in patients with Lyme meningitis [57]. In general, when the CNS is involved it is probably reasonable to use parenteral third generation cephalosporins such as cefotaxime or ceftriaxone [58,59]. Both are probably equally effective. Although 2-week regimens have statistically been shown to be as effective as 4-week courses [60,61], most centers treating large numbers of these patients have seen some individuals develop late CNS sequelae after shorter courses. Because of this, most currently use 4-week courses of ceftriaxone (and on rare occasion 6 weeks). However, treatment for longer periods of time has no rational basis.

As in any illness causing neurologic damage, recovery is slow. Peripheral axons must slowly regenerate or remyelinate. Although damaged CNS axons cannot regenerate, some clinical recovery does typically occur after even severe damage such as occurs in stroke; however, this is a slow process. Therefore, clinical response cannot be used as an indicator of the adequacy of antibiotic treatment. This is as true with neuroborreliosis as it is with a brain abscess or other CNS-damaging disorder. Therefore treating until symptoms completely resolve is illogical and unnecessary. Second, serologic results—even in the CSF—do not

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revert to negative after successful treatment, and therefore treating until the patient becomes seronegative typically is an exercise in futility. Finally, extended courses of antibiotics expose patients to substantial risk beyond the simple expense.

CONCLUSIONS

Lyme disease, a multisystem infection, frequently affects nervous system function. Involvement usually follows one of several distinct patterns. Peripheral nerve is affected frequently and is involved in a mononeuropathy multiplex that may present clinically as a mononeuropathy, a monoradiculopathy, a cranial neuropathy, or a more disseminated process. CNS involvement tends to be more clinically complex, but can be conceptualized as occurring as either a meningitis (common), a uni- or multifocal white matter disease (rare), or a mild encephalopathy or confusional state. The last can be caused by either a mild encephalitis (rare) or a ''toxic-metabolic'' encephalopathy (common) similar to that seen in many other inflammatory or infectious conditions. In most circumstances, antimicrobial therapy for 2 to 4 weeks is microbiologically curative. In some patients, clinical residua may remain, either as a consequence of neurologic damage or because of a protracted immune response. An animal model of neuroborreliosis, the rhesus macaque monkey, now exists and hopefully will permit a much better understanding of the interactions between this organism and the nervous system.

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Cardiovascular Manifestations of Lyme Disease

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INTRODUCTION

The cardiac manifestations of Lyme disease were first described in 1980 by Steere, who reported a series of 20 cases of Lyme carditis [1]. Cardiac involvement has since been observed in up to 10% of cases of Lyme disease. Clinical manifestations include atrioventricular block, myopericarditis, and transient left ventricular dysfunction. In addition, Lyme disease has been implicated as a cause of chronic dilated cardiomyopathy. This chapter reviews the epidemiology, clinical manifestations, diagnosis, and treatment of Lyme carditis.

EPIDEMIOLOGY

Cardiac Lyme disease is estimated to occur in 2 to 10% of cases of Lyme disease in the United States [1–3]. Involvement of the heart is probably less common in Europe. The exact frequency probably remains unknown because of the underdiagnosis of infection, the subclinical nature of cardiac involvement, and the lack of population-based studies. In a laboratory-based surveillance study identifying 1149 Lyme cases in Connecticut in 1984–1985, 2% of patients had cardiac manifestations [3]. Cardiac involvement was found unrelated to age (relative risk 0.5, 95% CI 0.1–1.7), whereas arthritis was more common in persons less than 20 years old. Moreover, a seasonal variation was observed with a peak incidence of Lyme cases during June and July.

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Lyme carditis occurs during the early disseminated or second stage of infection. Clinical manifestations appear between 4 days and 7 months (mean 4.8 weeks) after the acute illness, which occurs within 3 to 30 days of tick bite [2]. Comparative studies of Lyme disease with and without cardiac involvement are lacking. In 1991, van de Linde reviewed 105 cases of Lyme carditis and compared cases from Europe with those from the United States [4]. In both regions, Lyme carditis was predominant among males (3:1), whereas Lyme disease has no gender predilection. In the United States, only 13% of patients had a known history of tick bite whereas 61% experienced joint involvement and 28% developed neurologic illness. Eighty-two percent of cases in the United States had erythema migrans, which is similar to other reported series of Lyme disease. In these patients, carditis presented a median of 3 weeks after the onset of rash [1].

PATHOLOGY

Once *B. burgdorferi* is injected into a host by tick bite, the spirochete can spread through lymphatics and the blood stream to invade the heart. Lyme carditis appears to be a pancarditis as lymphocytes, plasma cells, and macrophages infiltrate the myocardial interstitium from epicardium to endocardium [5,6]. Other histopathologic findings include myocardial necrosis, neutrophil aggregation, vasculitis, and inflammatory changes of the pericardium. Spirochetes have been identified within the myocardium by immunohistochemical staining [6–9]. It remains unknown as to whether Lyme carditis is the result of local infection or is an inflammatory response. It is hypothesized that the spirochete persistence within the myocardium is a requisite for clinical carditis [2]. However, the number of spirochetes in the heart is typically few and does not correlate with the extent of the inflammatory response [7,8]. This suggests the heart to be a target organ of immune injury. It remains unknown if carditis can occur in the absence of *B. burgdorferi* invasion into the myocardium.

CLINICAL MANIFESTATIONS

Cardiovascular manifestations include atrioventricular block, myopericarditis, and transient left ventricular dysfunction. In addition, *B. burgdorferi* has been implicated as a cause of chronic dilated cardiomyopathy.

ATRIOVENTRICULAR BLOCK

The most common cardiac manifestation of Lyme disease is atrioventricular (AV) block, which accounts for 87% of cardiac involvement [2]. Although heart block is the most commonly appreciated cardiac manifestation, its incidence may not

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be as high as previously described. In a prospective study of 61 patients with early Lyme disease as evidenced by erythema migrans, 1.6% of patients developed heart block [10]. The site of block is variable but most commonly occurs at the level of the AV node as evidenced by narrow complex escape rhythms and electrophysiologic testing [1,6,8]. Among patients with AV conduction disturbances, 98% have first-degree AV block, 40% show Wenckebach rhythm, and 50% develop complete heart block [2]. First-degree heart block may progress to complete heart block within several minutes to several hours. Patients with prolonged first-degree AV block (PR interval >0.3 sec) are at greater risk of developing complete heart block [1,2]. Although the majority of patients (69%) with Lyme carditis experience palpitations [11], complete heart block often results in syncope and light headedness, as there is depression of ventricular response rhythm. In McAlister's series, 20% of patients had escape rates of less than 40 beats/min [2]. Complete heart block may occur in the absence of other symptoms [12,13]. Complete heart block usually resolves within 1 to 2 weeks but may last longer [2]. There are also isolated case reports of atrial tachyarrhythmias and ventricular tachycardia occurring in patients with Lyme disease [14,15]. However, there are too few of these observations to understand their clinical significance.

MYOPERICARDITIS

Myopericarditis of Lyme disease has not been well characterized. Steere reported mild impairment of left ventricular function in one third of patients who underwent assessment of left ventricular function [1]. Although probably uncommon, Lyme carditis has been observed to markedly decrease left ventricular systolic function [9]. Accordingly, patients with Lyme carditis may show an asymptomatic fall in left ventricular ejection fraction. Although Steere noted cardiomegaly on chest radiograph in one patient, left ventricular dysfunction generally occurred in the absence of ventricular dilation on echocardiography. The significance of this finding remains uncertain. Right ventricular dysfunction and reduced left ventricular compliance have been demonstrated in the setting of Lyme carditis and may also contribute to cardiac symptoms [16]. Approximately 5 to 15% of patients with Lyme carditis develop clinical heart failure [4,11]. Signs and symptoms of left ventricular failure include dyspnea, orthopnea, paroxysmal nocturnal dyspnea, and fatigue. The duration of symptoms is brief in most patients. Constitutional symptoms are not specific to Lyme carditis and are commonly seen in other causes of myopericarditis. Physical findings of left ventricular failure may include leftward displacement of the apical impulse, the presence of third and fourth heart sound, systolic murmur of mitral regurgitation, and rales on pulmonary auscultation. Improvement in left ventricular ejection fraction has been observed after approximately a week [2,9].

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Contiguous pericarditis is characterized by chest pain, pericardial friction rub, and electrocardiographic changes. In the United States, clinical findings of pericarditis are seen in 5% of Lyme carditis cases [4]. As heart block is less common in Europe, the relative proportion of cases with pericarditis is significantly higher (23%) than in the United States.

DILATED CARDIOMYOPATHY

Although Lyme carditis was originally described as a self-limiting disorder primarily involving the conduction system, recent reports suggest it to be a cause of long-standing cardiomyopathy and chronic heart failure [15,17]. The isolation of *B. burgdorferi* from endomyocardial biopsy samples of patients with long-standing dilated cardiomyopathy suggested borrelia to be a cause of chronic heart failure. A later study found 33% of 54 patients with dilated cardiomyopathy to be seropositive for antibodies to *B. burgdorferi*. The same group found 72 patients with dilated cardiomyopathy more likely to be seropositive for *B. burgdorferi* than were 55 patients with coronary disease as well as 61 healthy blood donors (26% vs. 23% vs. 8%) [17]. Although these studies suggest a link between Lyme disease and cardiomyopathy, they are inconclusive because positive serology to *B. burgdorferi* only indicates possible exposure. Additional evidence was found in a Dutch study of 42 patients with dilated cardiomyopathy. Of nine patients who were seropositive to *B. burgdorferi* and treated with ceftriaxone, six fully recovered and one showed partial response [18]. Further studies are needed to understand the possible link between Lyme carditis and dilated cardiomyopathy.

DIAGNOSIS

The diagnosis of Lyme carditis is clinical, and based on history, physical examination, and serology. A high index of suspicion is required. The diagnosis of Lyme carditis is difficult to make because its presenting features overlap with other conditions. The presence of flulike illness characterized by fever, chills, malaise, and headache is suggestive of the diagnosis in the setting of AV block. Although relatively few patients report tick bite, the presentation of myocarditis and heart block in areas endemic for Lyme disease or in young patients may prompt suspicion. Seasonal occurrence during the summer months may support the diagnosis. Cutaneous manifestations such as erythema migrans occur in the majority of patients.

Serologic testing is supportive but not diagnostic of Lyme carditis [1,19]. An early rise in IgM titers is more specific than IgG response. Although IgM antibody levels peak between 3 to 6 weeks of symptom onset, they may rise slowly and cardiac involvement can occur early in the course of illness. Therefore, antibody levels may rise after heart block is present [13,18]. Other labora-

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tory abnormalities found in Lyme disease are less specific and include an elevated erythrocyte sedimentation rate, mild anemia, and elevated white blood cell count [19].

Although the electrocardiogram may show T-wave flattening or inversion, ST segment depression, and intraventricular conduction delay, it is still rather nonspecific [1]. Cardiomegaly and pulmonary edema may be evident on chest radiograph. Echocardiography and radionuclide blood pool imaging may show diffuse and regional wall motion abnormalities. Cardiac inflammation has been detected by magnetic resonance and Gallium-67 imaging [8,16,20–23]. Indium-111 monoclonal antibody scans have shown diffuse myocardial uptake during active carditis, which normalized 6 weeks after treatment [12,16]. However, large-scale studies evaluating the utility of these modalities are lacking. Although cardiac involvement has been confirmed by silver staining of endomyocardial biopsy specimen, histologic confirmation does not generally alter management.

Systemic illnesses, which commonly include myocarditis (Rheumatic fever, Rocky Mountain spotted fever, Yersinia, and coxsackie virus), do not typically result in heart block [24]. Yersinia typically results in gastrointestinal symptoms. Rheumatic fever involves the valves of the heart, whereas Lyme carditis does not [24]. Recently, nonspirochete subacute bacterial endocarditis and cardiac myxoma were found to mimic Lyme disease in patients with constitutional symptoms such as skin rash, neurologic symptoms, and seropositivity to *B. burgdorferi* [25,26].

TREATMENT

Cardiac Lyme disease can usually be treated successfully with antibiotics. Although antibiotic therapy has been advocated for treatment in the early stages of Lyme disease, controlled studies of antibiotic therapy on the natural history of cardiac involvement are lacking. Therefore, it remains unknown if intravenous administration is superior to oral antibiotics. Cardiac involvement appears to be self-limiting with resolution of heart block within 2 to 6 weeks in untreated patients. Patients who develop prolonged first-degree or high-grade AV block should be hospitalized and monitored carefully. Although temporary pacemakers are needed in up to 38% of cases, permanent pacemaker implantation is rarely required [27].

Although glucocorticoids and other anti-inflammatory agents have been advocated for some patients [1,4,8,18], the effects of these agents on cardiac manifestations have not been well studied. In Steere's original series, there were no differences in the duration or long-term outcome of heart block in patients treated with antibiotics as compared with those treated with anti-inflammatory agents alone [1]. Glucocorticoid withdrawal resulted in recurrence of neurologic and musculoskeletal symptoms. Until additional studies become available, corti-

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costeroid therapy has been advocated in patients not responding to intravenous antibiotic therapy and in those with high-grade AV block.

After successful antibiotic treatment of Lyme disease, the long-term prognosis appears to be excellent from a cardiac standpoint. As previously mentioned, permanent pacing is rarely required. Sangha studied 176 persons who were previously treated for Lyme disease and found a similar prevalence of ECG abnormalities 5 years after disease onset, as compared with controls [28]. However, another study suggested heart block to be a chronic sequelae of Lyme carditis as *B. burgdorferi* titers were elevated in men with heart block of unknown origin having received pacemakers [29].

SUMMARY

Cardiac involvement occurs in 10% of cases of Lyme disease and is variable. Clinical manifestations include varying degrees of heart block, myopericarditis, and left ventricular failure. The diagnosis of Lyme carditis is clinical and requires a high index of suspicion. Serologic testing may be supportive. Management includes antibiotic administration and supportive therapy with medications for heart failure and temporary pacing. The role of anti-inflammatory agents is unknown. Although half of patients develop complete heart block, permanent pacemaker placement is rarely necessary as cardiac abnormalities resolve within 3 to 6 weeks. *B. burgdorferi* may also play a role in the onset of dilated cardiomyopathy.

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Lyme Arthritis

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INTRODUCTION

This chapter will focus on the musculoskeletal aspects of Lyme disease. Case reports dating back to 1941 describe meningitis and polyneuritis preceded by transient migratory and chronic arthritis [1]. The earliest reports of the disease in the United States eminated from Lyme, Connecticut from the year 1972 [2]. During the course of untreated Lyme disease, the muskuloskeletal symptoms follow distinct patterns.

Steere and colleagues have chronicled information regarding the natural history of muskuloskeletal Lyme disease [3]. In 1987, they reported that about 60% of untreated patients developed transient arthritis after an average of 6 months, and 10% of these patients developed a chronic arthropathy. Thus, along with skin, central nervous system, and cardiac involvement, involvement of the muskuloskeletal system is a major feature of this disease.

EPIDEMIOLOGY

Lyme arthritis has a worldwide distribution, but predominates in temperate climates. It has been reported in much of the United States, northern Europe, Scandinavia, Russia, China, Japan, and Australia [4,5]. In the United States, the three main endemic areas are the following [6,7]: Northeast from Massachusetts to

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Maryland, Midwest in Wisconsin and Minnesota, and West along the California coast. Lyme arthritis is the most common vectorborne arthritis in the United States. There are no studies to suggest gender variation and Lyme disease can occur at any age, although the highest incidence is seen in children under 15 years of age where it may mimic juvenile rheumatoid arthritis (JRA) [2]. There is also higher incidence in middle-aged adults which may possibly be related to vacation-related and recreational activities. There are no studies to suggest any differences in manifestations among various age groups and geographical areas.

PATHOGENESIS

After inoculating the skin, *B. burgdorferi* can spread to any site such as lymph nodes, skin, blood, cerebrospinal fluid (CSF), myocardium, retina, muscles, bone, spleen, liver, meninges, and brain [8–12]. Spirochetes have been identified in these tissues by silverstain or immunofluorescence [13–18]. Spirochetal DNA has been detected by PCR in the joint fluid of most untreated patients throughout the period of arthritis [19,20].

B. burgdorferi evade phagocytosis host defense by two mechanisms: lack of ingestion after adhesion to phagocytes and lack of actual spirochetal degradation after ingestion [21–23]. Specific receptor–ligand interactions may explain the ability of *B. burgdorferi* to bind to various mammalian cells [24]. The platelet integrin receptor αIIb3 is responsible for the localization of *B. burgdorferi* to sites of vascular injury. The predilection of the organism for the joints may be explained by its ability to bind to proteoglycans, including dextran sulfate and heparin [25].

The role of circulating immune complexes in mediating tissue injury has also been studied by Steere and colleagues. Increases in IgM and IgM containing cryoglobulins [26] in association with low serum C3 and C4 levels are found in patients who tend to develop additional manifestations after erythema chronicum migrans (ECM) [27]. Genetic susceptibility plays a role in many rheumatologic disorders. HLA-B27 is associated with ankylosing spondylitis and HLA-DR4–related antigens are associated with rheumatoid arthritis. Chronic Lyme arthritis has been shown in some studies to be associated with HLA-DR2, whereas others have shown an association with the DR4 phenotype [28]. In these genetically predisposed individuals, circulating immune complexes containing spirochetal antigens may localize to the synovium and cause endothelial cell activation manifested by expression of cellular adhesion molecules such as E selectin, ICAM, and VCAM [29]. Cellular and humoral immune responses are initated and proinflammatory cytokines, including IL-1, IL-6, and TNF alpha, are released [30]. Synovial cell hyperplasia occurs, as can be seen on histopathological sections. Vascular proliferation and infiltration of lymphocytes, plasma cells, and mononuclear cells are also seen. Synoviocytes obtained from the pannus by synovectomy

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and grown in tissue culture produce large amounts of collagenase and PGE2 which cause erosion of cartilage and bone as seen on roentgenograms and histopathological sections [31].

PCR has been used to study plasmid DNA targets (eg, DNA encoding outer surface proteins, Osp A or Osp B) and genomic DNA targets (eg, DNA encoding flagellin or 16S ribosomal RNA) [32]. It has been observed that the former are positive more often than the latter and this phenomenon has been called ''target imbalance.''

It is noted that spirochetes when grown in culture develop membrane blebs that are shed into the culture medium. Thus, it is hypothesized that these plasmid-rich DNA blebs may be shed into the synovial fluid along with membrane proteins. These blebs and spirochetal proteins enhance the inflammatory response even in the absence of intact spirochetes which are known to be difficult to isolate [33].

CLINICAL MANIFESTATIONS

The clinical course of Lyme Disease can be divided into three stages. Musculoskeletal manifestations occur during Stages II and III and represent early and late Lyme arthropathies (Table 1).

- Stage I: 3–30 days. Localized erythema migrans [34].
- Stage II: Days–weeks. Dissemination of infection occurs, at which time skin, CNS, and muskuloskeletal systems become involved [35]. Patients may experience migratory pain in the joints, bursae, tendons, muscles, and bone that may last for hours or days at a particular site. Alone with these symptoms, patients may be quite ill with malaise and debilitating fatigue. These symptoms improve or resolve within weeks during the natural course of the disease. Other reported manifestations at this stage

TABLE 1 Musculoskeletal Manifestations of Lyme Disease

Early
• Neck stiffness
• Migratory arthralgias
• Diffuse myalgias
• Backache
Late
• Transient oligoarthritis (knee, shoulder, ankle, elbow, TMJ, wrist, hip, or small joints).
• Chronic arthropathy (knee, shoulder, hip)

are less common and include osteomyelitis [36], myositis [37], panniculitis, and even significant involvement of ocular tissues [38].

- Stage III: Weeks–years, average 6 months. Late Lyme disease. About 60–70% of patients who develop late Lyme disease experience joint swelling and pain especially of the large joints, including the knees [39,40]. The attacks involve one or two joints at a time and last for a few weeks to a few months. Small joints can be involved; however, this arthropathy is rarely symmetrical as the more common pattern is asymmetric large-joint involvement. Knee effusions may progress to form Baker's cysts which may dissect into the calf and rupture. The arthritis may be accompanied by symptoms of fatigue; however, fever and other constitutional symptoms are lacking. These attacks usually decrease in frequency at the rate of 10 to 20% each year even without antibiotic treatment [40]. Joint symptoms may be accompanied by neurologic symptoms, including memory defects, neuropathy, and encephalomyelitis [41–43].

DIAGNOSTIC TESTING [Table 2]

The diagnosis of Lyme arthritis is based on the characteristic clinical picture, exposure in an endemic area, and an elevated antibody response to *B. burgdorferi*. The specific immune response mounts slowly, usually 4 weeks after active infection [44]. An early IgM response is most helpful, and later on, usually after 4 weeks, IgG antibodies can be detected. Enzyme-linked immunoabsorbent assay (ELISA) is used to screen for these antibodies; however, because there is a high

TABLE 2 Laboratory Testing

Nonspecific
• Mild anemia
• Elevated ESR
• Elevated WBC
• Synovial fluid leukocytosis (500–100,000 WBCs)
• Radiography (juxtra-articular osteopenia, erosions or osteophytes and sclerosis)
Specific
• ELISA
• Western blot
• PCR

Abbreviations: ESR, erythrocyte sedimentation rate; WBC, white blood cells; ELISA, enzyme-linked immunoabsorbent assay; PCR, polymerase chain reaction.

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rate of false positivity, the Centers for Disease Control and Prevention recommend that all equivocal or positive results be confirmed by Western blot [45].

Patients with Lyme arthritis usually have the highest levels of antibody to the spirochete, with responses to 12 or more spirochetal proteins. These antibody titres fall gradually over the years and thus are not helpful in assessing the adequacy of treatment. On the other hand, about 5 to 10% of individuals may have asymptomatic infection with *B. burgdorferi*. If these patients and those with a previous history of Lyme infection develop another rheumatic illness, the persisting Lyme antibodies may cause confusion in diagnosis.

PCR has recently been studied as an adjunct to diagnosis in patients with Lyme arthritis. Joint fluids studied were all virtually positive by PCR for genomic and plasmid targets before antibiotic therapy and became negative shortly after treatment [46]. The greatest drawback of PCR is the risk of exogenous contamination leading to false positive results [47]. The exact role of PCR in diagnosis and management of a patient with Lyme arthritis is yet to be defined.

Laboratory abnormalities that especially occur early in the illness include an elevated erythrocyte sedimentation rate, mild anemia, and elevated WBC with shift to the left [48]. Rheumatoid factor and antinuclear antibodies are usually negative. A few patients may have anticardiolipin antibodies but no associated coagulopathy. C3 and C4 levels are generally normal or elevated although it has been reported that patients who develop systemic manifestations after ECM may have low C3 and C4 [27].

Joint fluid exam may show leukocytosis ranging from 500 to 100,000 cells per mm^3 with a polymorphonuclear cell predominence (about 80%), although eosinophilia has rarely been reported [49]. Cryoglobulins are commonly present in the joint fluid [50]. Complement is normal and the antinuclear antibody and rheumatoid factor have been reported to be negative in the synovial fluid.

Radiographic changes are most often seen in the knee(s) and usually occur after arthritis of 1-year duration. In 40% of cases, findings are consistent with inflammatory arthritis manifested by juxta-articular osteopenia and erosions, accompanied by soft-tissue swelling and effusions. In 30%, findings are consistent with degenerative arthritis manifested by osteophytes and subchondral sclerosis. In 30%, radiographic findings are a mixture of inflammatory and degenerative changes [51,52].

DIFFERENTIAL DIAGNOSIS

This section will focus on the differential diagnosis of the muskuloskeletal aspect of the disease (Table 3). During the stage of disseminated skin lesions, a malar rash may mimic systemic lupus erythematosus; however, a careful look into the associated signs and symptoms will help differentiate the two entities.

Later on in the course of the disease, the arthropathy may mimic several

TABLE 3 Differential Diagnosis

Systemic lupus erythematosus
Acute rheumatic fever
Disseminated gonococcal infection
Reiter's syndrome
Rheumatoid arthritis (Palindromic Rheumatism)
Juvenile rheumatoid arthritis
Fibromyalgia
Septic arthritis
Osteomyelitis
Acute podagra
Osteoarthritis
Viral arthropathy

disorders. Acute rheumatic fever presents with preceding sore throat, migratory polyarthritis, and carditis; however, Lyme does not have evidence of previoius streptococcal infection. Valvular involvement does not occur in Lyme disease and joint symptoms are usually restricted to the large joints. Migratory pains in the joints and tendons may also resemble disseminated gonococcal infection. Reiter's syndrome results in large knee effusions; however, a history of exposure to ticks in a Lyme-endemic area and serological evidence will help differentiate the two conditions. In addition, Lyme disease, unlike Reiter's, does not have associated uretheritis, sacroiliitis, and chronic enthesopathy. Occasionally, Lyme arthritis presents as symmetrical polyarthritis mimicking rheumatoid arthritis. In children, confusion with juvenile rheumatoid arthritis is common. Attacks of Lyme arthritis are brief in duration and an antibody response to *B. burgdorferi* with reactivity to 10 or more spirochetal polypeptides helps differentiate between the two entities [53].

Very commonly, differentiation between fibromyalgia and Lyme disease becomes a clinical dilemma. Symptoms of fibromyalgia include diffuse musculoskeletal aching accompanied by fatigue. There are no focal joint symptoms, unlike the arthropathy of Lyme disease. Fibromyalgia is a common disorder that mainly affects women aged 30–60 years. Lyme disease can be one of the many triggers of fibromyalgia but the aches and pains of fibromyalgia are not indicative of active Lyme disease or persistent Lyme infection [54–56]. This should be kept in mind because failure to do so results in overdiagnosis of Lyme disease, particularly in patients with fibromyalgia and low-level positive serological tests which may have a relatively low specificity [57–60]. The treatment of fibromyalgia includes low-dose tricyclic antidepressants, nonsteroidal anti-inflammatory drugs (NSAIDs), and exercise.

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The acute presentation of an inflamed joint may be mistaken for pyogenic arthritis. Negative routine synovial fluid cultures will exclude bacterial infection. Cases of Lyme osteomyelitis have been reported. Bone biopsy has shown *B. burgdorferi*, thus aiding in the diagnosis [61]. Occasionally, Lyme arthritis involving the great toe can resemble acute podagra; however, lack of crystals in the joint aspirate will help exclude crystalline arthritis.

Chronic knee arthropathy associated with Lyme disease can have features of osteoarthritis and chondrocalcinosis, thus making it difficult to determine whether osteoarthritic changes are incidental or truly secondary to chronic inflammation [52].

Treatment

The majority of the musculoskeletal manifestations of Lyme disease can be treated with oral antibiotics unless associated objective neurological involvement necessitates intravenous antibiotics [61]. During early Lyme disease, dissemination occurs and arthralgias and myalgias are the chief musculoskeletal symptoms. These are often accompanied by fever, neck stiffness, skin lesions, photophobia, and headaches. Antibiotic treatment should be initiated. The choice between oral and parenteral should be made after careful neurologic evaluation [62–67].

Once the diagnosis of Lyme arthritis is established, oral antibiotics can be used but a longer duration of therapy is recommended. Doxycycline (100 mg every 12 hours) and amoxicillin/probenecid (500 mg every 4 hours) can be used for 30 to 60 days [68]. Intravenous ceftriaxone can be used to treat arthritis especially when accompanied by CNS involvement. Therapy is recommended for at least 2 weeks [69]. Parenteral penicillin has not been shown to be as effective as oral amoxicillin or doxycycline [70].

It has been shown that a subset of patients with chronic arthritis does not respond well to antibiotics. These patients have been shown to be HLA-DR4 positive and to have antibody reactivity to the OspA and OspB proteins of the spirochete [71]. In these patients, nonsteroidal anti-inflammatory agents and intra-articular steroids have been used to suppress inflammation. Arthroscopic synovectomy may be necessary when the above measures fail to control persistent synovitis [72]. The role of PCR in documenting complete eradication of infection from the joint has yet to be defined [73]. Fibromyalgia, whether accompanying or following Lyme disease, must be recognized and treated appropriately. Otherwise, repeated and prolonged courses of antibiotics will be used without clinical improvement [55,74].

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6

Lyme Vaccine

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INTRODUCTION

Lyme borreliosis is the most common vectorborne disease in the United States; other parts of the world in which this infection occurs include Europe, Russia, Japan, and China [1]. The causal agent of infection is a spirochete, *Borrelia burgdorferi*, which is transmitted by *Ixodes* ticks. Clinical manifestations ascribed to this multisystem disease include erythema migrans, borrelial lymphocytoma, acrodermatitis chronica atrophicans, carditis, arthritis, and neurologic conditions such as seventh nerve palsy [2]. Lyme disease was first reported in the United States in 1977 [3,4]. Today, almost 25 years later, Lyme disease remains a significant cause of morbidity with an increasing incidence in some established endemic regions along with geographic spread to new areas [5]. In the United States alone, approximately 10,000 cases of Lyme disease are reported annually [6].

Substantial antigenic diversity exists among the Lyme borrelia. At the time of this writing, three genospecies that cause disease in humans have been recognized: *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*. *Borrelia burgdorferi* sensu stricto is the only genospecies present in North America, whereas all three species exist in Europe. Interestingly, the heterogeneity of the Borrelia species may explain divergent clinical manifestations on the two continents [2]. Genetic variability is of importance when considering the potential for a cross-protective vaccine formulation.

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BACKGROUND

The first attempt at immunoprophylaxis to prevent Lyme disease began in 1986 when Johnson and colleagues showed that passive immunization protected Syrian hamsters (Table 1). New Zealand White rabbits were immunized with viable cells of either one of two isolates of *Borrelia burgdorferi*, one from Connecticut and the other from Minnesota. The respective immune sera were then injected into separate groups of hamsters 18 hours before introduction of an intraperitoneal challenge with *Borrelia burgdorferi* [7]. The study showed that passive immunization gave cross-protection when the hamsters were challenged with either the Connecticut or Minnesota isolate, but the animals were not protected when challenged with a *Borrelia burgdorferi* isolate from Germany [8]. The German isolate was not characterized but may have had substantial antigenic diversity compared with the U.S. isolates of *Borrelia burgdorferi*, as discussed above. Johnson and colleagues went on to immunize hamsters with an inactivated whole-cell vaccine of *Borrelia burgdorferi* that conferred immunity against challenge with the homologous strain of *Borrelia burgdorferi*. Antibody titers to *Borrelia burgdorferi* measured in hamster sera suggested that peak levels occurred 30 days after receipt of the vaccination, and subsequently an appreciable drop in titer occurred between 30 and 90 days [9, 10]. These studies paved the way for development of an inactivated whole-cell vaccine for use in dogs [11]. Two such preparations are currently licensed in the United States.

A whole-cell vaccine, however, has not been developed for use in humans. The reasons for this are largely theoretical. A whole-cell vaccine potentially might result in unintended immunologic sequelae because antibodies to certain *Borrelia burgdorferi* antigens are known to cross-react in vitro with nerve cell axons, hepatocytes, synovial cells, cardiac, and skeletal muscle proteins. Therefore, a whole-cell vaccine could potentially result in detrimental effects for the human vaccine recipient. Human immune responses to spirochetal antigens might ultimately lead to chronic inflammatory conditions and tissue destruction. The *Borrelia burgdorferi* antigen felt to be responsible for the molecular mimicry is

TABLE 1 Chronology of Lyme Vaccine Development

1977	Disease description
1982	Causal agent linked to disease
1986	Immunoprophylaxis successful in Syrian hamsters
1990	Whole-cell dog vaccine
1990	OspA vaccine studies in mice
1994	OspA vaccine human trials
1998	OspA vaccine granted FDA approval

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the 41 kd flagellin subunit. Under certain specific experimental conditions, hamsters immunized with whole-cell preparations have developed destructive arthritis [12].

Development of a Subunit Vaccine

Subsequent considerations dealt with the possibility of a subunit vaccine. Leading candidates were outer surface proteins (Osp) of *Borrelia burgdorferi*, such as OspA, OspB, and OspC. All of these outer surface proteins could be expressed via recombinant DNA technology in *Escherichia coli* and were highly protective immunogens when injected into experimental animals, particularly mice [13]. Of those studied, OspA has been the most extensively investigated, and was elected the most likely candidate for a successful recombinant protein vaccine. Although animal experiments showed that OspA was immunogenic and protective, there were also concerns with regard to human vaccine development. Substantial heterogeneity in this protein among *Borrelial* isolates in nature exists even among strains geographically restricted to North America. A central question was whether a single OspA protein vaccine would provide cross-protection against diverse borrelial strains. Even if an OspA vaccine were found to be cross-protective for strains of *Borrelia burgdorferi* in the United States, it would most probably falter in Europe because of the greater diversity of the borrelial strains there.

OspA Vaccine: How Does It Work?

The mechanism of action of the OspA vaccine is interrelated to the tick feeding process and natural mode of transmission of *Borrelia burgdorferi* [14]. *Borrelia burgdorferi* is found within the midgut of an unfed vector *Ixodes* tick. In taking a blood meal on an animal host, the *Ixodes* tick will feed for approximately 3 to 4 days. During this interval, secondary to stimulation by the nutrient blood meal, the borrelia within the tick begin to proliferate in number. Also, during the process of tick feeding, *Borrelia* within the tick's midgut migrate to the salivary glands. After reaching the salivary glands, transmission of the spirochete to the host may occur. Interestingly, the time necessary for the chain of events to take place serves as the explanation for why the tick needs to be attached for at least a 48-hour duration for successful transmission of *Borrelia burgdorferi*.

Borrelia burgdorferi present in the midgut of the tick express copious quantities of OspA. During the feeding process, down-regulation of OspA expression occurs concomitant with up-regulation of OspC [14]. What induces this major change in phenotype expression is unknown. By the time the borrelia have arrived at the salivary glands, there is much greater expression of OspC than of OspA. As might be anticipated from this sequence of events, tickborne infection regularly elicits antibodies to OspC in the host with little or no production of OspA antibodies. With down regulation of OspA of the infecting *Borrelia*, it is difficult

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to conceptualize why an OspA vaccine would be successful in preventing *Borrelial* infection in the human host. The answer to this paradox lies in the midgut of the feeding tick. During the 48-hour or more window period from commencement of feeding to transmission of the *Borrelia*, the spirochetes are reduced in number or eliminated from the midgut of ticks that feed on an OspA immunized host [15]. The opportunity for migration to the salivary glands is interrupted. The mechanism for killing of spirochetes in the tick midgut is presumably the OspA antibodies in the ingested blood meal, possibly in conjunction with other serum factors such as complement. This mechanism of action confers high potency because it extends immunity to diverse borrelial strains. It must be kept in mind that the potential for vaccine failure also exists if the *Borrelial* microorganisms have already migrated to the salivary glands of the tick before attachment to the human host, thus precluding the necessary step of elimination at the midgut level. Although the frequency of this event is probably small, it is theoretically possible.

Another approach to developing a human Lyme disease vaccine is using an outer surface protein, such as OspC, that is expressed in early human infection. Furthermore, it has been argued that OspC may be instrumental in facilitating transmission of the spirochete to its human host [1]. A major drawback to this approach is the substantial degree of heterogeneity of OspC protein among isolates of *Borrelia burgdorferi* sensu stricto. OspC protein is even more heterogeneous than OspA, which limits its utility as a suitable vaccine candidate.

Recombinant OspA Vaccine Preparations

Two separate single-protein recombinant OspA vaccine preparations have been administered to human subjects with evaluation in phase I to III trials. These vaccines have been formulated and introduced for human studies by Connaught Laboratories, Inc., and SmithKline Beecham Pharmaceuticals, respectively. The origin of the Connaught vaccine is *Borrelia burgdorferi* sensu stricto strain B31; the SmithKline Beecham vaccine is derived from sensu stricto strain ZS7. In contrast to the Connaught formulation, the SmithKline Beecham preparation is adsorbed with aluminum hydroxide as adjuvant to increase the immunogenicity of the vaccine preparation. At the time of this writing, only the SmithKline Beecham vaccine has been approved by the FDA for prevention of Lyme disease in humans.

A large-scale, multicenter, double-blinded, placebo-controlled efficacy trial of the SmithKline Beecham vaccine preparation was conducted involving adult volunteers from highly endemic areas for Lyme disease in the Northeast [16]. The enrollees, whose ages ranged from 15 to 70 years, were vaccinated intramuscularly with three 30 mcg doses of the recombinant OspA vaccine preparation (*LYMErix*; SmithKline Beecham, King of Prussia, PA) or placebo at 0, 1, and 12 months. A total of 10,936 subjects were enrolled, with 5469 receiving

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vaccine and 5467 receiving placebo. The subjects were then followed for 20 months from the time of the first injection (January 1995 to November 1996). Primary endpoints were the incidence of definite and asymptomatic Lyme disease. Patients with definite Lyme disease had to have objective clinical manifestations such as erythema migrans, neurologic, cardiovascular, or musculoskeletal manifestations, plus laboratory confirmation, which could include a positive skin biopsy culture for *Borrelia burgdorferi*, detection of borrelial DNA by polymerase chain reaction in a skin biopsy specimen, or seroconversion by immunoblot. Subjects were defined as having asymptomatic infection if there were no recognizable clinical manifestations, but IgG seroconversion by immunoblot had occurred between months 2 and 12 of the first year, or between months 12 and 20 of the second year. A secondary endpoint was the incidence of possible Lyme disease defined according to the study as an influenza-like illness with IgM or IgG immunoblot seroconversion, or physician-diagnosed erythema migrans but no corroboratory laboratory results. All participants were asked to inform the investigators if they had any symptoms consistent with Lyme disease throughout the study period.

During the first year, after administration of two intramuscular doses, 22 subjects in the vaccine group and 43 in the placebo group were classified as having definite Lyme disease (Table 2). In the second year of the study after administration of the third vaccine dose, 16 vaccine recipients and 66 placebo recipients were classified as having definite Lyme disease (intent-to-treat analysis). The OspA vaccine preparation was significantly more effective than placebo for the prevention of this category of illness in both year 1, 49% efficacy ($p = 0.009$), and year 2, 76% efficacy ($p < 0.001$). Determination of the incidence of asymptomatic infection was based on IgG immunoblot seroconversion on serum samples collected at 12 and 20 months after entry from each volunteer, in comparison with baseline serum samples collected before vaccination. After two vaccine doses, two subjects in the vaccine group and 13 in the placebo group were regarded as having asymptomatic infection (Table 2). In the second year, after three doses of vaccine all of the 15 seroconverters came from the placebo group (Table 2). The efficacy rate for protection of asymptomatic infection was 83% in year one ($p = 0.004$) and 100% in year two ($p = 0.001$).

Thirty study participants in year 1 and 33 in year 2 were designated as having possible Lyme disease based on the development of a flu-like illness and IgM or IgG immunoblot seroconversion. In addition, 16 patients were considered to have possible Lyme disease based on physician-diagnosed erythema migrans during year 1, and 13 in year 2. The vaccine preparation was not significantly more effective than placebo for this category of Lyme disease, raising the possibility of misdiagnosis. For example, human granulocytic ehrlichiosis without Lyme disease may be associated with IgM seroconversion for *Borrelia burgdorferi* antibodies by immunoblot [17].

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TABLE 2 Lyme Disease and Vaccine Efficacy Rates in Study Population

Lyme Disease	Vaccine (N = 5469)	Placebo (N = 5467)	Vaccine Efficacy (95% CI)	*P* value
Year 1				
Definite[1]	22	43	49% (15 to 69)	0.009
Asymptomatic[2]	2	13	83% (32 to 97)	0.004
Possible[3]				
Influenza-like illness	13	17	24% (−57 to 63)	0.46
Physician-diagnosed erythema migrans	7	9	22% (−109 to 71)	0.61
Year 2				
Definite[1]	16	66	76% (58 to 68)	<0.001
Asymptomatic[2]	0	15	100% (26 to 100)	0.001
Possible[3]				
Influenza-like illness	12	21	43% (−16 to 72)	0.12
Physician-diagnosed erythema migrans	7	6	−17% (−247 to 61)	0.12

[1] Definite Lyme disease: objective clinical manifestations such as erythema migrans, neurologic, cardiovascular, or musculoskeletal manifestations, plus laboratory confirmation by positive culture for *Borrelia burgdorferi*, detection of borrelial DNA by polymerase chain reaction, or seroconversion by immunoblot.

[2] Asymptomatic Lyme disease: no recognizable clinical manifestations, but IgG seroconversion by immunoblot occurring between months 2 and 12 of year 1, or between 12 and 20 months of year 2.

[3] Possible Lyme disease: influenza-like illness with IgM or IgG immunoblot seroconversion, or physician-diagnosed erythema migrans but no corroboratory laboratory results.

The improvement in vaccine efficacy from year 1 to 2 may be explained by the enhanced antibody response after three compared with two doses of the OspA vaccine preparation. When antibody titers were measured at month 2, 1 month after completion of the second dose, 95% of the vaccine recipients had a positive test result for LA-2-equivalent (protective epitope of OspA) antibody (100 ng/ml or greater). Repeat antibody titers at month 13, 1 month after the third vaccine dose, revealed that 99% of recipients had positive results associated with a marked anamnestic response to OspA [16].

The potential for vaccine failure occurs with waning levels of circulating antibodies in an immunized host. Protection of the host will depend on sufficient circulating levels of OspA antibodies at the time of tick attachment, but precisely

what titer of OspA antibodies is adequate to confer immunity and what the rate of OspA antibody loss is after completing the three-dose vaccination series are still unknown. It is likely that OspA vaccine booster doses will be required to sustain appreciable titers of antibody for maintenance of immunity [14], but the proper timing for administration of these boosters and their safety and tolerability are unknown.

Side effects of the SmithKline Beecham OspA vaccine preparation in the efficacy trial were primarily limited to discomfort at the injection site and self-limited systemic reactions. Significantly more subjects in the vaccine group reported soreness and erythema at the injection site (26.8%) when compared with those who received placebo (8.3%), ($p < 0.001$). Similarly, vaccine recipients were significantly more likely to report systemic complaints (19.4%) such as fever, chills, and myalgias in comparison with the placebo group (15.1%), ($p < 0.001$). These systemic complaints occurred within 48 hours after vaccination and lasted a median of 3 days. Of note, there was no significant increase in the frequency of arthritis or neurologic events in vaccine recipients, as compared with the placebo group. This was an important finding because in natural infection OspA antibodies are principally found in patients with Lyme arthritis [18]. Therefore, concern had been raised that an immune response to OspA might cause joint inflammation [19].

There was also no significant difference in the frequency of reported systemic complaints between vaccine and placebo groups, 30 days after vaccination ($p = 0.48$). These data show that immunization with this recombinant OspA vaccine preparation is safe and will reduce but not eliminate Lyme disease in adults. The FDA-approved dosage schedule is identical to that used in the efficacy study of 0, 1, and 12 months; however, this schedule is likely to become shorter based on the findings of additional immunogenicity studies. A randomized, multicenter, open-label study compared the reactogenicity and immunogenicity of a 0-, 1-, 2-month schedule with a 0-, 1-, 12-month schedule. Three doses of recombinant OspA vaccine, administered on either schedule, were equally tolerated and provided a very similar OspA antibody response [20].

The vaccine is not approved for children under 15 years of age, but safety and immunogenicity studies in pediatric populations are in progress. Lyme disease often affects children, therefore a safe and effective vaccine would be a welcome development for this age group. In a recent study, 250 children aged 5 to 15 years were randomized to receive either 15 or 30 mcg of OspA intramuscularly, on a 0-, 1-, 2-month schedule [21]. Preliminary results of this study showed that both dosages elicited what was considered a satisfactory antibody response. Similar to the experience with adults, the majority of adverse events were local injection site pain and discomfort. These results suggest that this recombinant OspA vaccine may also be safe and effective in prevention of Lyme disease in children.

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Less than a quarter of a century after the first report of Lyme disease in the United States, there is now a FDA-approved recombinant OspA vaccine preparation that has been shown to be safe and efficacious for prevention of Lyme disease in adults. Unresolved questions include those pertaining to safety and efficacy in children, and the timing, safety, and tolerability of booster doses.

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7

Tick Paralysis

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The earliest references to tick paralysis are in the diary of Captain William Hovell, who described in his ''Diary of a Journey to Port Phillips in 1824–1825'' the phenomenon of ticks causing paralysis in sheep and cattle in the Illawarra District of Australia [1]. Hovell reported that ''the tick buries itself in the flesh and would in time destroy either man or beast if not removed in time.'' In ensuing decades, British travelers to Australia described the paralysis of sheep, calves, and dogs caused by tick bites. In 1898, the earliest human deaths were recorded, two infants who seized and died with engorged wood ticks found on their necks. In 1912, Todd surveyed over 150 physicians in southern British Columbia; at least 10 cases of paralysis in children after the bites of ticks were described. In 1913 tick paralysis was eventually experimentally produced in animals [1,2].

It is currently recognized that tick paralysis is a worldwide disease seen in humans and other mammals, including cattle, sheep, horses, dogs, cats, deer, bison, and bandicoots. In the United States, it is predominantly seen in the Pacific Northwest and the Rocky Mountain states, but occasional cases have been recorded in Florida, Georgia, Mississippi, North Carolina, South Carolinia, Oklahoma, Texas, Virginia, and as far north as Washington, D.C., Pennsylvania, and New York. Other cases in North America are seen in western Canada. In Australia, the cases of tick paralysis are concentrated along the east and west coastal strips. The disease is also seen in South Africa, Crete, and Eastern and Western Europe [1,3–7].

Most patients are children, predominantly girls; this is attributed to their

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long hair which can hide a tick lurking on the scalp. However, the disease is also seen in adults, predominately in men, presumably because of occupational and recreational exposure.

Consonant with the life cycle of the tick, most cases are seen between April and June. Ticks tend to attach to the skin of the scalp, forehead, behind the ears, the external auditory canal, the neck and hairline, the genitalia, and the upper thigh. The tick does not cause pain and has been mistaken for a blister, mole, or wart. Some patients develop allergic reactions to the tick's toxin. This may manifest as anaphylaxis or a focal reaction with swelling and edema. In Australia, studies have shown patients with local reactions to demonstrate skin test reactivity and IgE antibody directed against salivary gland extracts of *Ixodes holocyclus* [8,9].

More than 60 species of ticks cause paralysis in humans or animals. In North America, six species have been implicated, *Dermacentor andersoni, Dermacentor variabilis, Amblyomma americanum, Amblyomma maculatum, Ixodes scapularis*, and *Ixodes pacificus* [10–12]. Additional genera reported from Europe, Crete, South Africa, and Australia include Rhinincephalus, Hyalomma, Boophilus, and Haemophysalis. In the vast majority of cases, the disease seems to be spread by an engorged pregnant female tick that has fed for at least 5 days, although cases attributed to male ticks have also been reported [13,14].

The toxin that causes paralysis is produced in the salivary glands of the tick, where it has been located by monoclonal antibody directed against the toxin. Within the salivary glands it appears to be located in cytoplasmic granules and chromatin. There are many different toxins among the paralysis-inducing ticks, but similarities suggest the potential for cross protection with a single vaccine. The toxins appear to have three subunits, with toxic effects seen only in the trimeric form. The toxins appear to block acetylcholine release and inhibit motor conduction. A defect is demonstrable in presynaptic release, possibly involving ion channel function. Measurements of neuromuscular function suggest that (1) motor nerve conduction velocity is normal or slightly slowed, (2) compound muscle action potentials are diminished, (3) sensory nerve action potential is normal (although it appears to increase after the tick is removed, suggesting there may be subclinical abnormalities), and (4) EMG is normal, very rarely showing fibrillation [15–18].

The toxin from the Australian paralysis tick *Ixodes holocyclus* has been purified and can cause paralysis in dogs. An antiserum and toxoid have been shown to confer protection in dogs. Antivenum directed against *Ixodes holocyclus* has been used in humans in clinically desperate situations; it may provide some benefit but probably only in bites caused by *Ixodes holocyclus*. Immunologic protection appears possible in that repeated bites have been shown to render animals immune to the subsequent administration of toxin. A functional significance of these toxins remains elusive, but suggestions have included stimulation of respiration to attract ticks by carbon dioxide expiration, local anesthesia, reduction of host motility, anticoagulation, feeding stimulation, and a down-regulation

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of protein synthesis during feeding (toxin production peaks on day 4 of feeding and declines thereafter). Thus, the paralytic effects of the toxin may be incidental to its original purpose [15–18].

Five to 7 days after tick attachment the illness characteristically begins. In a child this is manifested by an initial period of irritability and lethargy for 12 to 36 hours, followed by the onset of bilateral leg weakness. Occasionally, paresthesias are noted but sensory exam is within normal limits (WNL). Because of the weakness, the child appears uncoordinated and has difficulty walking. Paralysis develops over hours and is usually complete within 12 to 24 hours. In many patients it ascends to involve the upper extremities as well. Additionally, some develop the complication of bulbar paralysis, with dysphagia, dysarthria, lingual and facial paralysis, and ocular symptoms, although the sensorium remains clear. Cranial nerve involvement also takes an ascending path, involving the sternomastoid, trapezius, tongue and pharyngeal musculature, followed by the face and extraocular muscles. Respiratory paralysis and death may eventually supervene. Typically, patients are afebrile [1,6,14,15,19,20]. Clinical signs and symptoms that are characteristic of tick paralysis are listed in Table 1.

It has been suggested that the closer the tick is to the central nervous system, ie, if located on the back of the head or the neck, the shorter the incubation period and the more severe the paralytic insult [21].

What has just been described is the classic presentation. However, less-common onsets of illness may be extremely misleading to the clinician. For example, because of the tick's location it may produce only a localized weakness. Thus, patients have presented with unilateral Bell's Palsy attributable to a tick behind the ear or in the external auditory canal. Ticks on the forehead have produced weakness of the frontalis and obicularis oculi muscles. Unilateral leg weakness has been caused by a tick on the genitalia, and a tick on the upper arm has caused a unilateral bracheal plexus neuropathy [3,8,15,22–25].

Table 1 Characteristic Features of Tick Paralysis

Paralysis symmetric and flaccid
Lack of normal and pathologic reflexes
Normal sensory exam
Normal pupils
Preserved sphincter function
Clear sensorium
Lack of febrile response
Normal CSF
No autonomic dysfunction

Abbreviation: CSF, cerebrospinal fluid.

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Table 2 Unusual Presentations of Tick Paralysis

Bell's Palsy
Extraocular muscle palsies
Diplopia
Leg weakness
Arm weakness
Ataxia
Myoclonus
Chorea

Another misleading presentation is the development of ataxia. Although many patients with tick paralysis appear uncoordinated or even ataxic because of the extreme muscle weakness, ataxia per se, even without muscle weakness, may be a presentation of tick paralysis. In addition, patients have been described with myoclonus, choreiform movement, and diplopia from extraocular muscle involvement. Thus, the localized and atypical neurologic complications may pose a difficult challenge (Table 2) [14,26,27].

Non-neurologic complications include myocarditis and other tickborne diseases that may be spread by the tick in addition to tick paralysis [5,28]. Such additional infections should be considered if patients thought to have tick paralysis also have fever, which is not a part of the tick paralysis syndrome. For instance, a 4-year-old boy in western Colorado contracted both tick paralysis and Colorado Tick Fever from a single tick bite [29]. In the United States, the potential exists for other such double infections; Table 3 lists tickborne infections for which the tick vector also causes tick paralysis.

Treatment of tick paralysis comprises supportive care and removal of the tick. Ticks should be removed by gentle traction, with care not to leave the mouth parts (hypostome). Gloves should be worn if there is hand contact with the tick. Removal is facilitated by first applying chloroform, fingernail polish, ether, alcohol, gasoline, kerosine, glycerol, ethyl chloride, or a glowing match or cigarette to the tick [5]. The tick should not be squeezed. If the hypostome is left in the skin, it should be removed surgically.

A patient usually feels better within hours after tick removal. The rapidity of response is somewhat dependent on geographic location because the toxin seems to vary in potency among different ticks. Thus, in North America after removal of the tick the patient usually recovers in hours or days. The paralysis may continue for a few hours after the tick removal but thereafter most patients steadily improve. However, in Australia, where illness due to *I. holocyclus* tends to be more acute, paralysis may continue for 48 hours after the tick is removed

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TABLE 3 Classic Tickborne Infections for which Tick Vector May Also Cause Tick Paralysis

Tickborne infection	Tick vector that may also cause tick paralysis
Lyme disease	*I. scapularis*
	I. pacificus
Tularemia	*A. americanum*
	D. andersoni
	D. variabilis
Rocky Mountain Spotted Fever	*D. variabilis*
	D. andersoni
Ehrlichiosis	*D. variabilis*
	A. americanum
Colorado Tick Fever	*D. andersoni*
Babesiosis	*I. scapularis*
	I. pacificus

and recovery may take weeks. This is attributed to a more potent toxin. In general, if the tick is removed before symptoms appear, tick paralysis does not occur. If it is removed during the illness but before bulbar weakness, patients tend to recover completely within hours or days. However, if bulbar weakness has supervened, respiratory paralysis may develop with an overall mortality of 10 to 12%. Rarely, patients have prolonged weakness after removal of the tick, sometimes for as long as 6 months [1,7,14,30–35].

It is crucial to look for additional ticks, especially if there is no improvement or if there is an exacerbation of illness after the tick is removed. It is not unusual for patients to have more than one tick, and consequences are disastrous if only one paralysis-inducing tick is removed.

The differential diagnosis of tick paralysis is extensive. The illnesses most commonly confused with tick paralysis are Guillain-Barré Syndrome, polyradiculomyelitis, botulism, poliomyelitis, transverse myelitis, cord compression, and myasthenis gravis. Helpful differentiating points are presented in Table 4. A more comprehensive secondary list of differential diagnostic considerations would include additional peripheral neuropathies (porphyria, diphtheria, medication-induced, poisoning caused by organophosphates, arsenic, and thallium), myopathies (medication-induced, myositis, hypophosphatemia), and spinal cord processes (syringomyelia, AIDS-related vascular myelopathy, ischemia) [1, 3,14,19,20].

Clinicians should include tick paralysis in their differential diagnosis of any patient who has visited tick-endemic areas in the spring or summer and pre-

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TABLE 4 Major Differential Diagnoses

	Guillain-Barré	Botulism	Polio	Spinal cord compression	CMV polyradiculo-myelitis	Myasthenia gravis	Transverse myelitis	Tick paralysis
Ascending weakness	+	−	−	+	−	−	+	+
Symmetric weakness	+	±	−	±	+	±	+	+
Bulbar involvement	−	+	+	−	−	−	−	+
DTRs	↓	WNL or ↓	↓ (↑Early)	↑ (↓Early)	↓	WNL	↑ (↓ Early)	↓
Parasthesias	+	±	+	+	+	−	+	+
Sensory loss	±	−	−	+	+	−	+	−
CSF	↑ ↑ Protein	WNL	↑ Protein; pleocytosis (lymphs)	↑ Protein; pleocytosis (lymphs)	↑ Protein ↓ glucose pleocytosis (polys)	WNL	↑ Protein; pleocytosis (lymphs)	WNL
Bladder/bowel dys-function	−	+	+	+	+	−	+	−
Important in DX	EMG	EMG	Culture; serol-ogy	MRI	MRI PCR	Response to cholinergic drugs. EMG	MRI	Find the ticks
Additional com-ments	Preceding in-fection, ESP with *Campy-lobacter je-juni*	Dilated pupils; GI symp-toms; dys-phonia; dys-phagia; diplopia	Fever; menin-gismus; un-immunized patients	Flexor plantars	Seen in AIDs; may re-spond to antiviral Rx	Ocular weak-ness; fluc-tuates; anti-body to AChR	Antecedent infection; flexor plantars	May be ataxic

Abbreviations: CMV, cytomegalovirus; DTRs, deep tendon reflexes; WNL, within normal limits; CSF, cerebrospinal fluid; DX, diagnosis; EMG, electromy-ography MRI, magnetic resonance imaging; PCR, polymerase chain reaction; GI, gastrointestinal.
From Refs. [1,3,14,19,20,36].

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sents with symmetric paralysis, acute ataxia, or the atypical presentations listed in Table 2. Avoidance of ticks is maximized by wearing long pants tucked into socks and long sleeves, avoiding heavily wooded paths, and the use of chemical deterrents. After potential exposure, the skin and hair should be inspected carefully and any ticks removed as previously described. Tick paralysis is uncommon but important to consider because, while potentially fatal, it is a thoroughly curable disease.

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8

Babesiosis

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Babesiosis is a malaria-like illness caused by an intraerythrocytic protozoan that is transmitted by *Ixodes* ticks. This zoonotic infection occurs in a variety of wild and domestic animals, including mice, rodents, deer, dogs, and cattle. Humans are opportunistic hosts because they are infected only when the regular cycle of transmission between the tick vector and the animal host is interrupted.

The first historical mention of babesial infection may have been the biblical reference to a plague of murrain (hemoglobinuria) among cattle and other domestic animals (Exodus 9:3). Victor Babes first identified the causative microorganism of babesiosis in 1888 when he described the presence of intraerythrocytic parasites in cattle with febrile hemoglobinuria [1]. Five years later, Smith and Kilbourne discovered that *Babesia bigemina*, the Texas cattle fever pathogen, was transmitted by ticks [2]. The first human case of babesiosis was reported in 1957 in a 33-year-old asplenic cattle farmer from Yugoslavia [3]. In 1969, a case of babesiosis in a patient with an intact spleen was reported from Nantucket Island, Massachusetts [4]. Since then, babesiosis has been recognized as an emerging endemic disease in the northeastern and northern-midwestern United States with sporadic cases reported in the far west as well as in Europe and Asia.

MORPHOLOGY

There are approximately 100 species of *Babesia* [5]. Most are small (1.0–5.0 m in length) and oval, round, or pear-shaped. A few features distinguish babesia from the plasmodium species that cause malaria. These include formation of a

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tetrad known as a ''Maltese cross,'' the absence of pigment granules in infected erythrocytes, and the presence of extracellular merozoites. Unlike plasmodium merozoites, which are released from erythrocytes in synchrony, babesia species reproduce by asynchronous, asexual budding. This asynchrony decreases the possibility of sudden extensive hemolysis and explains the lack of periodicity of symptoms that characterizes malaria. An additional difference is the lack of an exoerythrocytic stage for babesia in the vertebrate host.

EPIDEMIOLOGY

Babesia species have a wide host and geographic range. In general, *B. bovis, B. bigemina, B. divergens*, and *B. major* infect cattle, *B. equi* infect horses, *B. canis* infect dogs, *B. felis* infect cats, and *B. microti* infect rodents. Five species are known to cause disease in humans. *B. microti*, WA-1, a strain originally isolated from a resident of Washington state, and MO-1, a strain isolated from a resident in Missouri, are the causative agents in the United States, whereas *B. divergens* and *B. bovis* are found in Europe. The clustering of cases of human *B. microti* infection in the United States contrasts with sporadic occurrence of the disease in other regions of the world. In the United States, most human cases have been reported from the coastal areas of southern New England and eastern Long Is-

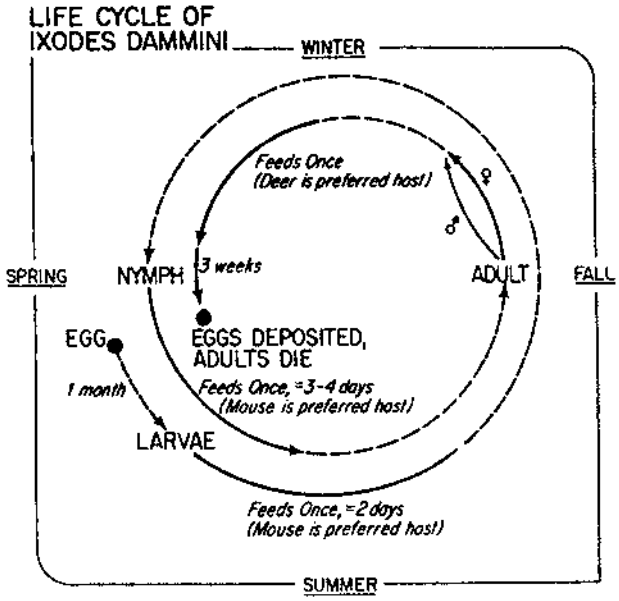

FIGURE 1 Life cycle of *Ixodes dammini.*

land [6]. However, cases have also been reported in California, Minnesota, Missouri, Wisconsin, and Washington State [6–10].

The parasites are usually transmitted by hard-bodied ticks of the *Ixodes* genus. In the northeastern United States, *B. microti* is transmitted by *I. scapularis* (also known as *I. dammini*) [11]. The precise vector for WA-1 has yet to be identified but it is suspected to be *I. pacificus* [8]. Bovine babesiosis is transmitted by *I. ricinus* in Europe [12]. The life cycle of *I. dammini* spans 2 years and has three active stages: larva, nymph, and adult (Fig. 1). Each takes a blood meal from a vertebrate host in order to mature to the next stage. Disease is transmitted to humans by the nymph between May and September and occasionally by the adult tick outside the usual transmission season. The major reservoir host of *B. microti* in the northeastern United States is the white-footed mouse (*Peromyscus leucopus*). The white-tailed deer (*Odocoileus virginianus*) is the principal host of the adult tick. Restocking of deer populations, curtailment of hunting, and elimination of natural predators have increased the deer herds, resulting in an increase in the tick population and the incidence of human babesiosis [12].

Rarely, babesiosis is acquired through blood transfusion [13,14]. Whole blood, frozen erythrocytes, and platelets have been implicated. The incubation period in these cases appears to be between 6 to 9 weeks. Transplacental/perinatal transmission of *B. microti* leading to clinically evident infection in infants has also been reported [15].

PATHOGENESIS

Erythrocyte lysis is thought to be responsible for many of the clinical manifestations and complications of the disease, including fever, hemolytic anemia, jaundice, hemoglobinemia, hemoglobinuria, and renal insufficiency [16]. Ischemia and necrosis result from obstruction of blood vessels by parasitized erythrocytes, and may cause hepatomegaly and hepatic dysfunction, splenomegaly, and cerebral abnormalities. The mechanism of erythrocyte hemolysis remains unknown. Electron microscopic studies have shown damage to the red blood cell membrane, including protrusions, inclusions, perforations, acanthocytosis, and stomatocytosis. *B. microti* probably reduces the deformability of the red blood cells it infects, thereby facilitating their removal by way of the spleen [16].

Patients with acute babesiosis have a significant increase in B cell number with polyclonal hypergammaglobulinemia. The levels of T lymphocytes bearing the IgG Fc receptor are also significantly elevated, and responses to nonspecific mitogens are suppressed. Increased levels of circulating immune complexes and reductions in C3, C4, and hemolytic activity suggest activation of the classical complement pathway [17]. The spleen is thought to protect against babesial infections by reticuloendothelial cell removal of parasites from infected erythrocytes

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through ''pitting'' (ingestion of infected cells) and through the production of antibabesial antibody.

SYMPTOMS

The clinical spectrum of *B. microti* infection ranges from subclinical or self-limited flu-like illness to fulminant disease resulting in death. In normal hosts, babesial infection is frequently asymptomatic and detected only by retrospective serological surveys [18]. Symptoms typically begin within 1 to 6 weeks after a tick bite [19]. There is often no recollection of tick bite by the patient because the engorged *I. dammini* is only 2 mm in length. A gradual onset of malaise, anorexia, and fatigue are commonly observed in the early stages of the disease, followed by fever ranging from 37.8°C to 40°C. Other common symptoms include nausea, vomiting, headache, chills, sweats, myalgia, and arthralgia [3–4,7–10,13,15,18–19]. In our review of 162 patients in southern New England, the most frequently reported symptoms in children and adults were fever and fatigue (Table 1). Less common symptoms of babesiosis are lability, depression, hyperesthesia, sore throat, abdominal pain, conjunctival injection, photophobia, weight loss, and unproductive cough. Despite an occasional protracted course, the majority of patients with *B. microti* infection eventually recover completely. By contrast, severe infection and death following *B. divergens* infection in Europe are common [20]. All reported cases in Europe have been in asplenic individuals.

The findings on physical examination generally are minimal, often consisting of fever alone. Splenomegaly or hepatomegaly may be noted. Slight pharyngeal erythema, petechiae, ecchymoses, jaundice, and retinopathy have also been reported. Rash similar to erythema chronicum migrans has been described but is probably caused by concurrent Lyme disease. Nonspecific laboratory findings may include a decreased hematocrit and platelet count, a normal or decreased white blood cell count, and an elevated erythrocyte sedimentation rate (ESR).

TABLE 1 Frequency of Clinical Manifestations of Babesiosis (%)

	Fever	Fatigue	Headache	Aches	Chills	Joint pain	Sweats	Joint swelling
Children	100	75	75	50	50	25	0	0
Adults	84	97	62	76	68	38	54	5

* Data are based on a review of 162 patients with babesiosis from southern New England (Sharan KP, Krause PJ, Sikand VJ, et al. Babesiosis and babesiosis/Lyme disease coinfection in children and adults. The Society for Pediatric Research Annual Meeting, New Orleans, LA, 1998.)

Liver enzymes and blood urea nitrogen are sometimes elevated. Urinalysis may reveal proteinuria and hemoglobinuria.

Patients at risk for severe babesiosis include those who lack a spleen, are immunocompromised because of infection with HIV or corticosteroid therapy, are over the age of 40, are coinfected with the agents of Lyme disease or ehrlichiosis, or have received repeated blood transfusions. These patients may have serious illness characterized by high fever and severe hemolytic anemia resulting in death or a prolonged convalescence. Severe hemolytic anemia and hemoglobinuria are sometimes associated with renal failure. Seven cases of acute respiratory failure have been reported in patients with severe babesiosis [21] who had acute noncardiac pulmonary edema secondary to an increase in capillary membrane permeability. Cerebral babesiosis has been reported as a major complication in patients with fulminant babesial illness, but the pathophysiology is poorly understood. Coinfection with babesiosis and HIV infection may result in severe febrile illness followed by recurrent parasitemia and a chronic infection that may require therapy to prevent relapse of disease [22]. Coinfection with babesiosis and Lyme borreliosis or ehrlichiosis is commonly observed [23,24]. These three organisms are maintained naturally in the environment by the same tick vector and reservoir host. Coinfection with two or three agents usually results in a more severe and prolonged illness. Children are infected with *B. microti* as frequently as adults. They generally have a more benign clinical course than adults, although severe infection that requires hospitalization may occur.

DIAGNOSIS

Babesiosis should be suspected in any patient with unexplained febrile illness who has recently lived or traveled to an endemic region during the months of May to September, with or without a history of tick bite.

During the acute phase of babesiosis, definitive laboratory diagnosis can be made by direct identification of the causative agent in Giemsa-stained peripheral blood smears. Multiple examination of both thick and thin smears is preferable because most documented cases of human babesiosis have had a low parasitemia [25]. The level of parasitemia usually ranges between 1 and 10% in normal hosts and up to 85% in asplenic and other high-risk individuals. The predominant forms in most of the blood smears are small, round to oval ring-shaped intraerythrocytic structures that closely resemble those of *Plasmodium* species (Fig. 2). Although uncommon, tetrad forms (Maltese cross) are pathognomonic of the disease, which together with the absence of pigment granules in infected erythrocytes distinguish them from *Plasmodium*. Reliable identification of babesia has been reported in peripheral blood using the Quantitative Buffy Coat technique [26].

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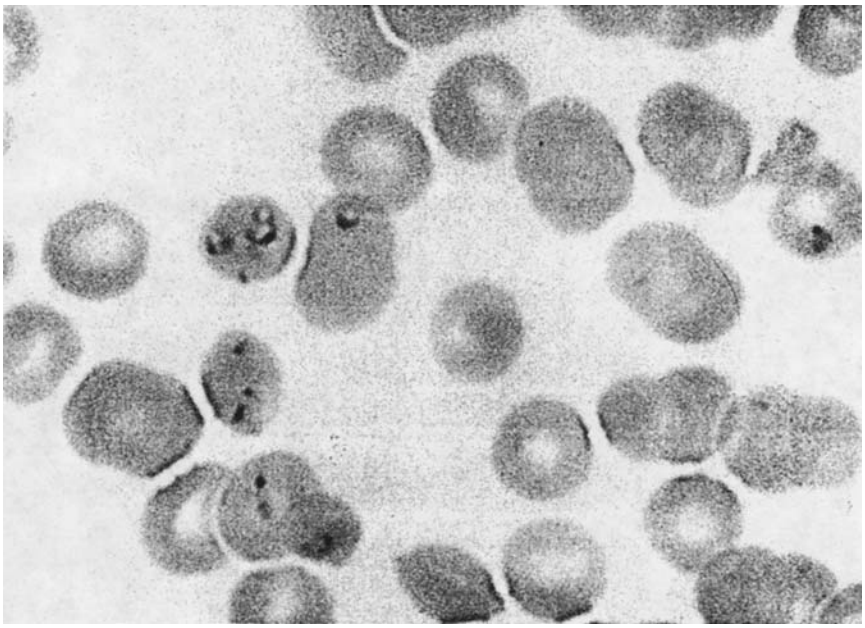

FIGURE 2 ***Babesia microti*** **on thin blood smear. The predominant forms in most of the blood smears are small, round to oval ring-shaped intraerythrocytic structures that closely resemble those of** ***Plasmodium*** **species.**

Specific diagnosis of babesiosis can be made by serological evaluation [27,28]. Of the commonly used serologic tests, indirect immunofluorescent assay (IFA) is the most reliable. Patients with a babesial antibody titer of 1 to 64 or higher are generally considered to be seropositive, whereas those with a titer of 1 to 1024 or greater are usually actively or recently infected. Cross-reactivity with other *Babesia* species and *Plasmodium* can occur.

In cases that are difficult to diagnose by smear or serology, detection of even mild parasitemia can be accomplished by inoculating the patient's blood into a hamster. Parasitemia is amplified to detectable levels within the hamster from 2 to 4 weeks after inoculation. This method of confirmatory diagnosis requires a specialized laboratory. The *B. microti* polymerase chain reaction (PCR) is more sensitive and equally specific, and should supplant hamster inoculation [29,30]. This method can detect *B. microti* within a day and can also be used to monitor infection or carry out epidemiological surveillance.

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THERAPY

In the past, symptomatic cases of babesiosis were treated with chloroquine because of frequent misdiagnosis as *P. falciparum* infection. Chloroquine provides only symptomatic improvement with no reduction in the degree or duration of parasitemia and is therefore not recommended for treating babesiosis. The current therapy of choice is the combination of clindamycin (20 mg/kg/day) and quinine (25 mg/kg/day). The combination was first used in an 8-week-old infant who acquired babesiosis through blood transfusion and failed treatment with chloroquine [31]. Her favorable outcome led to the subsequent use of clindamycin and quinine in many children and adult patients with prompt clearing of parasitemia and resolution of signs and symptoms. However, this combination frequently produces untoward reactions. About a fifth of treated subjects fail to complete the prescribed 7-day-regimen because of tinnitus and abdominal distress [32]. Several other drugs have been tried with varying degrees of success. A pentamidine and trimethoprim-sulfamethoxazole combination has proved to be moderately effective in decreasing symptoms and parasitemia [33]. Potential adverse reactions with pentamidine therapy, including pain at the site of injection, formation of sterile abscess, and nephrotoxicity, limit the efficacy of this combination. Recent observations suggest that atovaquone and azithromycin may effectively clear parasitemia with few side effects [32].

Treatment failures have been reported with clindamycin and quinine in patients with splenectomy, HIV infection, or those receiving concurrent corticosteroid therapy. Red blood cell exchange transfusion, combined with clindamycin and quinine, was found to be curative in these cases. Exchange transfusion should be considered only in severe cases of babesiosis with rising parasitemia [34]. In combination with clindamycin and quinine, it is the treatment of choice for all cases of *B. divergens* babesiosis.

PREVENTION AND CONTROL

Prevention of babesiosis can be accomplished by avoiding ticks and tick-infested areas during the transmission season. High-risk groups should be especially careful to avoid areas where deer ticks are found in abundance. When exposure is unavoidable in endemic areas, clothing that covers the lower part of the body should be used. The use of an insect repellent containing diethyl toluamide is recommended. A search for ticks on people and pets should be carried out. If found, the ticks should be removed as soon as possible by grasping the mouth parts with tweezers without squeezing the body. No data exists to recommend administration of prophylactic antibiotics after a tick bite to prevent babesiosis.

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The risk of a community acquiring babesiosis is directly related to the intensity of the vector tick population. In general, tick control methods fall into three major categories [35]. Ecological control involves vegetation management such as brush cutting and removal; controlled burns may also prove useful because deer ticks are dependent on humid microclimates. Chemical control involves killing the tick population by application of acaricide to vegetation or by targeted application using impregnated cotton that mice carry back to their nests. Biological control involves use of vaccines. Effective *B. bovis* and *B. bigemina* vaccines have been developed for use in cattle but no vaccine has been developed for use in humans. Transfusion-related cases can be prevented by excluding prospective blood donors who reside in endemic areas and present with a history of fever within the preceding 1 to 2 months or who have a history of babesiosis.

FUTURE DIRECTIONS

Studies have been undertaken to determine whether treatment with low doses of natural human interferon alpha administered by various routes inhibits the development of the *Babesia* protozoan. In one such study, human interferon alpha given intramuscularly significantly inhibited development of parasitemia compared with infections in control mice [36]. Attenuated vaccines may provide effective protection against infection.

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9

Rocky Mountain Spotted Fever

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INTRODUCTION

Rocky Mountain spotted fever (RMSF) is the primary rickettsial spotted fever seen in North America. Related rickettsial spotted fevers are known by various names in Latin America: Sao Paolo typhus in Brazil, Fievre Manchada in Mexico, Fievre Petequal in Columbia, and Fievre Maculosa in other countries. In Britain, it is termed tick typhus. Although RMSF of North America appears to be identical to the spotted fevers of South America, the tickborne rickettsiosis of Africa, Asia, Europe, and Australia have all been termed tick typhus. These rickettsiae are all immunologically related to *R. rickettsii,* the agent of RMSF. They differ in clinical presentation from RMSF in having an ulcer or eschar present at the primary site of inoculation, in contrast to RMSF which has no ulcer or eschar [1,2].

RMSF was first recognized and recorded in the Rocky Mountain region of the United States by Major W. W. Wood in 1896. Wood described several mild cases in Idaho with a mild mortality and morbidity. In 1899, Maxcy, also describing cases from Idaho, provided the first clinical description of the symptoms and signs of RMSF. Wilson and Chownings in 1902 were the first to suggest that the illness described by Maxcy in Idaho and McCullough in Montana was transmitted by the wood tick. Ricketts in 1906 confirmed the wood tick as the vector of RMSF. Ricketts also showed that the organism could be transmitted to guinea pigs from infected humans. In 1919, Wolbach described the pathology of the

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disease in humans and ticks, and was the first to show intranuclear multiplication of rickettsia in cells from ticks. Wolbach was also the first to show the key pathophysiological feature of RMSF, ie, vasculitis, in his classic monograph published in 1917. Blank was the first to culture rickettsia in mammalian cells, eg, fertile hens' eggs [2–4].

The initial serological diagnosis of RMSF was based on the observations of Weil and Felix. In 1916, Weil and Felix isolated a gram-negative bacillus from cultures of patient materials infected with typhus. The gram-negative bacillus was a *Proteus* and not agglutinated by antityphoid sera or from sera from paratyphoid cases of *Shigella* dysentery. Weil and Felix found that the sera from their typhus patients was agglutinated by suspensions of *Proteus. Proteus* agglutinin titers cross-reacting with the patients' typhus persisted for 1 to 2 weeks and rose as the rash reached its peak. These studies became the basis of the Weil-Felix reaction formerly used to diagnose RMSF and other rickettsial spotted fevers. Although *Proteus* is not at all related to the genus *Rickettsiae,* some strains of *Proteus,* fortuitously in this case, have antigen determinants in common with the *Rickettsiae* that is the basis for the Weil-Felix reaction. Serological diagnosis was critical because working with rickettsial organisms was dangerous. Von Prowazek and Ricketts died from working with the *Rickettsiae* that causes epidemic typhus. Later, Da Rocha-Lima named the causative organism of epidemic typhus *R. prowazekii* in honor of Ricketts and von Prowazek. Da Rocha-Lima was among the first to differentiate typhus from typhoid fever and show that rickettsial organisms multiplied in the cytoplasm of infected mammalian cells. Although more specific serological tests are available today, a positive Weil-Felix reaction is helpful. Rickettsiae are readily grown in the laboratory in chicken eggs [2–5].

Aside from showing the tick insect vector, ie, the wood tick, the pathophysiology of the disease, eg, vasculitis, the obligate cellular nature of the pathogen, eg, growth in egg yolk cultures, RSMF remained an often lethal disease until the advent of antibiotics with antirickettsial activity. In 1948, chloramphenicol and subsequently the introduction of tetracyclines greatly reduced the mortality and morbidity of patients with RMSF who were treated early. Our appreciation of the epidemiology of RMSF has changed from the initial early descriptions because most cases of RMSF are no longer found in the Rocky Mountain area. The distribution of RMSF is presently dependent on the distribution of various tick vectors [1–3].

MICROBIOLOGY

Rickettsiae are intermediate in size between viruses and bacteria and are often paired in a shape resembling pneumococci, eg, Lancelot-shaped diplococci. Like pneumococci, *R. rickettsii* are often surrounded by a clear zone resembling the

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capsule of pneumococci when stained. Rickettsiae stain poorly by the Gram stain method and are better seen using Giemsa or other stains. They develop primarily in the cytoplasm of infected mammalian cells. In ticks, *R. rickettsii* is more pleomorphic and tends to stain more deeply. Optimal culture temperature for rickettsiae is 35°C in chick embryo cells and at 32°C in mammalian tissue culture cells. Rickettsiae are easily inactivated by heat and destroyed by exposure to a temperature of 55°C for 10 minutes. They are susceptible to drying and do not survive drying for more than 10 hours. Rickettsiae are preserved and not destroyed by freezing. The rickettsiae are viable when tissue specimens are thawed [1,6,7].

EPIDEMIOLOGY

In 1902, Wilson and Chownings recognized *Dermacentor andersoni* as the insect vector of RMSF. *Dermacentor variabilis,* the dog tick, is the primary vector of the East Coast, Southeast, and Central Plains. *Amblyomma americanum,* the Lone Star tick, is the primary vector in the south central states. Ticks feed on a variety of small mammals and rodents, some of which are susceptible to RMSF. The nymph hibernates through the winter as do the adult ticks. Adult ticks become active in the spring and early summer and feed through the late fall and early winter. Ticks in the South and in warmer climates actively feed throughout most of the year. Wood ticks live on shrub and bush-covered ground, which are feeding areas for the ticks' mammalian hosts. Ticks position themselves on the lower branches of bushes and shrubs and attach to passing mammals in contact with the vegetation. The dog tick transmits the infection from dogs to humans. The activation or virulence in the tick is temperature dependent. Virulence increases as temperature increases, and therefore *R. rickettsii* are maximally virulent from spring to fall. The period of attachment of the tick on the mammalian host is also greatest during this period. These two factors account not only for the seasonal distribution of RMSF but its increased virulence in the summer months [8–11].

Dogs and many small mammals, including wild rabbits, are the primary animal reservoirs and may be naturally infected. Tick bites are painless and produce no local ulcer or eschar. For this reason, humans, especially children, may not be aware of the tick bite. Rickettsiae may gain entrance into the blood of the infected mammalian host via contamination by saliva or tick feces. *Rickettsiae* remain alive in fresh or dried tick feces for hours. If a tick does not remain in place on the mammalian host for long, the organism in the tick feces may infect the wound after the tick has dropped off or been removed. In general, the longer the tick is in place in feeding, the more likely the RMSF will be transmitted in the organism is present. The geographical distribution of RMSF mimics the distribution of the tick vectors and is most common in the northeast/mid-Atlantic states.

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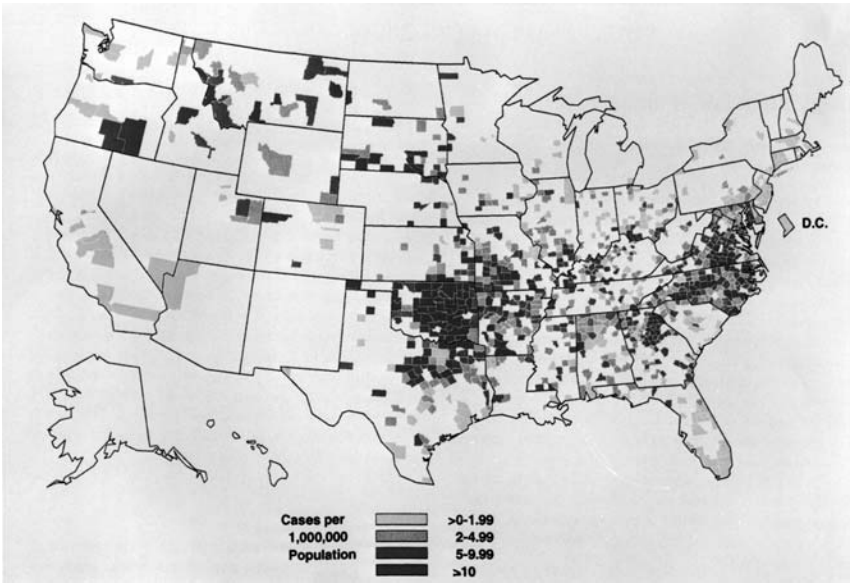

FIGURE 1 Geographical distribution of RMSF in the United States. From Ref. 30.

Initially recognized in the Rocky Mountain states, RMSF occurs in practically all states (except Maine, Hawaii, and Alaska) in the United States, especially the Atlantic states. It is limited to the western hemisphere. From May to September, when adult ticks are active, human infections are most likely to occur in tick-infested areas. Cases may occur in southern states throughout the year. The incidence is high in children less than 15-years-old and in others who frequent tick-infested areas for work or recreation (Fig. 1) [12–22].

PATHOPHYSIOLOGY

R. rickettsii have a predilection for blood vessels. RMSF is pathophysiologically an endovasculitis involving the small blood vessels. The clinical findings are out of proportion to the extent of anatomical lesions. Rickettsial organisms are thought to be disseminated via the bloodstream after the tick bite. The organisms have a predilection for the brain, central nervous system, skin, and heart, but all organs may be involved in the infective process. The liver is frequently grossly involved in about one third of cases, presenting with a nutmeg appearance or yellow color with some intracellular swelling of the liver parenchyma. The spleen

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is usually enlarged and the splenic pulp later becomes friable with prolonged infection. The kidneys are slightly swollen and the cortex is pale. Petechial hemorrhages may be noted in the capsule medulla or renal pyramids. The gastrointestinal tract is involved in about 50% of cases of RMSF at autopsy. Gastrointestinal hemorrhage may occur from petechiae from the small intestine or colon. The skin and subcutaneous tissues best show the characteristic lesions of the rickettsiae in blood vessels. Initially, rickettsiae invade the nuclei of endothelial cells and capillaries. In the endothelial cells, the rickettsiae multiply and destroy the cells resulting in the death of the cells. The initial endothelial lesion extends centrifugally along the intima eventually invading the smooth muscle cells of the media. Extension to large arteries is more common with RMSF than with other rickettsial infections. Lymphatics and venous involvement are not a feature of RMSF. Arterial necrosis and thrombosis result in thrombus formation and micro-infarcts. These occur primarily in the skin, subcutaneous tissues, and central nervous system. The petechial lesions characteristic of RMSF are responsible for hemorrhages in organs having endovascular damage manifested by petechiae on gross anatomical examination [1–3]. The fundamental site of invasion by the rickettsiae are the capillaries, often followed by a perivascular inflammatory response mediated by macrophages. This periphery reaction may be seen pathologically. Ordinarily the lungs are only minimally involved in RMSF, and limited areas of focal interstitial infiltration occur. Later in the course of the disease, a diffuse capillary involvement may result in ARDS. The genitalia and the adrenals are not involved in the diffuse vasculitis of RMSF. Right ventricular and right atrial enlargement are the usual cardiac findings; epicardial petechiae are not uncommon. Myocarditis of varying severity is common. Coronary artery thrombosis, or myocardial infarction, is not a feature of RMSF. Myocarditis is the most common cause of death in RMSF [23–27].

CLINICAL PRESENTATION

Tick bites are reported in about 70% of patients. The incubation period averages 7 days but varies from 3 to 12 days; the shorter the incubation period, the more severe the infection. After an abrupt onset, severe headache, chills, prostration, and myalgia develop. Fever reaches 39.5 or 40°C (103 or 104°F) within several days and remains high (for 15 to 20 days in severe cases), although morning remissions may occur. An unproductive cough later develops. A macular rash develops between the second and sixth days of fever on the wrists, ankles, palms, soles, and forearms. It may rapidly extend to the neck, face, axilla, buttocks, and trunk. Often a warm water or alcohol compress make it more obvious. Initially macular and pink, it later becomes maculopapular and darker.

In about 4 days, the lesions become petechial and form large, hemorrhagic

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TABLE 1 Clinical Features of Rocky Mountain Spotted Fever (*Rickettsia rickettsii*)

Incubation Period	Symptoms	Signs	Laboratory Abnormalities	Complications
7 days (3–12 days)	Photophobia Mental confusion Severe frontal headache Restlessness/insomnia Severe myalgias (abdomen/back/legs) Back stiffness Nonproductive cough Nausea/vomiting Abdominal pain Severe prostration	Usually temperature ≥102°F Relative bradycardia (after 1 week) Maculopapular rash (blanching, beginning day 1–2) Petechial/purpuric rash (days 2–3) Hypotension (after 1 week) Noncardiac pulmonary edema Acute deafness Periorbital edema Conjunctival suffusion Abdominal distention Splenomegaly Hepatomegaly Peripheral cyanosis Coma Edema of the dorsum of the hands/feet	CBC Normal WBC count No anemia Thrombocytopenia ESR Moderately elevated (≤100 mm/hr) LFTs Moderately ↑ serum transaminases Hypoalbuminemia ↑ CPK CSF Mild pleocytosis with mononuclear predominance Normal glucose Normal lactic acid Elevated protein EKG Low voltage ST/T segment abnormalities Varying degrees of AV block Chest Radiograph On admission, no infiltrate (ARDS/pulmonary edema 2° excessive fluids may occur later during hospitalization) KUB Nonspecific changes/generalized ileus Serological Tests Usually negative until convalescence	Coma Seizures Encephalopathy Muscular rigidity DIC GI hemorrhage ATN (2° to oliguria/anuria) Bronchopneumonia Pulmonary edema ARDS Hypoproteinemia/generalized edema Otitis media Parotitis Permanent deafness Gangrene (face/hands/feet) Myocarditis (hypotension/ventricular arrhythmias) Death

Source: Ref. 34.

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areas that later ulcerate. Headache, restlessness, insomnia, delirium, and coma are all indicative of encephalitis. Prominent abdominal pain or headache may lead the unwary clinician away from the diagnosis of RMSF. There may be hepatomegaly and splenomegaly, but jaundice is infrequent. Localized pneumonitis rarely occurs. Untreated patients may develop bronchiolitis, pneumonia, tissue necrosis, and circulatory failure with such sequelae as brain and heart damage. Hypotension develops in severe cases. Cardiac arrest with sudden death from myocarditis occasionally occurs in fulminant cases (Table 1) [3,7,28–46].

DIFFERENTIAL DIAGNOSIS OF RMSF

Differentiating the RMSF from other acute infectious diseases is difficult during the first several days before the rash appears. A history of tick bite in known endemic areas of RMSF is helpful. Any seriously ill patient who lives in or near a wooded area and has unexplained fever, headache, and prostration, with or without a history of tick contact, should be suspected of having RMSF [1,20,29].

In meningococcemia, the rash may be pink, macular, maculopapular, or petechial in the acute form and petechially confluent or ecchymotic in the fulminant form; it resembles RMSF. The meningococcal rash develops rapidly in acute cases and is tender on palpation; the rash usually appears on or about the fourth febrile day and gradually becomes petechial or ecchymotic over several days.

In rubeola, the rash begins on the face, spreads to the trunk and arms, and soon becomes confluent; it may be confused with RMSF symptoms. In rubella, the rash usually remains discrete. Postauricular lymph nodes and lack of toxicity favor rubella.

In murine typhus, which is milder than RMSF or epidemic typhus, the rash is nonpurpuric, nonconfluent, and less extensive; renal and vascular complications are uncommon. However, differentiating RMSF from murine typhus may be difficult, and specific serologic testing may be required. Treatment should not be delayed until this distinction is made [1,29,32,40].

Epidemic louseborne typhus causes all the profound physiological and pathological abnormalities of RMSF, including peripheral vascular collapse, shock, cyanosis, ecchymotic skin necrosis, digital gangrene, aztoemia, renal failure, delirium, and coma. The rash of epidemic typhus usually appears first over the axillary folds and trunk; later it spreads peripherally, rarely involving the palms, soles, and face.

Small encrusted lesions (eschars) occur in patients with scrub typhus, rickettsial pox, and occasionally spotted fever. Here, the epidemiological history is important. The rash in rickettsial pox is vesicular; in tickborne typhus, it is often maculopapular. In Q fever, a rash is unusual; in trench fever, sparse. There is no exanthem in ulceroglandular tularemia (often associated with an eschar) and other

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TABLE 2 Differential Diagnosis of Rocky Mountain Spotted Fever

	RMSF	Atypical Measles	MC	Enteroviruses	Measles	Typhoid Fever	Typhus Fever
Symptoms							
Acute severe headache	+	−	−*	±	−	+	+
Photophobia	−	−	−*	−*	+	−	−
Coryza (early)	−	−	−	−	+	+	−
Sore throat	−	−	±	−	+	+	+
Nausea/vomiting/ diarrhea	+	−	−	±	+	−	−
Abdominal pain	±	−	±	−	± (RLQ)	±	−
Mental confusion	±	−	−	±	−	−	−
Signs							
Temp ≥102°F	±	±	+	±	±	+	+
Relative bradycardia	+	−	−	−	−	+	+
Macular rash (early)	+	−	−	+	−	±	−
Petechial rash (late)	+	±	+	±	−	−	+
Vesicular rash	−	+	−	−	−	−	−
Truncal rash (early)	−	+	−	+	+	+	+
Rash on palms and soles (late)	+	−	−	−	−	−	−
Periorbital edema	+	−	−	−	−	−	−
Conjunctival suffusion	+	−	−	−	+	−	+

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Hypotension/shock (early)	−	−	±	−	−	−	−
Hepatic tenderness	±	−	−	−	−	±	−
Splenomegaly	±	−	−	−	−	±	−
Cardiomegaly (late)	±	−	−	−	−	−	−
Edema, dorsum of hands/feet (early)	+	−	−	−	−	−	−
Laboratory Findings							
WBC count	N	N	↓	N/↓	N	↓	N
Anemia	−	−	−	−	−	−	−
Eosinophilia	−	+	−	−	−	−	−
Thrombocytopenia	+	−	−	−	−	−	−
Mild ↑ serum transaminases	+	−	−	±	−	+	±
Hyponatremia	±	−	−	−	−	−	−
↑ CPK	±	−	−	−	−	−	−
↑ Interleukin-1	+	−	−	−	−	−	−
↑ C3	+	−	−	−	−	−	−
↓ Transferrin	+	−	−	−	−	−	−
↑ α-1 antitrypsin	+	−	−	−	−	+	+
↑ Haptoglobin	+	−	−	−	−	−	−
↑ Factor VIII	+	−	−	−	−	−	−
↑ Fibrinogen	+	−	−	−	−	−	−
Hypoalbuminemia	±	−	−	−	−	−	−
Chest radiography (admission)	−	+	−	−	−	−	−
CSF (mild pleocytosis)	±	−	−*	+	−	−	−

* Positive if meningococcemia accompanied by meningococcal meningitis.

Abbreviations: MC, meningococcemia; RMSF, Rocky Mountain Spotted Fever; RLQ, right lower quadrant; WBC, white blood cell; CSF, cerebrospinal fluid.

Source: Ref. 34.

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forms of tularemia. Lyme disease, in which the characteristic erythema chronicum migrans rash often occurs, should also be considered. Rickettsial pox is mild; usually an initial eschar occurs at the point of mite attachment, and the vesicular rash, with surrounding erythema, is sparse. Because similar lesions occur in varicella, it must be ruled out.

Scrub typhus occurs in different geographical areas, particularly in Asia, Malaya, and Northern Thailand. These patients have all the clinical and pathologic manifestations of RMSF. Frequently, an eschar develops with satellite adenopathy (Table 2) [40–47].

Patients who present with symptoms/signs of RMSF without a rash, ie, ''spotless RMSF,'' should be considered as having ehrlichiosis until proven otherwise.

Nonspecific Laboratory Tests

There are a variety of nonspecific laboratory abnormalities that are associated with RMSF. The nonspecific laboratory abnormalities may suggest the diagnosis of RMSF, or be helpful in considering/ruling out other diagnostic possibilities. Thrombocytopenia is a common finding, and is usually accompanied with a normal WBC count and no anemia. The ESR is variably elevated.

Serum transaminases and the CPK are often elevated. Serum creatinine elevations are secondary to prerenal azotemia/retentive intravascular volume insufficiency. Decreased serum sodium attributable to dilutional hyponatremia often follows overzealous hypotonic fluid replacement. Patients with RMSF presenting with CNS symptoms should have a lumbar puncture performed to rule out meningeal pathogens. The cerebrospinal fluid in RMSF usually shows increased pressure and minimal pleocytosis with mononuclear cells. CSF glucose and lactic acid levels are usually normal, and protein levels may be minimally elevated [29,31]

Chest radiographs are clear early in RMSF. Overaggressive fluid replacement may result in pulmonary edema and typical infiltrates in chest radiograph, ARDS with low lung volume, microatelectases, bilateral infiltrates, and severe hypoxemia may complicate RMSF [43]

The EKG may show ST, and nonspecific ST-T wave abnormalities. Ventricular arrhythmias suggest the presence of myocarditis [2,47].

Specific Laboratory Tests

Serologic tests, isolation, and identification of *R. rickettsii* from blood or tissues, and identification of the agent in skin or other tissues by immunofluorescence help confirm the diagnosis. To be useful, serologic tests require three serum samples, taken during the first, second, and fourth to sixth week of illness (Table 3) [48–54].

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TABLE 3 Serologic Diagnosis of Rocky Mountain Spotted Fever

Serologic Test	Positive Titer	Diagnostic Titers After Onset	Antibody Persistence	Comments
Weil-Felix	≥1:320	2–4 weeks	Months	Lacks both sensitivity and specificity, but the traditional serologic test
Indirect immunofluorescence (IFA)	≥1:16	IgM positive in 1–2 weeks IgG positive in 4–6 weeks	IgM ~ 6 months IgG ≥ 1 year	Most widely used serologic test; relatively good sensitivity
Complement Fixation (CF)	≥1:16	3–4 weeks	≥1 year	Lacks sensitivity compared with IFA and ELISA, but is specific
ELISA	≥50	≥2 weeks	≥1 year	Useful in early diagnosis
Latex Agglutination (LA)	≥1:5	≥2 weeks	Months	Lacks sensitivity in late convalescence
Indirect Hemagglutination (IHA)	≥1:40	≥2 weeks	Months	Sensitivity comparable to IFA
Microagglutination	≥1:8	≥2 weeks	Several months	Less sensitive than IHA

Source: Adapted from Refs. 51–53.

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TREATMENT OF RMSF

All rickettsioses, particularly RMSF, require specific chemotherapy and supportive care. Clinical manifestations are promptly alleviated if therapy begins early, when the rash first appears. Untreated patients with RMSF may become moribund or die before definitive serologic data are available. Specific treatment should begin as soon as a presumptive diagnosis is made. Obvious clinical improvement is usually noted within 36 to 48 hours, with defervescence in 2 to 3 days.

The tetracyclines and chloramphenicol are specifically effective; they are rickettsiostatic, not rickettsicidal. Optimal regimens include doxycycline 200 mg (IV/PO) q12h, or chloramphenicol 50 mg/kg or 500mg (IV/PO) q6h. Double drug therapy has no advantage over monotherapy in the treatment of RMSF. Intravenous preparations are used in patients too ill to take oral medication. Quinolones, eg, levofloxacin, have antirickettsial activity and may be used in patients unable to take chloramphenicol or doxycycline. Antibiotic treatment is usually given for 2 weeks. Effective antibiotic treatment may blunt, delay, or eliminate the serologic response in RMSF (Table 4) [58–67].

In patients first treated during the later stages, improvement is slower and fever is of longer duration. Patients seriously ill with a rickettsial disease of the spotted fever group often have circulatory collapse, oliguria, anuria, azotemia, anemia, hyponatremia, hypochloremia, edema, and coma. In mildly and moderately ill patients, these aberrations are absent which makes management less complicated. Corticosteroids may be given for critically ill patients first encountered in the late stages of illness in combination with specific antibiotics, but their effectiveness has not been confirmed (Fig. 2).

Severely ill patients in the late stages of RMSF often manifest marked

TABLE 4 Antimicrobial Therapy of Rocky Mountain Spotted Fever*

Antimicrobial	Dose/Dosing Interval
Chloramphenicol**	500 mg (IV/PO) q6h
Tetracycline	500 mg (PO) q6h
Doxycycline**	200 mg (IV/PO) q12h
Ciprofloxacin	750 mg (IV) q12h or 500 mg (PO) q12h
Levofloxacin**	500 mg (IV/PO) q24h

* Duration of therapy is usually 2 weeks.
** Preferred antibiotics.

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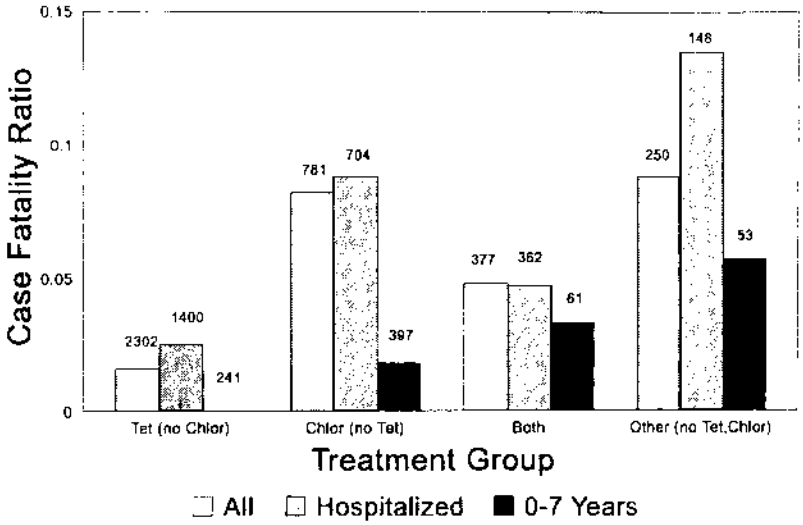

FIGURE 2 Relative efficacy of tetracycline versus chloramphenicol therapy in RMSF. From Ref. 30.

increase in capillary permeability. Isotonic intravenous fluids should be given cautiously to avoid worsening pulmonary and cerebral edema. Heparin is not recommended despite manifestations suggesting disseminated intravascular coagulation.

PROPHYLAXIS

Early antibiotic treatment has significantly reduced mortality from about 20 to 5% and prevented most complications. No effective vaccines are available. We lack practical means to rid entire areas of ticks. Tick populations may be reduced in endemic areas by controlling small-animal populations; spraying the area with DDT, dieldrin, or chlordane is also helpful. Those who live or work in tick-infested areas should use tick repellents [eg, dimethyl phthalate or diethyltoluamide (Deet)] which with pyrethrin on clothing effectively repels ticks, although toxic reactions have been reported in children. Engorged ticks should be removed with care and not crushed between the fingers because of the danger of transmission. Gradual traction of the head with a small forceps dislodges the tick. The point of attachment should be swabbed with alcohol.

Antibiotics should not be given immediately when a tick bite occurs in a known endemic area and clinical manifestations are absent. Rather, the patient or parent should be cautioned about early clinical signs. If fever, headache, and malaise occur, with or without a rash, antibiotics should be started promptly [47,68].

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10

Ehrlichiosis

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INTRODUCTION

Tickborne rickettsiae in the genus *Ehrlichia* are intracellular pathogens of wild and domestic mammals and, more recently, man [84,88,103,121,122]. At least ten separate *Ehrlichia* species are currently recognized, and distinctive infectious syndromes have been described for canids, equines, ruminants, as well as humans (Table 1) [44,56,103,121]. Part of the life cycle for many *Ehrlichia* species involve arthropod hosts (ixodid or hard-shelled ticks), and *Ehrlichia* were previously considered species specific in their invertebrate and vertebrate host requirements. Ehrlichioses have been described on all major continents [48,121]. Recent developments in molecular biology have shown close genetic relationships between several veterinary pathogenic Gram negative bacteria and the *Ehrlichia* species that cause human disease (Fig. 1). With the exception of the documented infections by *Ehrlichia sennetsu*, prior to the 1980s *Ehrlichia* species were thought to cause infections only in certain animals. However, within the last decade two distinctive *Ehrlichia* species have been identified as important causes of morbidity and occasional mortality in humans [6,44,51,56,83]. This chapter will focus on the human pathogens *Ehrlichia chaffeensis* that causes human monocytotropic ehrlichiosis (HME), and a yet to be fully characterized granulocytic *Ehrlichia* species that causes human granulocytotropic ehrlichiosis (HGE). Several excellent reviews of human ehrlichioses [44,48,121], as well as HME [51,56,61,66,88] and HGE [1,6,7], have been published recently and the interested reader is referred to these reports for additional information.

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TABLE 1 *Ehrlichiae* Genogroups and Species Distribution

Genogroup	Relevant Mammalian Hosts	Mammal Target Cells
I. *Ehrlichia canis*	Canids, humans	Monocyte/macrophages
E. chaffeensis	Humans, deer, dogs	Monocytes/macrophages
E. ewingii	Canids	Polymorphonuclear leukocytes
E. muris	Voles	Monocytes/macrophages
II. *E. equi*	Horses, dogs	Neutrophils
E. phagocytophila	Sheep, goats, deer	Neutrophils
	Agent of HGE*	
	Humans, deer, rodents, horses, dogs	Neutrophils
E. platys	Canids	Platelets
III. *E. sennetsu*	Humans	Monocytes/macrophages
E. risticii	Horses	Monocytes

* Species not yet defined.
Source: Modified from Ref. 121.

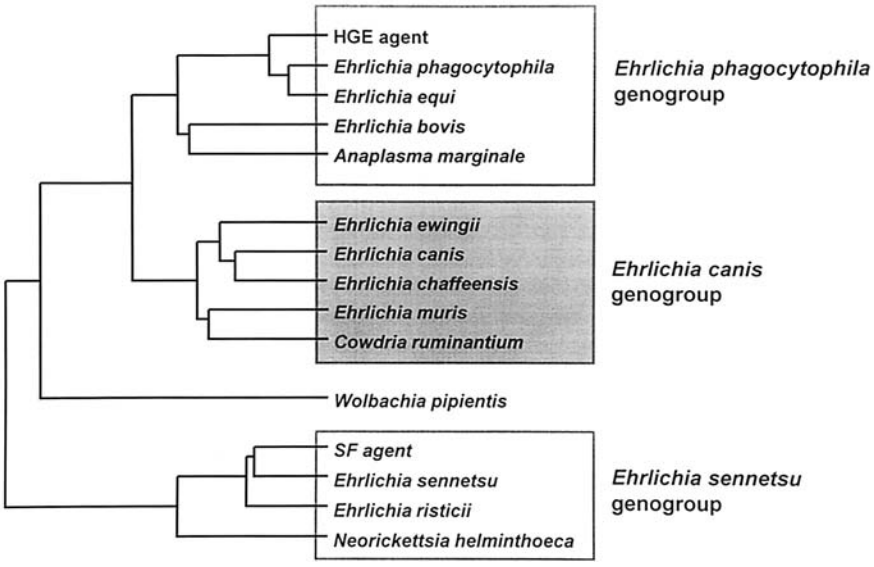

FIGURE 1 Unrooted dendrogram representing the phylogenetic relationships of ehrlichiae as determined by 16S rRNA gene sequence similarity. The three clusters of bacteria enclosed within the rectangles include organisms currently designated within the order *Rickettsiales* (family *Rickettsiaceae*, tribes *Ehrlichieae* and *Wolbachieae*, genera *Ehrlichia, Cowdria, Wolbachia*, and *Neorickettsia*, and the family *Anaplasmataceae*, genus *Anaplasma*).

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HUMAN EHRLICHIAE IN THE UNITED STATES

Ehrlichia species are obligate intracytoplasmic bacteria with a cell diameter of about 0.2 to 2.0 μm. Electronmicrographs show a characteristic bilamellar cell wall structure that is typical of Gram negative bacteria [6,83,101,121]. *Ehrlichia* species were previously classified by (1) morphologic features, (2) infected host cell, (3) infected host species, (4) geographic location of natural infection, and (5) serologic cross-reactivity. More recent classification schemes use 16S rDNA gene sequence analysis as the primary taxonomic tool [57], and three genogroups, each containing species that infect humans, are now recognized [44,48,121] (Table 1). *Ehrlichia chaffeensis* [2,35] and *Ehrlichia canis* [97] belong to the *Ehrlichia canis* genogroup [44,103,121], whereas the human granulocytic *Ehrlichia* agent [6], which appears closely related to or identical with *Ehrlichia equi* and *Ehrlichia phagocytophila*, belongs in the *Ehrlichia phagocytophila* genogroup [30]. *Ehrlichia sennetsu*, which belongs in the *Ehrlichia sennetsu* genogroup [121] and the illness that is caused by this bacteria have hitherto only been encountered in Asia and will not be discussed further.

Parts of the life cycles of *Ehrlichia chaffeensis* and the human granulocytic *Ehrlichia* involve arthropod hosts [121]. Transovarial passage of *Ehrlichia* from the adult tick stage to eggs has not been reported, but transstadial propagation of the infectious agents occurs readily in the life cycles of both *Ehrlichia chaffeensis* and the human granulocytic *Ehrlichia*. Mammal hosts therefore play a crucial role in the maintenance and propagation of these infectious agents in nature. HME and HGE are reportable illnesses in only a few states, and accurate figures for disease incidence and prevalence have largely relied on passive reporting to health authorities. Even though HME and HGE present as similar febrile illnesses, the geographic areas where these illnesses are contracted overlap only in a few places [10,44,48]. Epidemiologic, clinical, and laboratory features differ between *Ehrlichia chaffeensis* and the human granulocytic *Ehrlichia* agent, thus each species and corresponding ehrlichial illness will be discussed separately.

HUMAN MONOCYTOTROPIC EHRLICHIOSIS (HME)

Microbiology

Both *Ehrlichia chaffeensis* and *Ehrlichia canis* can be grown in vitro in several tissue culture cell lines, such as the canine macrophage tissue culture cell line DH82 [29,36]. The ehrlichial cells divide by binary fission and multiply within the confines of the host cell lysosomal vacuole to form clusters of cells with the shape of a mulberry (*Latin morula*) made up of three to 50 individual ehrlichial cells. Human mononuclear phagocytes (macrophages, monocytes, and occasion-

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ally lymphocytes) are preferentially infected in vivo by *E. chaffeensis*, although infection of human peripheral blood neutrophils have also been described [60,83,121]. Small *Ehrlichia* inclusions (morulae) can be detected in the host cell cytoplasmic space after 3 to 7 days [35]. Eventually the spatial constraints placed on the host cell presumably leads to rupture of the cytoplasmic membrane and the released ehrlichial cells attach to and enter new host cells. *Ehrlichia chaffeensis* can infect mice [115], dogs [26], and white tailed deer (*Odocoileus virginianus*) [38,80,81]. Only a few research laboratories are currently equipped to perform ehrlichial cultures.

Major antigenic outer cell membrane constituents of *Ehrlichia chaffeensis* were recently described by Chen and coworkers, and include a heat-labile 22 kD protein band, as well as heatstable protein bands of molecular weight 44, 55, and 66 kD, respectively [29]. Other protein antigenic determinants include bands of molecular weight 27 and 29 kD [23], and 40, 47, and 64 kD [104]. A 120 kD band has recently been identified and is felt to play an important role in the pathogenesis of *Ehrlichia chaffeensis* and protective immunity of infected hosts [129,130]. Many of these immunoreactive antigens cross-react with antisera from other *Ehrlichia* species, but show closest antigenic relationship with *Ehrlichia canis*.

Epidemiology

Seroepidemiologic surveys and clinical studies strongly implicate the Lone Star tick (*Amblyomma americanum*) as the principal tick vector of *Ehrlichia chaffeensis*, and the geographic areas where tick bites have been reported to occur before onset of HME have closely correlated with the area of distribution of the Lone Star tick [36,44,51,52,56,66,121] (Table 2). *Ehrlichia chaffeensis* has been demonstrated in Lone Star ticks by immunofluorescent staining technique and polymerase chain reaction (PCR) analysis using the specific 16S rDNA specific primer set HE1/HE3 [3]. *Ehrlichia chaffeensis* specific antigen has also been demonstrated by immunofluorescent technique (IFA) in a single nonfeeding *Dermacentor variabilis* tick (American dog tick, or wood tick) [3], although this tick species has never been shown to be involved in the passage of the infectious agent to humans after a tick bite. The range of distribution of the Lone Star tick extends to the south of an imaginary line extending from downstate New York to Texas and the heaviest concentrations of cases of HME have been reported from Oklahoma [67,69], Texas [56], Arkansas [35], and Missouri [52]. More than 465 cases of HME from 34 US states have been reported to the Center for Disease Control and Prevention (CDC) at the time of this writing (Fig. 2) [56; JE Dawson, personal communication, 1997]. HME has also been reported from regions outside of the United States, including Spain [63], Portugal [89], Belgium [100], as well as Mali [117]. The white tailed deer (*Odocoileus virginianus*) sup-

TABLE 2 Distinguishing Microbiological and Epidemiologic Features Between HME and HGE

Characteristic	HME	HGE
Pathogen	*Ehrlichia chaffeensis*	HGE agent/*Ehrlichia equi*/*Ehrlichia phagocytophila*?
Tick vector	*Amblyomma americanum*	*Ixodes persulcatus group*§
Host target cells	Monocytes/macrophages	Neutrophils
Infected host cells (%)	0–1	0–42
IFA* antigen	*Ehrlichia chaffeensis*	*Ehrlichia equi*/HGE agent
16S rDNA PCR† primer set	HE1/HE3	ge9f/ge10r
Predominant in-vitro cell culture line	DH-82 canine macrophage¶	HL 60 human promyelocyte IDE8 *I. scapularis* embryo
WB‡ antigenic outer cell membrane determinants	22-, 25-, **27-**, **29-**, **44-**, 55-, 66-, 120-kD	25-, **40-**, 42-, **44-**, 46-, **65-**, 110-kD

* Indirect immunofluorescent antibody test.
† Polymerase chain reaction test.
‡ Protein bands detected by Western blot analysis. Major determinant bands in bold typeset.
§ *Ixodes scapularis*, *Ixodes pacificus*, *Ixodes ricinus*, *Ixodes persulcatus*.
¶ Many other cell lines also available.
(HME data from Refs. 104,129; HGE data from Refs. 5,45,74,132).

ports growth of all stages of the Lone Star tick, can be experimentally infected, and is found naturally infected with *E. chaffeensis* in regions where Lone Star ticks are present, and represents the most important reservoir host, in particular for the adult tick stage [38,80,81]. Simultaneous feeding by all stages of *A. americanum* permits the opportunity for amplification of *E. chaffeensis* infection.

Clinical Manifestations

Seroepidemiologic surveys have indicated that HME may be a mild illness or a largely asymptomatic infection [58,98,112,127]. Most reported cases of HME have typically begun as a nonspecific acute febrile influenza-like illness, and it is likely that many cases have been misdiagnosed or missed entirely in the past. Indeed, before specific indirect immunofluorescent antibody (IFA) serologic testing became available, HME was diagnosed as a clinical variant of Rocky Mountain spotted fever without the characteristic rash [51]. More than 80% of patients give a history of tick exposure, and three quarters of interviewed patients recall one or more tick bites during the month before onset of clinical illness (Table 3) [51,52,56,61,67,112].

The median incubation period from time of tick bite until onset of clinical symptoms is approximately 1 week (Table 3). Ownership of dogs may increase

FIGURE 2 Distribution of states where 466 cases of human monocytotropic ehrlichiosis (HME) have been reported to the Centers for Disease Control and Prevention per April 1, 1997. Some cases were infected in states other than the reported state of residence. Lighter shading indicates states reporting any case. Darker shading indicates states where patients have acquired their infection. (Data courtesy of J. Childs, Centers for Disease Control and Prevention.)

the risk for acquiring HME [51]. Most clinically ill patients have reported the onset of symptoms during May through July, and acute HME is encountered infrequently during the late fall and winter months [51,56]. Males are infected 3 to 4 times more frequently than females [51,52,56,112], and gender-specific incidence rates for males exceed those of females in all age groups [57]. Symptoms of HME have typically been present for 3 to 4 days for most patients at the time of the initial physician visit [52,56,112]. Median patient age has ranged from 37 to 66 years in reported series, but patients as young as 3 years and as old as 87 years have been reported [51,56,67,112]. Approximately 75% of patients have been admitted to a hospital for a median duration of 5 to 7 days [51,56,67,112], and hospitalized patients were older (median, 50.5 years) than those who were not hospitalized (median, 37.6 years) in a large case series reported by Fishbein and coworkers [56]. Case fatality rates were initially reported to be as high as 5.3% [51], but more recent data analysis suggests that the case fatality rate is approximately 1 to 2% [44,56,112]. Elderly patients are more prone to severe illness and death, and an adverse outcome has been associated with delayed diagnosis and treatment [44,56].

Most patients have reported sudden onset of fever, chills, generalized myalgias, severe headache, and malaise early in the course of acute HME (Table 4).

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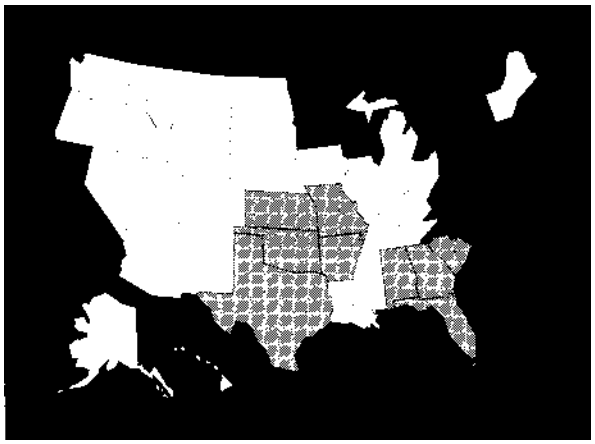

FIGURE 3 Distribution of states where 345 cases of human granulocytotropic ehrlichiosis (HGE) have been reported to the Centers for Disease Control and Prevention per April 1, 1997. Some cases were infected in states other than the reported state of residence. Lighter shading indicates states reporting any case. Darker shading indicates states where patients have acquired their infection. (Data courtesy of J. Childs, Centers for Disease Control and Prevention.)

TABLE 3 Demographic Characteristics of Patients Infected with *Ehrlichia chaffeensis* (HME) and the Human Granulocytic *Ehrlichia* agent (HGE)

Characteristic	HME	HGE
Patients with tick exposure (%)	80	85–95
Patients with tick bite (%)	75	40–75
Median patient age (years)	42–66	47–59
Patients ≤ 16 years of age (%)	<5	<5
Ratio (males:females)	3–4:1	1.2–4:1
Median incubation period* (days)	7–9	5.5–8
Median duration of symptoms† (days)	3–4	6–7
Patients hospitalized (%)	38–85	28–52
Median duration of hospital stay (days)	5–7	6
Reported case fatality rate (%)	0–5.3	1.2–5.9

* Days after tick bite to onset of systemic illness.
† Days of clinical illness until diagnosis recognized and specific therapy started.
HME data from Refs. 51,52,56,61,67,112; HGE data from Refs. 1,6,7.

Table 4 Common Signs and Symptoms Reported by Patients Infected with Acute HME and HGE

Sign or Symptom	HME (%)	HGE (%)
Temperature ≥ 37.6°C	85–97	94–100
Median temperature (°C)	39.9	39.7
Rigors	61–65	39–98
Myalgias	43–53	78–98
Sweats	85	98
Headache	63–85	61–85
Nausea	42–73	39
Anorexia	27–50	37
Vomiting	36–49	34
Cough	10–39	29
Arthralgias	9–33	27
Rash (any)	0–36	Rare

HME data from Refs. 51,52,56,112; HGE data from Refs. 1,6,7.

Less common complaints include nausea, vomiting, diarrhea, abdominal pain, arthralgias, dry cough, and confusion. Some investigators have reported presence of nonspecific rashes [51,56,71], although no rash was noted among 11 patients reported by Standaert [112]. A case control study by Harkess concluded that rashes were present more commonly in the control patients (50%) than the study cohort (35%) [67]. However, rash may occur more frequently in the pediatric age group than in adults [69].

Fishbein reported that the risk for a more severe illness was proportional to age [56]. More severe clinical manifestations that have been reported include pulmonary infiltrates, pneumonia, acute respiratory distress syndrome (ARDS) [51,96], congestive heart failure [118], culture negative endocarditis [25], myocarditis [123], toxic shock–like syndrome [53], acute renal failure [51], encephalopathy, and meningitis [40,49,51,68,102]. Four patients reported by Eng et al. had laboratory evidence of disseminated intravascular coagulation [51]. Roland and coworkers described persistence of fever in patients with HME ranging from 17 to 51 days before starting specific antibiotic therapy with a tetracycline drug [106], and persistent infection has also been reported [42]. Fulminant infection has been documented in individuals who are immunocompromised by immunosuppressive therapy after organ transplantation [4], corticosteroid usage [87], or HIV [93,94].

LABORATORY FINDINGS

The laboratory findings observed in patients with HME are as nonspecific as their clinical manifestations (Table 5). Most clinically ill patients present with variable

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TABLE 5 Frequency (%) of Laboratory Abnormalities Observed Among Patients with Acute HME and HGE

Laboratory finding*¶	Reference range	HME (%)	HGE (%)
Leukopenia*	4.0–10.0 $\times$ 10^9/L	60–74	44–59
Anemia*	13.0–17.0 g/dl	45–50	50
Thrombocytopenia*	150–400 $\times$ 10^9/L	72–80	67–90
AST or ALT¶	15–40 IU/L	75–88	61–92
Creatinine¶	0.5–1.3 mg/dl	24–86	15
ESR¶	1–21 mm/h	ND	85
CRP¶	0.4–0.8 mg/dl	ND	100

* Value below normal reference range.
¶ Value above normal reference range.
Abbreviation: ND, No data.
HME data from Ref. 51,56,112; HGE data from Ref. 1,6,7.

degrees of leukopenia and thrombocytopenia during the first week of illness, and patients who have been ill for more than a week have frequently been reported to be anemic [51,56,112]. Absolute leukocyte concentrations (including neutrophils and lymphocytes) reach nadir values around day 7 (occasionally $\leq 1.0 \times 10^9$/L) and then gradually increase towards normal values during the second week of illness [56]. Increases in the percentage of neutrophils and neutrophil band forms with concomitant decreases in the relative and absolute proportions of lymphocytes have been observed in the differential leukocyte count during the first week of illness. During the second week of illness, at a time when recovery is beginning, relative and absolute lymphocytosis, predominated by γ/δ T-cell lymphocytes, and a gradual decrease in the relative proportion and absolute concentration of band neutrophils have been noted [27,56]. Atypical lymphocytosis may be observed at this time. The platelet count follows the same pattern as that of the leukocytes, reaching nadir values as low as 50 $\times$ 10^9/L or lower around day 7 and thereafter gradually increases towards normal concentrations over the next 2 weeks [51,52]. Because of occasional very severe headache and presence of abnormal neurological findings, some patients have undergone lumbar puncture. Cerebrospinal fluid (CSF) analysis has revealed evidence of inflammation with mononuclear pleocytosis and elevated protein values for most of these patients [40,51,68,102]. Several investigators have reported finding *Ehrlichia chaffeensis* morulae in CSF mononuclear leukocytes in patients with HME meningitis [49,102]. Bone marrow examinations during the acute phase of illness reveal hyperplasia in 75% of patients, and hypoplasia in only 13%; small noncaseating granulomas or histiocyte aggregates are present in many patients. Thus, the findings suggest peripheral sequestration, consumption, or destruction as the mechanism for the cytopenias that occur with HME [43].

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Diagnosis of HME

Light microscopic inspection of Wright's stained peripheral blood smears or buffy coat preparations have occasionally demonstrated characteristic mulberry-shaped inclusions (morulae) in monocytes and rarely in lymphocytes (Table 6) [44,51,56]. Morulae typically appear as dark blue, irregularly stained, rounded cytoplasmic densities, and identification may be difficult for the untrained microscopist (Fig. 4). The color hue of the morulae is usually darker than that of the cell nucleus, which tends to stain red-purple [60]. Examination of the peripheral blood smear is insensitive and nonspecific, and a negative bloodsmear evaluation should not rule out the diagnosis. Noncirculating mononuclear phagocytes in the liver, spleen, bone marrow, and lymph nodes may also become infected, and the infectious agent may be identified by use of in situ immunohistochemical and monoclonal antibody staining techniques on biopsied material from such sites [41,128].

Diagnosis of HME is most often based on the demonstration of a serologic response to *Ehrlichia chaffeensis*. Diagnostic rise in antibody titer usually occurs by the third week after onset of illness, but some patients with suspected acute HME based on diagnostic tests other than IFA have failed to generate antibodies during the convalescent phase (Table 6) [52,106]. The current CDC surveillance case definition for HME requires a clinically compatible history and a minimum IgG antibody titer of at least 64 or a fourfold or greater change in antibody titers using indirect IFA testing [108]. Earlier IFA testing used *Ehrlichia canis* as surrogate assay antigen, as this species readily crossreacts with sera from patients infected with *Ehrlichia chaffeensis* [34,36]. IFA assays using *Ehrlichia chaffeensis* antigen were subsequently shown to have higher sensitivity and specificity than assays performed with *Ehrlichia canis*, and *Ehrlichia chaffeensis* is now the preferred substrate antigen [35,44,48,56,121]. The major disadvantage with IFA testing is that diagnosis confirmation becomes retrospective. A degree of anti-

Table 6 Sensitivities of Tests Used to Diagnose Human Ehrlichioses

Testing method	HME (%)	HGE (%)
Presence of morulae	0–1	10–73
IFA high titer*	78	94–96
$\geq 4 \times$ IFA titer change	63	82
PCR¶	87	43–86

* See Table 7 for specific cut-off values.
¶ See Table 2 for specific PCR primer sets required.
HME data from Refs. 51,52; HGE data from Refs. 1,7,50.

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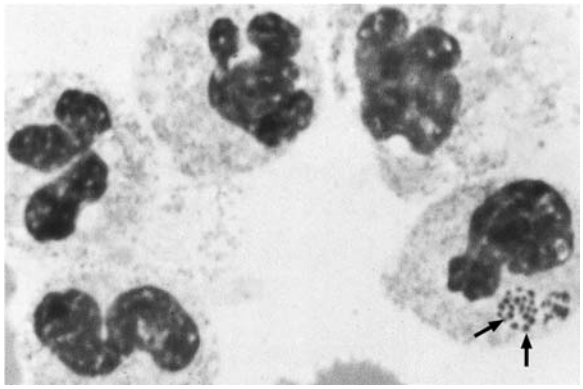
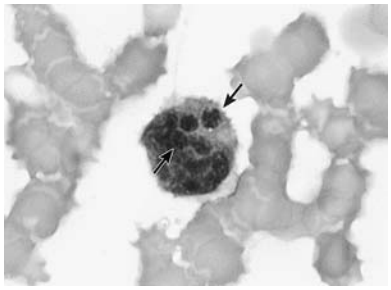


(a)



(b)

FIGURE 4 ***Ehrlichia*** **spp. morulae (arrows) in peripheral blood leukocytes from patients with HME or HGE. Note the heterogenous appearance of morulae in the different infected cells: morulae are generally stained basophilic and round, but may vary in size, shape, and color; the internal structure often appears slightly stippled, but may contain discernible individual** ***Ehrlichia*** **spp. bacteria [(a), small arrows]. (a) Morulae from a patient with HGE (*E. phagocytophila* group infection) and (b) morulae seen in a patient with HME (*E. chaffeensis* infection) (arrows). [Wright stains, approximate magnifications × 1000; (b) courtesy of Joan Barenfanger, M.D., Memorial Medical Center, Springfield, IL.]**

genic diversity exists among strains of *E. chaffeensis* isolated from patients; however, whether such diversity affects diagnostic serology is not known [131].

Ehrlichia chaffeensis antibodies may occur in conjunction with seroconversions for other bacterial pathogens, including the HGE agent, *Rickettsia rickettsii, Rickettsia typhi, Borrelia burgdorferi, Brucella* species, and perhaps others. *Ehrlichia chaffeensis*–reactive sera in patients with *B. burgdorferi* antibodies most

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TABLE 7 Proposed Case Definitions for Human Monocytotropic Ehrlichiosis (HME) and Human Granulocytotropic Ehrlichiosis (HGE)

Definition criteria	HME	HGE	Diagnosis category
Influenza-like illness, fever ≥ 37.6°C (100°F), and history of tick exposure	Yes	Yes	Presumptive
and IFA* single titer *or*	≥ 64	≥ 80	Probable
Leukocyte morulae	Monocytes/ lymphocytes	Neutrophils	Probable
and IFA ≥ 4X titer change‡ *or*	*E. chaffeensis*	HGE agent	Confirmed
PCR§ positive *or*	HE1/HE3	ge9f/ge10r	Confirmed
Ehrlichia-culture† positive	DH82	HL60	Confirmed

* Reverse of sero-dilution tested.
† Tissue cell culture lines that support growth of specific *Ehrlichia* species.
‡ Acute and convalescent serum-samples obtained at least 14 days apart.
§ Polymerase chain reaction 16S rDNA *Ehrlichia* species specific primer sets.
From Refs. 6,7,51,52.

likely result from antibodies to *Borrelia* species heat shock proteins that cross-react with those of *E. chaffeensis*, and not from coinfections in areas where both tick vectors are not present. Whether other serologic reactions represent cross-reactions, prior infections, or concurrent infection with other tickborne infectious agents is not known. There is currently no established diagnostic role for IgM serology [48].

Minute quantities of *Ehrlichia chaffeensis* DNA can be detected in biologic fluid by PCR using the specific 16S rDNA primer set HE1/HE3 or the 120 kD protein gene primer set PXCF3b/PXAR5 (Table 2) [130]. PCR offers rapid testing of infected blood for diagnosis confirmation, and the sensitivity of PCR for patients infected with *Ehrlichia chaffeensis* using the 16S rDNA amplification method was recently reported to be 87% (Table 6) [52]. PCR testing specificity is limited only by the possibility of assay contamination with ehrlichial DNA in laboratories where PCR is performed frequently [52,121]; however, prior doxycycline therapy will diminish the sensitivity by eliminating the infectious agent. Testing is currently restricted to research institutions and a few commercial laboratories, and may be fairly expensive [2,3,52,121]. Early clinical diagnosis is still imperative to institute potentially life-saving antimicrobial therapy.

Ehrlichia chaffeensis may also be cultivated in vitro by using tissue culture cell lines [36], but the procedure is laborious, insensitive, and it may take 7 to 35 days or longer before a positive result becomes apparent (Table 2). Western blotting (WB) of sera from infected patients has demonstrated several characteristic immunodominant antigenic protein bands (Table 2). Both tissue culture and WB testing are currently limited to a few research institutions, and the usefulness

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of these testing methods is limited by the lack of general availability, timeliness, and cost. A summary of tests used for diagnosing HME and reported outcomes is shown in Table 6.

The diagnosis of HME requires a compatible exposure history, suggestive clinical and laboratory findings, and diagnosis confirmation by use of one or several specific laboratory tests. Case definitions for presumed, probable, and confirmed HME are outlined in Table 7.

TREATMENT

All ehrlichial species recognized to date appear to be susceptible to tetracycline antibiotics and their derivatives [21,103]. In vitro susceptibility tests have rarely been performed because testing procedures are laborious and in vitro cultivation has only recently been described. Furthermore, methods for susceptibility testing have not been standardized, and results from different institutions may not be directly comparable [15]. Most treatment recommendations, therefore, have been primarily based on clinical treatment outcomes in animals and humans. Brouqui and Raoult found a single *Ehrlichia chaffeensis* strain to be susceptible to doxycycline and rifampin, but resistant to penicillin, chloramphenicol, ciprofloxacin, erythromycin, trimethoprimsulfamethoxazole, and gentamicin [21]. Tetracycline drugs have been used successfully to treat canine ehrlichiosis (*Ehrlichia canis*), but delayed therapy may allow for the development of chronic infection [26]. No posttreatment relapse has been described in humans to date. Doxycycline has been the preferred tetracycline derivative for treatment of humans because of excellent pharmacokinetic properties and good patient tolerance for this drug [15]. Doxycycline hyclate 100 mg tablets should be administered by mouth or intravenous infusion at 12-hour intervals for 7 to 10 days for adults infected with HME. Children should receive doxycycline 4.4 mg/kg body weight per day by intravenous or oral route for 7 to 10 days, or for at least 3 days after fever has abated to minimize the risk of dental staining in the very young child [15, 44,48,120].

Chloramphenicol has not been found to have in vitro activity against *Ehrlichia chaffeensis*, despite the close phylogenetic relationship between this *Ehrlichia* species and *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever [21]. Conflicting results have been published about the effectiveness of chloramphenicol for the treatment of HME. Some observers have reported rapid improvement with chloramphenicol therapy [18,56], and 41 of 47 reported patients (87.2%) resolved clinical symptoms of illness 2 to 6 days after initiating chloramphenicol treatment [15,51,53]. Six reported patients failed to improve, however, and three of these patients died despite chloramphenicol therapy [15]. The majority of chloramphenicol-treated patients are children, thus comparison of efficacy versus tetracycline therapy may be confounded. Thus, the

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TABLE 8 Antibiotic Treatment Recommendations for Human Monocytotropic Ehrlichiosis (HME) and Human Granulocytotropic Ehrlichiosis (HGE)

Antibiotic	Dose/dose frequency	Route of administration	Length of therapy	Expected effect	Ehrlichial infection
Doxycycline hyclate	Adults: 100 mg Q 12 h	PO or IV	7–10 days	Effective	HME HGE
	Child: 4.4 mg/kg/day	PO or IV	7–10 days *or* 3 days after fever resolved	Effective	HME HGE
Tetracycline hydrochloride	Adults: 500 mg QID	PO	7–10 days	Effective	HME HGE
	Child: 25–50 mg/kg/day	PO	7–10 days *or* 3 days after fever resolved	Effective	HME HGE
Chloramphenicol	Adults: 500 mg Q 6 h	PO or IV	3–10 days	Possibly effective	HME* HGE*
	Child: 50 mg/kg/day	PO or IV	3–10 days	Possibly effective	HME* HGE*
Rifampin†	Adults: 300 mg BID	PO	Unknown	Possibly effective	HME‡ HGE‡
	Child: 10 mg/kg/day	PO	Unknown	Possibly effective	HME‡ HGE‡

* No documented in vitro effect. In vivo experience is inconclusive.
† Isolated cases of fetal malformations have been reported with use during pregnancy.
‡ Documented in vitro activity. No published in vivo experience.
Source: From Refs. 1,7,15,51,56,112.

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role for chloramphenicol remains undefined and its use should be restricted to patients who are intolerant to or have strict contraindications to tetracycline drugs. There are no reports describing use of rifampin for the treatment of HME, but *Ehrlichia chaffeensis* appears to be very susceptible to this drug in vitro [21]. Thus, rifampin may be a suitable antibiotic for the treatment of children under the age of 8 years and patients who are allergic to doxycycline [15]. Rifampin probably should not be used to treat pregnant women because fetal malformations have been reported to occur in offspring of women who were treated with rifampin during pregnancy. Table 8 summarizes the antibiotic drugs that have been documented to be effective or possibly effective for the treatment of human ehrlichioses.

HUMAN GRANULOCYTOTROPIC EHRLICHIOSIS

Microbiology

The agent of human granulocytotropic ehrlichiosis (HGE) was considered to be uncultivable in vitro until Goodman described a successful method that used the human promyelocytic cell-line HL60 (Table 2) [62]. Morulae can typically be detected in the cytoplasm of infected HL-60 cells after 5 to 10 days in patients that are shown to have morulae in peripheral blood. In vitro growth of granulocytic *Ehrlichia* has also been supported by an IDE8 tick-embryo derived cell-culture line [90]. When present, these ehrlichiae are typically detected in circulating granulocytes [1,6,44]. Light microscopic examination of Wright-stained peripheral blood or buffy coat smears from infected patients reveal dark blue rounded inclusions inside cytoplasmic vacuoles of segmented and band neutrophils (Fig. 4) [6]. Examination of tissues taken from a patient who died has also demonstrated ehrlichiae in neutrophils in spleen and liver and in lung macrophages [6]. The human granulocytic *Ehrlichia* agent has been shown to naturally and experimentally infect the white footed mouse (*Peromyscus leucopus*) [114,119] and white tailed deer (*Odocoileus virginianus*) [39]. Neither of these animal species showed signs of clinical illness during the period of ehrlichiemia, which may last a week or longer. Both the white footed mouse [70,111] and white tailed deer [6,39,19] support growth of all stages of *Ixodes scapularis*, and therefore are presumed to be the definitive mammal hosts for the human granulocytic *Ehrlichia* agent. Madigan and coworkers have successfully infected horses with the human granulocytic *Ehrlichia*, resulting in clinical disease that is indistinguishable from that seen with *Ehrlichia equi* [82], and confer protection against challenge with *E. equi* during the convalescent phase [16].

The major outer membrane protein antigen determinants of the human granulocytic *Ehrlichia* have recently been elucidated (Table 3) [45,74]. Major protein antigen bands of molecular weights varying between 40 and 48 kD have been

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recognized with human sera, as well as with sera from infected horses, dogs, and mice [45,74]. Asanovich and Zhi independently demonstrated that antigenic structural diversity of the immunodominant bands exists among clinically indistinguishable granulocytic *Ehrlichia* isolates from various geographic regions [5,132]. These major outer membrane proteins possess some characteristics of the major surface protein (MSPs) of the genetically related *Anaplasma marginale* [132]. Lesser antigens of molecular weights 25, 65, and 80 kD have also been noted in convalescent sera from infected human patients [45,74,92]. Caturegli et al. recently cloned part of an HGE agent gene that encodes a 68 kD protein antigen with at least two repeated units with a predicted amino acid similarity to mammalian ankyrin proteins, and speculated that this protein may play an important role in protein–protein interactions between ehrlichiae and host cells [28].

Epidemiology

The first identified case of HGE was that of an 80-year-old man from Spooner, Wisconsin who died of multiorgan failure in June 1990 [6]. More than 30 additional cases were recognized among residents of northwestern Wisconsin and central Minnesota during the next few years [6]. Shortly thereafter Wormser and coworkers described an additional 29 patients from New York who met the case definition criteria for confirmed or probable HGE [126]. Although HGE has now been reported in residents from 23 states (JE Dawson, personal communication, 1997), most cases have occurred in Wisconsin, Minnesota [7], and New York [1]. HGE was recently reported in patients in northern California [59] and in Slovenia [99]. Seroepidemiologic surveys have shown that HGE for the most part is contracted in regions known to be endemic for Lyme borreliosis [1,6,7]. Furthermore, as many as 20% of patients in the United States and Europe who have Lyme borreliosis appear to have serologic evidence of coinfection with the HGE agent as well [46,91,95]. Figure 3 displays the U.S. states where HGE has been reported to state health agencies.

Ixodes scapularis (dammini), which is the tick vector for *Borrelia burgdorferi*, is thought to represent the main tick vector for HGE in the upper midwest and northeastern United States [33,44,95,114]. As many as 10.3% of *I. scapularis* ticks collected in northwestern Wisconsin and north central Minnesota during the 1980 to 1990s were found to contain the HGE agent [95]. Up to 50% of *I. scapularis* ticks collected in coastal Connecticut [86] and Westchester County, New York [107] contained HGE agent nucleic acids. *Ixodes pacificus* is believed to be the tick vector of the human granulocytic *Ehrlichia* in California. This tick species has been shown to transfer *Ehrlichia equi* from an experimentally infected horse to *Ehrlichia*-naive horses after feeding to repletion [17,82]. *Ixodes ricinus* is a known vector of *Ehrlichia phagocytophila*, and appears to be the candidate tick vector in Europe [65]. Although many patients have recalled bites by *Derma-*

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centor variabilis ticks before onset of HGE, there is currently no evidence in the literature that would support a role for these ticks as a vector for human granulocytic *Ehrlichia* [6,7].

As is the case with *Borrelia burgdorferi*, the major mammal host reservoir for the human granulocytic *Ehrlichia* in the United States is believed to be the white footed mouse (*Peromyscus leucopus*) [7,116]. The role of the white tailed deer (*Odocoileus virginianus*) as a reservoir host is unknown [39], although direct exposure to infected deer blood through skin cuts or nicks, inhalation of vaporized blood, or blood splashed into the conjunctival sac may have accounted for alternative routes of infection in three meat cutters [8]. Most cases of HGE have been reported to occur during late May, June, and July [1,7], which corresponds with the peak feeding period of nymphal *Ixodes scapularis* ticks [54]. *Ixodes scapularis* nymphs are difficult to see because of their small size, and human outdoor activity in the United States is probably higher during these months than any other period of the year, making nymphal ticks the most likely vector for passing granulocytic *Ehrlichia* species to humans.

Seroepidemiologic surveys for HGE among residents living in diverse geographical regions of the United States have shown prevalence rates ranging from 0.4% in northern California [58], 8.8% on Long Island, New York [109], to 14.9% in northwestern Wisconsin [14]. Clinical symptoms suggestive of HGE were not recalled any more frequently among the seropositive than the seronegative individuals in any of the investigated areas, suggesting the frequent occurrence of subclinical infections. IFA testing with *Ehrlichia equi* conducted on serum samples from patients with serologic evidence of Lyme borreliosis in Connecticut [86], Wisconsin [20,95], Sweden [47], Norway [9], United Kingdom [113], and Switzerland [24] have demonstrated antibodies to *Ehrlichia equi* among 8.6 to 17.1% of patients tested. Bakken et al. found an average HGE incidence rate of 16.1 cases/100,000 population in northwestern Wisconsin residents between 1990 and 1995, but rates as high as 58 cases/100,000 were observed for some counties [7]. Estimated incidence rates for several counties in downstate New York (Westchester County and others) may be considerably higher [7].

Clinical Manifestations

Bakken and coworkers initially described HGE as a severe illness [6]. Ten of their 12 patients required hospitalization for an average of 7 days, and two of the 12 patients died. Recent seroepidemiologic surveys would argue, however, that the majority of patients infected with HGE have a mild or asymptomatic illness [14,58,107,109]. Male patients have outnumbered females by a factor of 4 to 1, and 40 to 75% of surveyed patients have remembered a preceding tick bite [1,7,20]. Patients have described abrupt onset of symptoms starting approxi-

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mately 7 days after tick exposure or a recognized tick bite (Table 3) [1,7,20]. The patient median age is older than that of patients with HME. However, Aguero [1] found patients with HGE from New York to be significantly younger (median 43 years) compared with patients from the upper midwest reported by Bakken et al. (median 59 years) [7]. Clinical infection in children has been reported infrequently. The illness in children has been mild, and no fatal cases have been reported.

Three hundred and forty-five patients with HGE from 22 states had been reported to state health agencies by April 1997 (J. Childs, personal communication). Four patients are known to have died from complicating opportunistic infections consequent to HGE [1,95], allowing for an estimated case fatality rate of 1.2%. Autopsy findings from a 44-year-old man who died suddenly showed presence of granulocytic *Ehrlichia* in myocardial tissue (D.H. Persing, personal communication).

Characteristic signs and symptoms of acute HGE include rapid onset of fever, chills, malaise, rigors, headache, and generalized myalgias (Table 4). Many patients have also complained of anorexia, gastrointestinal upset with nausea, vomiting, diarrhea, and arthralgias. A nonspecific erythematous macular rash has been observed in only one of more than 120 patients serially followed by the authors [15] (J.S. Bakken, unpublished data), and was also described in one of 18 patients from New York reported by Aguero and coworkers [1]. Local skin reactions at the bite site have not been reported for patients who have recalled a tick bite before the onset of HGE.

Cough, and pulmonary infiltrates documented by chest radiograms, have been reported relatively frequently among patients with a more severe illness [7,124]. Four patients were reported to have severe headaches and abnormal neurologic findings [7]. One of these patients had a tonic-clonic seizure. The CSF analysis showed normal findings in each case [7]. Brachial plexopathy has been reported in a patient from New York [72].

It has been suspected that human granulocytic *Ehrlichia* induces a state of clinical immunosuppression in severely infected patients, as occurs in ruminants with *E. phagocytophila* infections [79]. One of the patients who died had not received specific antibiotic treatment (doxycycline) and developed progressive oliguric renal failure, ARDS, and hemorrhagic shock [6]. Complicating opportunistic viral infections (Herpes simplex virus, Cytomegalovirus) and fungal infections (*Cryptococcus neoformans, Aspergillus fumigatus*) have been identified as the causes of death of this patient and of two additional patients [6,95].

Acute HGE appeared to have a milder course in patients who had contracted their illness in New York than in patients living in the upper midwest, as judged by the reported rates of hospitalization (Table 3) [6,64]. Predictors of a severe illness have previously been shown to include older age, detection of morulae in the peripheral blood smear, as well as anemia, and the risk for severe disease

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also varies directly with the relative distribution of granulocytes (segmented and band neutrophils) and inversely with the relative distribution of lymphocytes (lymphopenia) in the peripheral blood [7]. Some patients have remained hospitalized for as long as 14 days [7]. Our group has confirmed HGE in approximately a dozen patients in the late convalescent phase of illness who were never treated with doxycycline (Bakken, unpublished data) [7]. Complete resolution of clinical symptoms usually had occurred by 14 days, and none of these patients manifested clinical symptoms that lasted beyond 60 days. The high number of asymptomatic seropositive residents living in areas that are endemic for HGE provides added support for the hypothesis that HGE is a self-limited illness most of the time [14]. Possible risk factors that allow for severe or fatal infections are poorly understood, but may include pre-existing mild degree of immunosuppression, previous hepatic dysfunction, and older age [7].

LABORATORY TESTS

The laboratory findings in patients infected with human granulocytic *Ehrlichia* are as nonspecific as those seen in patients with HME (Table 5). Leukopenia and thrombocytopenia have been reported to occur in most patients at some point during the course of their illness [1,6,7]. Recent studies have shown that blood leukocyte and platelet concentrations reach absolute nadir values in infected patients around day 7 of illness, and microscopic examination of peripheral blood-smears during the first week of illness typically shows a relative predominance of segmented and band neutrophils, and absolute and relative lymphopenia [13,73]. There is often a conspicuous absence of monocytes, eosinophils, and basophils in the peripheral blood smear during this period of illness. Thrombocytopenia appears to occur more frequently than leukopenia, and frank anemia develops as a late finding in patients with more prolonged illness (Table 5) [13]. Klein and coworkers have recently suggested that pancytopenia may be caused by myelosuppressive α- and β-chemokines produced by infected host cells [77].

Most patients who have been tested have shown elevated serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels approximately 2 to 4 times above baseline during the acute period of illness. Much higher values were observed in the patients who died, probably indicative of additional pathogenic mechanisms at play at the same time [6,64]. HGE elicits an inflammatory response as evidenced by elevated erythrocyte sedimentation rates (ESR) and C-reactive protein (CRP) concentrations (Table 5). Transient elevation of serum creatinine values has been described in patients who had normal renal function prior to their acute illness [1,7], and the few patients noted to have markedly elevated serum creatinine values all had pre-existing chronic diabetic nephropathy [6]. Abnormal coagulation parameters have been described in only two patients, both of whom died [6,64]. However, specific testing has been infrequently

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performed because, clinically significant bleeding has rarely been observed, even in patients with marked thrombocytopenia.

Diagnosis of HGE

HGE frequently presents as a nonspecific febrile illness, and confirmation of the diagnosis typically relies on demonstration of antibodies to the HGE agent or the surrogate antigen marker *Ehrlichia equi*. We have previously proposed case definition criteria for presumed, probable, and confirmed HGE (Table 7) [7]. Minimal presumptive diagnostic criteria for acute HGE are those of a patient presenting with an acute influenzalike illness and a temperature of at least 37.6°C. A confirmed case of HGE requires the aforementioned and, in addition, identification of 16 S rDNA sequences of the HGE agent in blood by polymerase chain reaction (PCR) amplification using *E. phagocytophila* group-specific primers, or a fourfold or greater change in *E. equi* IFA titer. Detection of morulae in cytoplasm of peripheral blood neutrophils without positive PCR or *Ehrlichia equi* serologic titer change, or a single *E. equi* IFA titer of at least 80 in a patient with fever, headache, and myalgias constitutes a probable case.

Patients who have been infected in the upper midwest have frequently presented with detectable morulae in neutrophilic leukocytes (Table 6) [7]. Examination of the peripheral bloodsmear may be time consuming in the initial few days of infection when the number of infected leukocytes may be few, and morulae may easily escape detection by the untrained eye [7]. Absence of morulae in the blood smear, however, does not rule out the diagnosis of HGE and should not preclude tetracycline therapy if the clinical suspicion of HGE is strong. Morulae have infrequently been recognized in the blood smear of patients from New York [1,126], which would support the conclusion by Aguero et al. that patients from the Atlantic coast region have a milder clinical course than patients from the upper midwest [1], or alternatively that patients have been identified earlier.

Antibodies that react in IFA assays with *Ehrlichia equi* or the human granulocytic *Ehrlichia* have been detected in serum samples from patients with acute HGE by as early as the second week of illness [7,11]. Peak antibody titers have been recorded 4 to 6 weeks after the onset of illness, and elevated IFA titer values were noted after 18 months or longer for more than 50% of patients [11]. However, patients who started doxycycline during the first week of illness were more likely to have negative IFA values after 12 months than patients who started treatment later, or not at all [11]. Protein immunoblotting of sera from infected patients have demonstrated presence of both IgM and IgG antibodies during the early convalescent period [74], and an immunoblot test kit that detects antibodies directed against major immunodominant protein bands in serum is currently being developed for commercial use.

PCR using the specific primer set ge9f/ge10r amplifies a 919-base pair

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DNA sequence of the human granulocytic *Ehrlichia* 16S rDNA [30]. PCR has proved to be a rapid, sensitive, and specific test method for confirming acute HGE (Table 6) [1,6,7,50,73]. Some commercial laboratories are now offering PCR as part of their diagnostic HGE repertoire, but expedient and careful sample handling is crucial and the test assay is costly.

The human granulocytic *Ehrlichia* agent was recently cultivated in vitro in an HL-60 promyelocyte cell line [62], and *Ehrlichia* were detected by culturing infected blood from patients reported from the upper midwest and New York (Table 6) [1,7]. Although cultures may provide a relatively rapid diagnosis ($\leq$7 days), only a limited number of research institutions are currently able to perform in vitro *Ehrlichia* cultures.

TREATMENT

Nonspecific acute febrile illnesses occur frequently, and many cases of HGE have undoubtedly gone by unrecognized and untreated. HGE is for the most part a self-limited illness that resolves within a few weeks even in the absence of antibiotic therapy. All patients diagnosed with HGE, however, should be treated because this illness has the potential for ending fatally [6,44,64]. Furthermore, prognosis may significantly worsen with delay in diagnosis and treatment [44,48,121].

The antibiotic treatment of HGE follows the same guidelines as for HME (Table 8). Klein et al. recently showed that a single strain of the human granulocytic *Ehrlichia* was susceptible in vitro to tetracycline, doxycycline, and rifampin, whereas beta-lactam agents, macrolides, chloramphenicol, aminoglycosides, and sulfonamides were all inactive [76]. Doxycycline is the recommended drug of choice for treatment of adults, based on excellent clinical outcomes of empiric treatment and because of the possibility of coinfection with *B. burgdorferi* [1,6,7,15,73] (Table 8). Doxycycline (100 mg) should be administered to adults by oral or intravenous route twice daily for a total of 14 days to ensure adequate treatment of HGE and Lyme borreliosis [15,120]. Patients respond quickly to doxycycline therapy, and most signs and symptoms resolve in 24 to 48 hours. One patient with HGE died while receiving doxycycline therapy [6], and another patient died after completed doxycycline treatment [64]. The causes of death were attributed to secondary opportunistic infections, and neither patient had any evidence of active ehrlichiosis at autopsy. Doxycycline is also recommended for the treatment of pediatric infections but the gravity of the clinical situation must dictate whether treatment should be instituted in children younger than 8 years and in pregnant women because of the potential for adverse drug reactions (Table 8) [15].

There are no published reports about the clinical efficacy of rifampin, but based on the in vitro activity, rifampin may be an effective alternative in patients who are intolerant to tetracycline drugs. One patient reported by Goodman et al.

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was treated with chloramphenicol and recovered uneventfully [62]. Subsequent in vitro susceptibility studies have shown chloramphenicol to be inactive against human granulocytic *Ehrlichia* at concentrations that are readily achieved in vivo. The role of chloramphenicol for the treatment of HGE therefore remains undefined [15].

PREVENTION OF HUMAN EHRLICHIOSES

Because HME and HGE are tickborne infections, prevention can be achieved by avoiding tick bites. The risk of tick bites can be reduced by use of long-legged light-colored pants tucked inside the socks. The light color will make it easier to spot deposited ticks [110]. Use of insect repellent skin spray containing 30% N, N-diethyl-m-toluamide (DEET) on exposed skin areas has been found to reduce the frequency of tick attachment. Permethrin (Permanone®) insect repellent spray on clothing is also helpful, but must not be used on naked skin. A daily inspection of all skin areas is an important preventive measure to avoid tick attachment that lasts beyond 24 hours. Epidemiological studies have suggested that the risk of developing Lyme borreliosis and HGE increases significantly in individuals who have ticks attached for longer than 24 to 36 hours [116].

SUMMARY

Both HME and HGE are nonspecific, febrile illnesses, and most patients recover uneventfully even in the absence of antibiotic therapy. Historical considerations must include the presence of illnesses that occur endemically in the area where patients have recently traveled, and differential diagnostic considerations may include other tickborne infections such as Lyme borreliosis, babesiosis, Rocky Mountain spotted fever, in addition to enteroviral infections, and acute hematologic malignancies. Influenza is usually not a diagnostic consideration because of the seasonal differences between human ehrlichioses (typically summer months) and influenza (typically winter months). Once the diagnostic suspicion of human ehrlichioses has been raised, patients should be considered for antibiotic treatment with doxycycline, because both HME and HGE have caused fatal infections in some cases. Laboratory confirmation of the diagnosis may not be possible until IFA seroconversion can be demonstrated, which usually takes 2 to 4 weeks after the onset of illness. However, patients with human ehrlichioses respond to doxycycline treatment within 24 to 48 hours. Failure to improve with doxycycline warrants re-evaluation of the clinical diagnosis. Laboratory tests should be specifically requested for the ehrlichial agent presumed to be responsible for the acute illness, because *Ehrlichia chaffeensis* and the human granulocytic *Ehrlichia* are antigenically different and require distinct methods for diagnosis by serology, PCR, and culture.

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Tickborne Relapsing Fever

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INTRODUCTION

Tickborne relapsing fever is a nonspecific clinical syndrome caused by a number of geographically defined species of borreliae. These organisms are distinct from *Borrelia burgdorferi*, the causal agent of Lyme disease, and *Borrelia recurrentis*, the agent of the more clinically severe louseborne relapsing fever. Most species are carried by soft-bodied ticks of the genus *Ornithodoros* and are pathogens of rodents and birds, infecting humans who come into contact with these ticks. Relapsing fever spirochetes, including tickborne species and *B. recurrentis*, have in common the ability to evade the host immune system by switching their surface antigens. This accounts for the typical clinical pattern of fever that lasts for several days only to abate and return days later when a new population of spirochetes bearing different surface antigens have emerged in the host. Tickborne relapsing fever borreliae are similar to other pathogenic borreliae in their ability to infect the central nervous system. Patients may present with neurologic manifestations as well as fever. Both louseborne and tickborne relapsing fever borreliae are sensitive to tetracyclines and beta-lactam antibiotics. Soon after the onset of appropriate antibiotic therapy, patients with tickborne relapsing fever can develop significant Jarisch-Herxheimer reactions, but to a lesser degree than those with louseborne relapsing fever. This reaction is thought to be mediated by TNFα and other cytokines. Present challenges in the study of these infections include improved methods of diagnosis, understanding the complex genetic mechanisms of serotype switching, mechanisms of pathogenesis, especially in the nervous

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system, optimal antimicrobial therapy, and management of the Jarisch-Herxheimer reaction.

History of Tickborne Relapsing Fever

Tickborne relapsing fever was probably described in 1857 by Livingston, and was shown by Ross and Milne to be caused by a bloodborne spirochete in Uganda in 1904 [1]. Others in the early part of the twentieth century, including Dutton and Todd in Central Africa, reported transovarial passage of spirochetes in the tick *Ornithodoros moubata* and the mechanism of infection in ticks [1]. Tickborne relapsing fever in the United States was first described in 1915 by Meader, who demonstrated spirochetemia in two out of five patients studied [2]. Tickborne relapsing fever is also known as endemic relapsing fever. Cases occur sporadically in clusters, or individually when patients are exposed to infected ticks. By contrast, louseborne relapsing fever is also known as epidemic relapsing fever and spreads through large numbers of people in settings of overcrowding, poverty, and war.

SPIROCHETE AND VECTOR

Tickborne Relapsing Fever Borreliae

The agents of tickborne relapsing fever belong to the order Spirochetales, within the family Spirochetaceae, which includes the genera *Spirochaeta* and *Treponema* as well as *Borrelia* [3]. Borreliae are motile organisms that measure 5 to 25 μm in length and 0.2 to 0.5 μm in width [3]. The motility and morphology of borreliae are conferred by periplasmic flagellae, which are attached to each terminus of the protoplasm of the spirochete [3]. Borreliae are distinguished from treponemes by the fact that borreliae have unsheathed flagellae and lack cytoplasmic microtubules [3]. These organisms are microaerophilic [3] and are able to synthesize purines [4]. Their genomes are arranged in a unique linear chromosome and a number of linear and circular plasmids. The plasmids of tickborne relapsing fever borreliae replicate in a fixed ratio with the linear chromosome and encode essential housekeeping genes, causing some to refer to them as minichromosomes [5].

All Borrelia species have arthropod vectors, most of which are soft-bodied ticks. *Borrelia recurrentis*, the causal agent of louseborne relapsing fever, is thus far the only borrelia transmitted by lice rather than ticks [3]. The majority of relapsing-fever borreliae are found in ticks from the family *Argasidae*, including the genus *Ornithodoros*. Table 1 lists the geographic distribution of these borreliae and the variety of their tick vectors and animal reservoirs.

The difficulty of in vitro cultivation of borrelia species has made the study of relapsing fever spirochetes challenging. The tickborne relapsing fever organ-

TABLE 1 Tickborne Relapsing Fever Borrelia Species

Disease	Borrelia species	Arthropod vector	Reservoir	Geographic distribution
East African tickborne relapsing fever	*Borrelia duttoni*	*Ornithodoros moubata*	Humans	Central, Eastern, Southern Africa
Hispano-African tickborne relapsing fever	*B. hispanica*	*O. erraticus* (large variety)	Rodents	Spain, Portugal, Morocco, Algeria, Tunisia
North African tickborne relapsing fever	*B. crocidurae*	*O. erraticus* (small variety)	Rodents	Morocco, Libya, Egypt, Iran, Turkey, Senegal, Kenya
	B. merionesi	*O. erraticus* (small variety)	Rodents	Kenya, Sahara, Dakar
	B. microti	*O. erraticus* (small variety)	Rodents	Iran
	B. dipodilli	*O. erraticus* (small variety)	Rodents	Kenya
Asiatic-African tickborne relapsing fever	*B. persica*	*O. tholozani* (also *O. papillipes*, *O. crossi*)	Rodents	West China, Kashmir, Iraq, Egypt, former USSR, India
Caucasian tickborne relapsing fever	*B. caucasica*	*O. verrucosus*	Rodents	Caucasus to Iraq
	B. latyschewii	*O. tartakowskyi*	Rodents	Iran, Central Asia
American tickborne relapsing fever	*B. hermsii*	*O. hermsii*	Rodents (chipmunks, tree squirrels)	Western United States; Virgin Islands?
	B. turicatae	*O. turicata*	Rodents	Southwestern United States
	B. parkeri	*O. parkeri*	Rodents	Western United States
	B. mazzottii	*O. talaje* (*O. dugesi*)	Rodents	Southern United States, Mexico, Central and South America
	B. venezuelensis	*O. rudis* (*O. venezuelensis*)	Rodents	Central and South America
Relapsing febrile illness with neurologic symptoms	Unnamed Spanish *Borrelia species*	*O. erraticus*	Unknown	Spain

Source: Refs. 1, 3, 40, 54

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ism *B. hermsii* was cultured in 1971, before cultivation of *B. burgdorferi* in 1984 and *B. recurrentis* in 1994 [6]. Before the development of enriched media for in vitro cultivation, strains had been maintained in rodents and laboratory ticks and could be cultured in embryonated eggs [3]. Media most commonly used to grow borreliae are BSK II or BSK H. These are both complex media that are usually incubated at temperatures between 30 and 37°C and monitored by dark-field microscopy for up to 6 weeks [3].

Biology in Ticks

The three species of borreliae responsible for tickborne relapsing fever in the United States are *B. hermsii, B. turicatae*, and *B. parkeri*. Their corresponding tick vectors are *O. hermsii, O. turicata*, and *O. parkeri*, which occupy remote niches in the West. *O. hermsii* is found in forested areas, usually at elevations above 3000 feet [2]. *O. turicata* lives in caves and on the plains at lower elevations. Finally, *O. parkeri* has a similar distribution and is uncommonly implicated in transmission of relapsing fever to humans [2].

These American relapsing fever borreliae, like borreliae in general, are extremely specific to their respective tick vectors and are not transmitted by ticks of a different species feeding on a spirochetemic host [3]. Borreliae have a somewhat restricted mammalian and avian host range as well.

Blood ingested by these ticks is digested within the epithelial layer of the midgut [7]. Borreliae move from the gut epithelia, multiply in the hemolymph of the tick, and are subsequently concentrated in the tick's salivary glands, reproductive tissues, and neural tissue [7]. Infection can be transmitted transovarially in ticks so that several succeeding generations of ticks can transmit borreliae without the need to feed on an infected host. There can also be venereal transfer of borrelia from male to female ticks, but the significance of this is unknown [7]. Borreliae are spread from the tick to the mammalian host by way of saliva or the fluid of the nearby coxal glands, which is released by some ticks during feeding [7].

Argasid ticks are quite long lived. They can survive several years of starvation and remain capable of transmitting borreliae at the time of their next blood meal. The infectious dose for a susceptible animal host can be exceedingly small. Laboratory studies have been aided by the fact that a single *B. hermsii* or *B. turicatae* can cause infection in a mouse, allowing for examination of clonal populations [7].

The incidence of human infection peaks in the summer months when human activity in tick habitat is at a maximum, although infection can occur in winter as well. Typically humans encounter the ticks when sleeping in structures that are not rodent-proof, or spend time in caves or near woodpiles or other potential rodent habitat. *Ornithodoros* ticks feed briefly, for not more than 20 to 30 minutes, then drop off their warm-blooded host. They feed at night and their

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bites are painless. Transmission of relapsing fever borreliae can occur during this short period of time. This contrasts with *Ixodes* ticks, which stay on their hosts for hours and are more likely to transmit *B. burgdorferi* infection the longer they stay attached.

MOLECULAR BIOLOGY OF TICKBORNE RELAPSING FEVER

The clinical manifestations of periodic fevers are determined by the mechanisms by which relapsing fever borrelia species undergo serotype switching. *B. hermsii* is the most studied of the American species. These spirochetes possess serotype-specific outer membrane proteins known as variable major proteins (Vmps). Over 25 different serotypes have been associated with the progeny of a single cell of *B. hermsii* strain HS1 [8,9]. The technical difficulties of studying an organism that switches surface antigens in response to the host immune system are overcome by immunosuppressing the animal host such that it cannot produce antibodies to the spirochete and thereby instigate antigenic switching. These strategies include irradiation of adult mice [10] or use of mice with severe combined immunodeficiency (scid mice) [11]. The frequency of serotype switching is estimated to be about 10^{-4} per generation both in vitro in broth and in vivo in mice [8]. If the level of spirochetemia in an infected animal can approach 10^{7} borrelia per milliliter [7], this means there may be significant numbers of spirochetes switching serotype at the peak of infection.

The genome of *B. hermsii* consists of a linear duplex DNA molecule of about 1 million base pairs; a single borrelia cell contains 10 to 20 copies of this chromosome and its own set of linear plasmids that contain both expressed and silent copies of the (variable major protein) *vmp* genes [5,12]. Some evidence indicates that there may be a circular intermediate form of one of these plasmids [5]. There are circular plasmids in other borreliae such as *B. burgdorferi*; the significance of linear plasmids in relapsing fever borreliae is unknown. In *B. hermsii*, there are approximately one to two linear plasmids per chromosome [13]. These plasmids are approximately 28 to 32 kilobase pairs in length [14]. Evidence suggests that replication of the plasmids and the chromosome may be tightly coupled in borreliae and, given that these plasmids contain essential housekeeping genes in addition to important virulence factors, that the linear plasmids be considered ''minichromosomes'' [4,5].

Each Vmp is encoded by a unique *vmp* gene [9]. The Vmp expressed by a specific serotype is expressed by its corresponding unique *vmp* gene. Other *vmp* genes encoding other silent Vmps are located in other regions of either the silent or expression plasmids. The active or expressed version of the *vmp* gene is only seen in cells that are expressing that Vmp from the expression locus on one of the plasmids. A particular borrelia has silent copies of multiple *vmp* genes at other loci. When serotype switching occurs, a silent copy of the next *vmp* gene to become active undergoes a recombinational event in which a copy of the silent

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vmp moves into the expression locus and the previously expressed *vmp* is lost. The expression locus is near the right telomere of the expression plasmid. The telomeres are formed by covalently closed terminal hairpin loops with short inverted repeats near the termini [14]. The expression site of a *vmp* gene is flanked at its 5′ end by a sigma-70 promoter and an upstream homology site (UHS), and at the 3′ end by a conserved region referred to as the downstream homology sequence (DHS) [9,14]. Downstream from the DHS is the covalently closed end (telomere) of the expression plasmid. The *vmp* genes are variable in length, and the length of the sequences between the expressed *vmp* and the telomere is also variable [9].

There are at least three mechanisms by which *vmp* genes may be expressed from the expression locus. The first is an interplasmidic recombination, which is a reciprocal event between the *vmp* at the expression locus and one at a silent locus. The recombination occurs between the 5′ end of the *vmp* and the DHS, and a new copy of the previously silent *vmp* occupies the expression site and the previously expressed *vmp* is lost [14]. Evidence that this recombination sometimes occurs in an incomplete fashion was suggested by the report of a chimeric Vmp in which a silent *vmp* had incompletely recombined into the expression locus and no silent gene for the resulting Vmp existed [10]. A second, intraplasmidic model for Vmp expression is activation of a pseudogene [15]. In this model, an adjacent silent *vmp* lacking a promoter, start codon, and a portion of its 5′ end (ie, an incomplete gene) moves into the expression locus. This recombination is mediated by a deletion between direct repeats in the previously active *vmp* and the pseudogene, and results in the pseudogene being placed directly downstream from the previous promoter and start codon, allowing it to be actively transcribed. The third mechanism for antigenic variation proposed is that of postswitch gene conversion [12]. In this model, after an intraplasmidic deletion, mutations appeared at the 5′ end of the gene that were not present in the silent gene. Certain positions appear to be sites most likely for these nucleotide changes to take place. Although postswitch mutations are noted to increase in frequency during infection [12], the relative contribution of each of these mechanisms in the natural course of infection is unclear.

Vmps can be separated into two groups based on size. Genes of about one kilobase pairs encode large Vmps, and small Vmps are encoded by genes of about 600 base pairs. Sequence of the small *vmp* gene is most unique within the middle 300 base pairs. Small *vmp* genes are felt to be 80% identical overall, but identity over similar lengths of small and large *vmp* genes is only 40 to 50% similar [9,12]. Small Vmps are felt to belong to a family of 20 kd cell surface proteins, which includes outer surface protein C (OspC), a major surface antigen in *Borrelia burgdorferi* [16,17]. Specific small Vmps may play a role in preferential localization of borreliae in the central nervous system in both *B. turicatae* and *B. burgdorferi* [11].

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RELAPSING FEVER

Clinical Spectrum of Disease

Tickborne and louseborne relapsing fever are diseases characterized by episodic high fever and accompanied by headache, chills, and fatigue. In general, tickborne disease, with its mortality rate of 0 to 8%, is a milder disease than louseborne relapsing fever, which carries a mortality rate of 40% or even higher in some epidemics [1]. Patients at risk for death include those at extremes of age [1,18]. Many patients with tickborne relapsing fever do not remember having an attached tick, because argasid ticks feed rapidly and drop off their vertebrate hosts after not more than 30 minutes [1]. Occasionally ''insect bites'' are seen or recalled [19–21], and sometimes a pruritic eschar may develop at the site of the bite [2]. The incubation period is approximately 1 week, with a range of 3 to 18 days [1,2,20]. The onset of illness is characterized by sudden high fever (over 103°F), chills, fatigue, and headache in the majority of patients. Other symptoms may include arthralgia, myalgia, diaphoresis, abdominal pain, nausea, vomiting, anorexia, diarrhea, cough, epistaxis, and sore throat [1,2,20,21]. Between 4 to 50% of patients develop a nonspecific rash, which has been described as macular, papular, petechial, or, rarely, as erythema multiformelike [1,19,21]. Physical findings may include liver tenderness, hepatomegaly, splenomegaly, lymphadenopathy, particularly of cervical and axillary nodes, and weight loss [1,19–22].

Without antibiotic therapy, the fever lasts 3 to 6 days. Lysis of the fever is marked by diaphoresis [2,20]. The patient remains afebrile for an interlude that can last an average of 8 days, but can range from 3 to 36 days [2,20]. The natural history of untreated tickborne relapsing fever is for an average of 3 to 5 relapses to occur, cycling through febrile episodes and afebrile periods [2,20]. Typically, subsequent febrile episodes are somewhat milder and of shorter duration than the initial one, although this is not always the case [2,20]. The length of time between febrile episodes tends to increase over the course of the illness [20].

Respiratory symptoms are more common in very young patients [1]. When clusters of relapsing fever cases occur, a few patients may report symptoms suggestive of upper respiratory tract infection [20]. Some patients present with an exacerbation of their underlying asthma [23]. Nonproductive cough has been reported in 9% of patients with tickborne relapsing fever, but pneumonia does not occur [24]. There has been one report of adult respiratory distress syndrome (ARDS) in a relapsing fever patient who was also pregnant [25].

Similar to other borreliae, tickborne relapsing fever borreliae have a propensity to infect the central nervous system. The rate of neurologic complications of relapsing fever is comparable to that of Lyme disease [26]. The incidence of neuroborreliosis during tickborne relapsing fever depends on the species of borrelia and appears to be highest with the African *B. duttoni* and the American *B.*

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turicatae [26]. Eight to 23% of relapsing fever patients have symptoms such as headache, vomiting, and neurologic findings, including meningismus, lethargy, cranial nerve palsies, hemiparesis, problems with word finding and concentration, delirium, mania, vertigo, radiculopathies, extrapyramidal symptoms, transverse myelitis, and, rarely, seizures [1,19,21,26–28]. The most commonly noted cranial nerve palsy is a seventh nerve palsy; fifth, sixth, or eighth cranial nerve involvement is occasionally reported [26]. Cranial nerve palsies usually appear not with the first febrile episode but with one of the subsequent episodes. These deficits usually resolve with or without antibiotic therapy, but persistent seventh and eighth nerve cranial palsies have been reported [26]. Prolonged depression, headaches, backaches, and fatigue are reported to be common in patients with infections attributable to *B. turicatae* [26].

Cerebrospinal fluid examination is abnormal more than half of the time and reveals pleocytosis with a lymphocytic predominance, increased protein, and occasionally the spirochetes themselves [1,26]. In cases studied, the mean total white blood count in the cerebrospinal fluid is between 100 to 300 cells per cubic millimeter [26]. The mean cerebrospinal fluid protein level in these studies was approximately 0.3 g/dL [26].

Ocular manifestations of tickborne relapsing fever include photophobia, eye pain, and conjunctival injection [19,21]. Iritis, uveitis, and endophthalmitis may also occur [1,19]. Eye involvement is bilateral in approximately a third of cases, and there are frequently residual defects [26]. Ocular manifestations in tickborne borreliosis tend to occur, like neurologic manifestations, during third or later febrile episodes [26]. The incidence of ocular involvement is species dependent, with *B. turicatae, B. duttoni*, and *B. hispanica* most frequently associated [26]. A newly described Borrelia species has also been found to cause uveitis in dogs [29].

Unique risks have been observed in pregnant patients with tickborne relapsing fever. There is increased incidence of spontaneous abortion, and transplacental transmission has been reported [19,30].

Laboratory test abnormalities occur during febrile crises and are nonspecific. These include modest leukocytosis, anemia, and increased erythrocyte sedimentation rate [23].

The differential diagnosis includes Lyme disease, Rocky Mountain Spotted Fever, Q fever, Colorado Tick fever, typhus, infectious mononucleosis, leptospirosis, influenza, brucellosis, psittacosis, and malaria [20,23,28,31].

Pathophysiology

In an animal host, the borreliae multiply rapidly in the blood until the host produces sufficient concentrations of serotype-specific antigen to remove them. Development of antibody to the spirochetes is a T-cell independent phenomenon

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[32]. After antibody binds to borreliae, the spirochetes undergo either phagocytosis or complement-mediated lysis [33]. Experimental evidence suggests that Kupffer cells in the mammalian liver actively ingest borreliae, and therefore may play a significant role in defense against relapsing fever [34]. Despite rapid clearance from the bloodstream, borreliae persist in multiple tissues, including the liver, spleen, kidneys, eyes, and, most notably, the central nervous system [7]. Silver staining of postmortem tissues has revealed spirochetes in splenic and brain tissue [30]. Rodents can shed viable borreliae in urine, saliva, and milk [7]. *B. hermsii* has been found to possess a glyceraldehyde-3-phosphate dehydrogenase homolog, which may act as an adhesin or cell membrane receptor and thereby play a role in pathogenesis [35].

Tickborne relapsing fever borreliae display varying degrees of neurotropism, depending on the species. In experimental animal models, borreliae have been recovered from animal brains up to 3 years after infection [7]. Experiments comparing association of *B. hermsii, B. turicatae*, and *B. burgdorferi* to endothelial cells and cultured neural cells show the relapsing fever agents to preferentially associate with neural tissue and the Lyme agent to associate with endothelial cells, although competition for neural cells was noted between both groups [36]. The serotype variation that characterizes bloodstream infection has also been shown to occur in the brain in immunocompetent animal models. Immunodeficient mice retain the original infecting serotype in the brain even after bloodstream borreliae have been cleared by administration of exogenous antibodies, further suggesting that borreliae found in the brain are secondary to central nervous system infection, rather than secondary to contamination by blood [37].

Histology of central nervous system infection caused by a newly described European tickborne relapsing fever species reveals perivascular infiltrates of typically mononuclear cells of the meninges and choroid plexus [38]. This pattern is also seen in infections caused by other tickborne relapsing fever species [26]. Borreliae have also been seen between neurons and glial cells in sections of cortical tissue; there is no evidence that borreliae directly invade neurons [26].

DIAGNOSIS

A definitive diagnosis of borrelia spirochetemia can be made by examining a peripheral blood thick or thin smear or buffy coat smear stained with Wright-Giemsa stains. Other spirochetes, including Leptospira and Treponema, do not stain with these dyes [21]. *B. burgdorferi* usually does not occur in high-enough concentrations in the blood to be revealed by smear [3]. At the height of a febrile episode of relapsing fever, patients usually have a sufficiently high level of spirochetemia to allow the borreliae to be seen on peripheral smears. Occasionally the spirochetes are recognized on examination of blood smears for malaria [23]. The sensitivity of Wright-Giemsa smears obtained during a febrile episode ap-

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proaches 70% [1]. If white blood cell differential counts are done in an automated fashion rather than manually, spirochetes will be missed. Therefore, in areas where tickborne relapsing fever occurs, patients with high-grade fever, headache, and other suggestive symptoms should have a manual differential count performed [31]. The spirochetes can also be visualized by dark-field of wet preparations of blood [3]. Acridine orange staining and examination of smears by fluorescence microscopy has also been demonstrated to be a sensitive tool in examining blood of patients who may have a relatively mild degree of spirochetemia [39].

Attempts have been made to develop immunofluorescent assays or ELISAs for diagnosis, but they are hampered by the antigenic switching the spirochetes undergo [28]. The Centers for Disease Control performs serology testing by ELISA, which can be of use in demonstrating a rise in antibody titers in paired acute and convalescent sera against *B. hermsii* or *B. turicatae* [27,40]. Cross-reactivity of Lyme antigens with relapsing fever antigens occurs and can make the diagnosis more difficult in areas of geographic overlap between both diseases [31]. Examination of bands in Western blots and comparison of relative titers of antibody can aid in differentiating these diseases for those experienced in interpretation of such data. An additional group of serologic tests developed to help distinguish between Lyme and relapsing fever organisms are immunofluorescence assays or ELISA to detect antibody to the *B. hermsii* glycerophosphodiester phosphodiesterase GlpQ [41]. This immunoreactive protein is conserved among many borrelia species with the exception of *B. burgdorferi*, and is recognized by mammalian antibodies following relapsing fever [41].

Culture of borrelia in Barbour-Stoenner-Kelly (BSK II) medium is most specific [3], but intraperitoneal inoculation of a mouse with blood from the patient and subsequent monitoring of the animal for spirochetemia also results in isolating the organism [21]. This approach is more useful if a suspected relapsing fever patient has a low level of spirochetemia and the borreliae are not found by staining [28].

Occasionally Proteus OXK agglutinins appear in some tickborne relapsing fever infections, but this is not a reliable finding [20,21]. If positive, titers to Weil-Felix tests tend to be low [21].

THERAPY AND PREVENTION OF TICKBORNE RELAPSING FEVER

Few controlled trials of therapy for tickborne relapsing fever have been reported. Based on previous experience, the drugs of choice are tetracycline and doxycycline [1,42]. Although single-dose therapy has been effectively used for louseborne relapsing fever, longer courses are needed in tickborne relapsing fe-

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ver. The relapse rate for single dose therapy for tickborne relapsing fever has been reported to be greater than 20% [26]. The dose and duration of therapy with doxycycline is usually 100 mg twice a day for 5 to 10 days [28]. Prudence may dictate a longer course of therapy in patients with neurologic manifestations [28]. Although doxycycline appears to be the most effective drug for treating tickborne relapsing fever, there have been rare reports of treatment failure [43]. Relapses are more commonly seen with penicillin G therapy [20,42]. The incidence of treatment failures may be higher in pregnant patients [19]. Third-generation cephalosporins, such as ceftriaxone, have been used to treat tickborne relapsing fever with neurologic manifestations [28]. In the absence of controlled studies, data from neuroborreliosis animal models suggest an effective length of therapy is 10 days [44]. Oral therapy is usually successful, but occasionally parenteral therapy is required as is hospitalization.

Erythromycin has been successfully used; however, treatment failures with erythromycin have also been reported [19,28,45]. Erythromycin is not known to be effective therapy for central nervous system complications of tickborne relapsing fever [26]. Despite the fact that vancomycin is bacteriocidal for tickborne relapsing fever borreliae [44,46] experiments in animal models of infection with *B. turicatae* showed that vancomycin was not always successful in treating central nervous system infections [44]. Although there have been reports of clinically effective therapy with streptomycin, borreliae are considered to be relatively resistant to the aminoglycosides [26]. Chloramphenicol does not consistently prevent persistent brain infection or treat relapses of tickborne relapsing fever in animal models, and there is little experience with the use of chloramphenicol to treat relapsing fever in humans [26].

Control of the incidence of tickborne relapsing fever can be accomplished through rodentproofing dwellings and campsites, as well as using insecticides directed against ticks [21,27]. Use of tick repellents on clothing seems a prudent measure as well [23].

JARISCH-HERXHEIMER REACTION FOLLOWING THERAPY OF TICKBORNE RELAPSING FEVER

The Jarisch-Herxheimer reaction is a sequella of antimicrobial therapy, usually of spirochetal infections. Although the reaction has been encountered with Lyme disease and syphilis, the most severe reactions occur with *B. recurrentis* infection [47]. The propensity to develop a Jarisch-Herxheimer reaction in tickborne borreliosis varies with the species; it has been reported as being common with *B. duttoni* and rare to nonexistent in infections attributable to *B. crocidurae* [28]. The incidence of the Jarisch-Herxheimer reaction with infection caused by the North American tickborne relapsing fever species is approximately 33% [19].

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Reactions are more commonly seen with tetracycline therapy, especially with higher doses, and occur within 90 minutes of administration of an appropriate antibiotic [19,45]. Manifestations consist of chills, heightened fever, tachycardia, and initial hypertension followed by hypotension. The latter may be profound [1]. This reaction is mediated by lysis of the spirochetes, followed by massive release of TNFα, IL-6, and IL-8 [45,47,48]. There may be some role played by endogenous opiates in the pathophysiology of this reaction [49].

No effective therapy for the Jarisch-Herxheimer reaction exists. Patients should receive supportive care, including close hemodynamic monitoring as the situation warrants. Multiple attempts have been made to find agents that will significantly attenuate the Jarisch-Herxheimer reaction, yet none has been found. Administration of corticosteriods or acetominophen did not prevent rigors [50]. Pentoxifylline failed to prevent or blunt the Jarisch-Herxheimer reaction in patients with louseborne disease, despite a favorable effect on serum cytokines [51]. An opiate agonist-antagonist, meptazinol, has been shown to modestly reduce the severity of the symptoms of the Jarisch-Herxheimer reaction, but not to abolish it [49]. Treatment with anti-TNF-α Fab antibody fragments before administration of antibiotics in louseborne relapsing fever may suppress the hemodynamic instability in the Jarisch-Herxheimer reaction and lowers concentrations of circulating cytokines in these patients; this approach is still investigational [52,53]. The strategy of waiting until the patient is afebrile to initiate therapy has not been proven to spare patients the Jarisch-Herxheimer reaction [19].

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12

Colorado Tick Fever

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Colorado tick fever (CTF) is a viral disease transmitted by ticks in the Rocky Mountain region and the Pacific slope of the United States and Canada. The characteristic course of CTF is the biphasic appearance of fever, headache, myalgias, and leukopenia.

ETIOLOGY

In the 1850s, Colorado tick fever was called ''mountain fever'' and was not clearly distinguished from Rocky Mountain spotted fever or tularemia until the 1930s. In the 1940s, Florio successfully transmitted CTF to human volunteers and hamsters by the injection of serum from patients with CTF [1]. The agent was able to pass through filters that retained bacteria, and the causative organism was subsequently shown to be a double-stranded RNA virus.

Colorado tick fever virus was initially classified in the genus Orbivius of the family Reoviridae but was recently assigned to the Coltivirus genus [2]. The virus genome contains 12 segments of double-stranded RNA [3]. There are three serotypes of CTF virus, each of which causes a similar illness: CTF virus, Eyach virus, and strain S6-14-03. The Eyach virus was isolated from *Ixodes ricinus* ticks in Germany and France from the *Ixodes ventalloi* tick in France [4]. The S6-14-03 strain was isolated in coastal California. The virus is relatively stable at room temperature, and is capable of infecting many types of mammalian cells in tissue culture [5].

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EPIDEMIOLOGY

In the United States, CTF occurs almost exclusively in the area of distribution of the *Dermacentor andersoni* tick, a mountain wood tick, and is acquired from the bite of virus-infected ticks. The virus has been recovered from other types of ticks in the Pacific and Rocky Mountain areas, but *D. andersoni* is the only tick known to transmit the disease to humans [6,7]. *D. andersoni* is found at elevations of 4,000 to 10,000 feet, predominantly on south-facing mountain sides. Sagebrush, juniper, pine trees, and shrubs on dry rocky surfaces are typical habitats for the ticks [8]. Infections in people who are visiting endemic areas can lead to the appearance of the disease outside of western North America. Rare cases of infection by contact with infected tissue have been reported, such as in laboratory accidents and blood transfusions [9].

D. andersoni is a hard-body tick that is abundant in areas where small mammals share habitats with wild and domestic large mammals. Female ticks lay eggs under dead vegetation. The larvae emerge and feed during the summer on small mammals, such as squirrels, chipmunks, and deer mice [10]. The larvae develop into nymphs and hibernate. In the spring, the nymphs feed on small mammals, fall off, and molt into adults. The newly emerged adults take a blood meal and may mate, but more commonly hide under debris near the surface of the soil for a second winter. They then come out of hibernation when the snow melts and climb to the top of low vegetation, where they have the opportunity to transfer to animals. Adult ticks usually feed on mammals such as porcupines [11], elk, deer, and marmots or, accidentally, on humans. Female ticks attach and feed for 6 to 13 days, drop off to lay eggs, and die. The male may feed for only a few hours before seeking an attached female. The virus is not transmitted transovarially; rather, the larvae and nymphs acquire the CTF virus from feeding on small mammals that are viremic. The virus survives the winter in hibernating nymphs or adults. The nymphs transmit the infection to other small mammals in the spring, perpetuating a reservoir of the virus. The virus may survive for 2 to 3 years before completing the three-host cycle.

Although most patients with CTF report exposure to ticks while working, hiking, or camping, only half are aware of a tick bite or attachment. Males between the ages of 20 and 39 years contract CTF more often than other subgroups of the population, probably because of more time spent outdoors. As many as 15% of forest rangers in endemic areas possess neutralizing antibody against the virus. The peak incidence of CTF in humans is April and May at low altitudes, and June and July at higher elevations. About 200 to 400 cases of CTF are reported to public health authorities in the United States each year.

PATHOGENESIS AND PATHOLOGY

Viremia is almost always found in patients with CTF and in animals experimentally infected with the virus. CTF virus may be recovered from the plasma during

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the first week of illness and from blood cells for up to 120 days after onset of illness. The peak levels of viremia occur in the second and third week after onset. The duration of viremia correlates to the normal life span of circulating erythrocytes. The mature erythrocyte lacks functional ribosomes essential to virus replication, and entry of virus into mature erythrocytes has not been shown. It is likely that infection of erythrocytes begins in hematopoietic cells, a hypothesis that is supported by the demonstration of virions and virus replication within erythrocyte precursors in the bone marrow [12,13].

Patients with CTF have concentrations of interferon alpha in their serum that is higher than the concentrations of interferon alpha in patients with other viral diseases. Double-stranded RNA viruses are known to be potent inducers of interferon. The concentrations of interferon correlate well to the degree of fever [14].

MANIFESTATIONS

The incubation period is usually 3 to 6 days, but can be as long as 14 days [7,10]. Patients often report chills, but no true rigors. Symptoms of fever up to 39.5°C, headache, lethargy, and myalgias of the back and legs are usually sudden in onset. Retroorbital pain and pain on orbital movement are commonly reported. About 20% of patients report abdominal pain or vomiting and a mild pharyngitis. A mild diffuse rash is seen in less than 10% of patients. The rash is macular, maculopapular, or petechial and can be confused with the rash of Rocky Mountain spotted fever [15].

About half of patients experience a characteristic biphasic or ''saddleback'' fever in which a 2- to 3-day febrile period is followed by a 1- to 2-day remission [16]. This is followed by another 2- to 3-day febrile period. The remaining patients have either a single febrile episode or, less frequently, three episodes of fever. Recovery usually occurs within 2 weeks but there are a few cases of prolonged weakness, malaise, and depression months after the initial infection. With recovery, lifelong immunity is the rule, but second attacks have been reported.

Physical findings are few. A mild transient rash may be seen [7,10]. Flushed facies, conjunctival injection, pharyngeal erythema, tachycardia, and hepatosplenomegaly may be observed. Occasionally, an imbedded tick is found.

Leukopenia is a characteristic finding during the course of CTF [17]. The leukocyte count typically reaches its nadir during the second febrile episode, with values as low as 1000/μl, and may continue to be depressed for up to 7 days after clinical recovery. A relative lymphocytosis occurs, along with an increase in immature forms of polymorphonuclear cells and, occasionally, thrombocytopenia. The bone marrow reveals a maturation arrest of neutrophils.

In the rare hemorrhagic form of the disease, thrombocytopenia and dissemination intravascular coagulation occur. Endothelial swelling with focal necrosis of capillaries was found in a fatal case.

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Encephalitis and meningitis in CTF occur in less than 100% of cases and probably result from viral invasion of the brain and meninges [10]. The virus was recovered from the cerebrospinal fluid of a patient who had encephalitis as well as from experimentally infected subjects who had no neurologic symptoms. Intracytoplasmic inclusion bodies were seen in neurons and Purkinje cells of the midbrain in a fatal case of encephalitis.

In adults, pericarditis and myocarditis may occur in association with CTF. Other unusual complications include epidiymo-orchitis, arthritis, pleuritis, chorioretinitis, and pneumonitis.

DIAGNOSIS

Fever, myalgias, and leukopenia occurring in a patient in late spring or summer with recent exposure to ticks in the northwestern United States or Canada should suggest CTF. A biphasic clinical course is highly specific. In a study in Colorado of febrile patients, abdominal pain, sore throat, and rash were associated with other viral origins [18].

The diagnosis of CTF can be confirmed by isolation of virus from blood, identification of the virus in erythrocytes in fluorescent antibody staining, or serology [18,19]. The titer of virus may be higher in the plasma than in the cellular fraction of the blood during the first few days of illness before neutralizing antibodies appear in the serum. For these reasons, whole blood should be examined during the first 3 days after onset of symptoms. Examination of the blood clot is preferred on days 4 to 10, and of a washed blood clot thereafter. For virus isolation, red cells are homogenized and injected into suckling mice. After 3 to 4 days, the heart blood of the mouse is examined by fluorescent antibody staining. Direct fluorescent antibody examination of a blood clot is positive in virtually all cases from 5 days to 2 months after onset. False negative tests may occur during the first few days of the disease [20].

A fourfold rise in neutralizing antibodies, complement fixation antibodies, or antibodies measured by either indirect immunofluorescence or enzyme immunoassay (EIA) is also diagnostic when using acute and convalescent sera [19,21]. The complement fixation antibodies are rarely performed but are diagnostic of recent infection. About 94% of patients develop neutralizing antibodies, although the assay for neutralizing antibody is time-consuming and costly. The EIA for either IgM or IgG antibody is less specific and does not become positive until over 2 weeks after the onset of illness, but is easy to perform and less expensive [20]. Diagnostic tests for CTF are available at most state health department laboratories in the endemic areas, along with the Division of Vector-Borne Viral Disease, Centers for Disease Control and Prevention, Fort Collins, Colorado (telephone number 904-221-6407).

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DIFFERENTIAL DIAGNOSIS

In the western United States, confusion of CTF and Rocky Mountain spotted fever is common. Rocky Mountain spotted fever can be distinguished from CTF by the progressive rash that appears 2 to 6 days after onset, a tendency for leukocytosis, and a more severe course. Although *Rickettsia rickettsii* may be recovered from *D. andersoni* in the western United States, the disease is relatively uncommon in the Rocky Mountains. Most cases are reported from the southeastern states. Simultaneous infections with both Rocky Mountain spotted fever and CTF have occurred, and ticks that carry both CTF and *R. rickettsii* have been found.

A biphasic fever can occur in dengue as well as in CTF. Dengue can usually be excluded on epidemiologic grounds. However, the migration of dengue northward from Mexico heralds the possibility for confusion.

Relapsing fever, a disease caused by *Borrelia* species and transmitted by soft-body ticks, also occurs in areas endemic for CTF. In this disease, a leukopenia is rare and the spirochetes are often seen in peripheral blood smears.

Tularemia may rarely be tickborne in the Rocky Mountain area. Regional lymphadenopathy and ulceration at the site of the tick bite are typical. The agglutination test for tularemia is reactive after 10 to 14 days.

Ehrlichiosis is a tickborne illness that occurs mainly in the eastern and southern United States and is characterized by fever, headache, leukopenia, and elevated transaminases. Treatment consists of tetracycline. Differentiation from CTF is based on the epidemiologic factor of onset of tick bite or exposure.

PROGNOSIS

Three deaths from CTF have been reported in children who had severe hemorrhage disease. Many patients complain of malaise and weakness during the convalescent phase. An association exists between the duration of viremia and the length of convalescence. Immunity is generally lifelong, but there have been documented cases of reinfection.

The CTF virus crosses the placenta and is teratogenic in laboratory animals. In 12 women who had CTF while pregnant, 10 delivered normal babies, one had a spontaneous abortion, and one infant had multiple congenital abnormalities.

THERAPY

Treatment is symptomatic. Analgesic and antipyretic drugs are usually adequate for control of the headaches and myalgias. In a patient with petechiae or other evidence of a hemorrhagic syndrome, salicylates should be avoided. When a patient is seriously ill and has a history of exposure to ticks in an endemic area,

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other treatable diseases such as Rocky Mountain spotted fever, tularemia, ehrlichiosis, and relapsing fever should be considered and treated empirically.

PREVENTION

Avoidance of tick bites is the cornerstone of prevention. Persons going into an area likely to have ticks should wear light clothing that fits tightly at the wrists, ankles, and waist. Repellents should be sprayed on exposed skin surfaces and clothing can be sprayed with permethrin. Clothing, hair, and skin should be inspected twice daily [18].

If ticks are found, they should be removed by using a 10-minute application of alcohol, nail polish remover, ether, acetone, or benzene to the tick using a saturated pledget of cloth. Gentle, straight-backward traction should be applied with forceps. Care should be taken not to crush the tick. The tip of the needle may be inserted under the imbedded head of the tick before applying traction.

An experimental vaccine prepared from inactivated virus was successful in producing high titers of neutralizing antibody in volunteers [22]. Possible use of a vaccine would appear to be justifiable only for laboratory personnel working with the virus and outdoor workers in highly endemic areas.

The patient with CTF does not pose a risk of contagion and need not be isolated. The patient's blood should be considered potentially hazardous and should not be used for transfusion for at least 6 months after the illness.

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13

Tickborne Encephalitides

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INTRODUCTION

The tickborne encephalitides are a group of widely distributed viral diseases of humans or ruminant animals that are exposed to ticks. Infection may manifest without symptoms, as a fever of unknown origin, as an acute neurologic disease with or without sequelae, or may terminate fatally. Tickborne encephalitis, or TBE, refers specifically to disease caused by a flavivirus transmitted by hard (ixodid) ticks, but there are various local designations such as Russian Spring-Summer encephalitis, Central European encephalitis, and Powassan fever. TBE was first recognized as a distinct nosologic entity in 1932 [1] following an epidemic in the Russian Far East, but retrospective analysis of the literature indicates that the clinical syndrome was endemic in lower Austria in the late 1920s [2]. The causal agent was isolated in 1937 [3]. A large literature has accumulated within the past half-century; Medline lists nearly 1500 references on the biology and clinical aspects of TBE since 1969. Until the recognition of Lyme disease as a pan-Holarctic zoonosis in the past two decades, TBE was the most prevalent tickborne disease affecting humans. In many parts of Russia, TBE may still be considered the most burdensome vectorborne infection because of its great morbidity and mortality. This chapter focuses on the epidemiology, clinical presentation, diagnosis, and prevention of TBE.

BIOLOGY OF THE AGENT

TBE viruses are members of the family Flaviviridae, which include major human pathogens such as those causing yellow fever, dengue, and Japanese B encephali-

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tis. The flaviviruses were previously known as the Group B arboviruses, defined by their serologic relationship in hemagglutination-inhibition assays [4]. There are at least 70 described flaviviruses in eight serologic subgroups, of which the tickborne agents (TBEV, to distinguish the agent from the disease) constitute a single subgroup. Flaviviruses are spherical lipid-enveloped particles 50 nm in diameter whose capsid contains a single-stranded positive-sense RNA of approximately 11 kilobases in size. The RNA encodes three structural proteins, C (capsid), M (membrane), and E (envelope), as well as seven nonstructural proteins required for viral replication [5]. TBEV is assumed to infect cells by receptor-mediated endocytosis, and the viral membrane fuses with the endosomal membrane to place the nucleocapsid into the host cell cytoplasm [6]. The positive sense RNA acts directly as the mRNA, and the entire TBEV genome is translated intracellularly starting at the 5′ end to produce a polyprotein including both nonstructural proteins and structural proteins C, E, and M. The separate proteins are cleaved posttranslationally by cellular signal peptidase within the endoplasmic reticulum. A complementary negative sense strand is produced with the aid of the nonstructural proteins, and this serves as template for genomic progeny RNA synthesis. Assembly of virions occurs during budding, which occurs in cytoplasmic vacuoles, and they are released as mature virions during cell lysis [7].

Glycoprotein E is immunodominant in TBE [8,9], and may serve in receptor binding and fusion [10]. Although glycoprotein E induces neutralizing antibody and confers protection when used as an immunogen, its amino acid sequence within TBEV isolates seems to vary little between geographic areas, suggesting functional constraints [9,11]. This glycoprotein has been crystallized and its three-dimensional structure elucidated [12]. Elegant analyses using infectious TBEV cDNA clones strongly suggest that virulence, in part, is associated with a tyrosine to histidine substitution at position 384 of the E amino acid sequence [13,14]. Variation in virulence that is observed between the various TBEV strains may be related to amino acid substitutions within this critical ''Domain B'' envelope region [15].

Like other viruses, TBEV comprises a number of different ''strains'' or subtypes. Some confusion is apparent in the terminology used to refer to these agents. Herein we use the term ''isolate'' to designate the propagation and maintenance in the laboratory of virus from a single source, ''strain'' to refer to such an isolate that is used as a standard, and ''subtype'' to refer to an isolate or strain that has been serologically characterized as different (fourfold difference in titer by any procedure using heterologous and homologous sera raised against other typical strains) from others, or molecularly characterized and differing by 15% of the nucleotides in a sequenced portion of a phylogenetically informative gene [16]. Existing subtypes, strains, and isolates that may be encountered in the literature are presented in Table 1, along with their known geographic areas of transmission.

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Ideally, virus ''species'' are defined by distinct natural groupings, or clades, in phylogenetic trees constructed by aligning and comparing nucleotide sequence information (Fig. 1). In such molecular analyses, three major clades are apparent; TBEV sensu lato, Tyuleniy, and Powassan, which correspond to previous classification schemes based on serologic typing. Six viruses have traditionally been recognized in TBEV sensu lato: Far Eastern or Russian Spring-Summer encephalitis (RSSE), Central European encephalitis (CEE), Louping Ill (LI), Omsk hemorrhagic fever (OHF), Kyasanur Forest disease (KFD), and Langat [17]. Turkish Sheep encephalitis (TSE) has recently been added to this list. OHF and KFD cause hemorrhagic syndromes and are not considered further in this chapter. Naturally acquired human infection by Langat or TSE are as yet undescribed, although Langat was used briefly as an attenuated viral vaccine against TBE in Czechoslovakia and Russia [18,19]. Louping Ill, an infection of major veterinary importance in the United Kingdom, has been reported in laboratory workers [20], with a clinical presentation similar to that of CEE. Tyuleniy (and ecologically similar Saumarez Reef virus) seems associated with seabirds, and the potential for human infection is unknown although serosurveys suggest exposure [21]. Powassan virus (POW) has caused two dozen cases of a devastating meningoencephalitis in residents of eastern Canada and the northeastern United States [22]. Of this diverse group of agents, we focus our subsequent discussion on RSSE, CEE, and Powassan.

TBEV in general seem relatively resistant to environmental inactivation; they may be stable at pH of less than 7 (with optimal stability at pH 7.4), and will survive 10 minutes at 60°C, 48 hours in 0.5% formalin, and 1% phenol for 10 days [23]. This stability explains the extravector modes of transmission, eg, epidemics attributable to TBEV in goat, sheep, or cow's milk [24], or laboratory accidents by way of aerosol during propagative or preparative procedures [25]. Because aerosol infection is well documented, the case fatality rate can be significant for certain subtypes, and TBEV other than Powassan are exotic, Biosafety Level 4 conditions are recommended for working with these pathogens within the United States [26]. Few such ''spacesuit'' facilities exist worldwide, and in the Americas are located at the Centers for Disease Control (Atlanta), USAMRIID (Ft. Detrick), Southwest Foundation for Biomedical Research (San Antonio), and the Canadian National Laboratory for Special Pathogens (Winnipeg).

As the name implies, the main mode of transmission is by tick bite. In particular, TBE is associated with a Holarctic species complex that includes *Ixodes persulcatus* and *I. ricinus* [27], the vectors of Lyme disease, babesiosis, and granulocytic ehrlichiosis. Although other ticks such as *Dermacentor* spp. and *Haemaphysalis* spp. are experimentally susceptible and occasionally found to be infected in nature, the vectorial capacity of *I. persulcatus* and *I. ricinus* seems much greater. The life cycle may be summarized as follows: small mammal reservoirs, particularly rodents such as the woodmouse, *Apodemus* spp., or the bank

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TABLE 1 Taxonomic Status and Distribution of Tickborne Encephalitis Complex Viruses*

Name of virus	Taxonomic status	Site of derivation; date	Isolated from	Known distribution	Human pathogen?	Comment
Central European Encephalitis (CEE)	TBE (species)	—	—	Europe except Spain and Portugal	Yes	Also known as TBE western subtype
Hanzalova	CEE (strain)	Beroun, Czechoslovakia, 1948	Human	—	Yes	''Stillerova'' strain isolated from accidental human infection with Hanzalova
Hypr	CEE (strain)	Brno, Czechoslovakia; 1953	Human	—	Yes	—
Kumlinge	CEE (strain)	Kumlinge, Finland; 1959	Ticks (*Ixodes ricinus*)	—	Yes	—
Neudoerfl	CEE (strain)	Austria; 1971	Ticks (*Ixodes ricinus*)	—	Yes	Proposed prototype for CEE (TBE western subtype)
Karshi (KSI)	TBE (species)	Uzbekistan; 1972	Ticks (*Ornithodoros papillipes*)	—	Not known	—
Kyasanur Forest Disease (KFD)	TBE (species)	Mysore, India; 1957	Monkey	—	Yes	Causes hemorrhagic fever
Langat (LGT)	TBE (species)	Malaysia; 1956	Ticks (*Ixodes granulatus*)	—	Not known	—
Louping Ill (LI)	TBE (species)	Scotland; 1929	Sheep	—	Yes	—
Negishi	LI (strain)	Tokyo, Japan; 1948	Human	—	Yes	—
Spanish Sheep Encephalitis (SSE)	LI (strain)	Basque region, Spain; 1987	Sheep	—	Not known	—
Omsk Hemorrhagic Fever (Omsk)	TBE (species)	Omsk Oblast, Russia; 1947	Human	Western Siberia	Yes	Causes hemorrhagic fever

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Powassan (POW)	TBE (species)	Ontario, Canada; 1958	Human	Southern Canada, Northern United States, Far Eastern Russia	Yes	
Deer Tick Virus	POW (strain)	Massachusetts; 1995	Ticks (*Ixodes dammini*)	—	Not known	—
Royal Farm (RF)	TBE (species)	Kabul, Afghanistan; 1968	Ticks (*Argas hermanni*)	—	Not known	—
Russian Spring-Summer Encephalitis (RSSE)	TBE (species)	—	—	Russia, where *Ixodes persulcatus* is endemic	Yes	Also known as TBE Far Eastern subtype
Absettarov	RSSE strain	Leningrad, Russia; 1951	Human	—	Yes	—
Sofjin	RSSE strain	Primorskii, Russia; 1937	Human	—	Yes	Prototype for TBE Far Eastern subtype
Saumarez Reef (SRU)	TBE (species)	Queensland, Australia; 1974	Ticks (*Ornithodoros capensis*)	—	Not known	—
Turkish Sheep Encephalitis (TSE)	TBE (species)	Gebze, Turkey; 1968	Sheep	—	Not known	—
Greek Goat Encephalitis (GGE)	TSE (strain)	Thessaloniki, Greece; 1969	Goat	—	Not known	—
Tyuleniy (TYU)	TBE (species)	Sea of Okhotsk, Russia; 1969	Ticks (*Ixodes uriae*)	—	Not known	—

* Diverse names are encountered in the literature, many of which simply represent strains or isolates of the main TBE complex virus species. Note that this table represents the authors' interpretations of a large body of literature. The International Committee on the Taxonomy of Viruses (www.life.anu.edu.au/viruses/ICTVdB/ictvdb.htm) or the *International Catalogue of Arboviruses* (American Society of Tropical Medicine and Hygiene, 1985) should be considered the final authority on virus taxonomy, but neither organization lists all the following names or issued final rulings on the validity of all the listed taxa.

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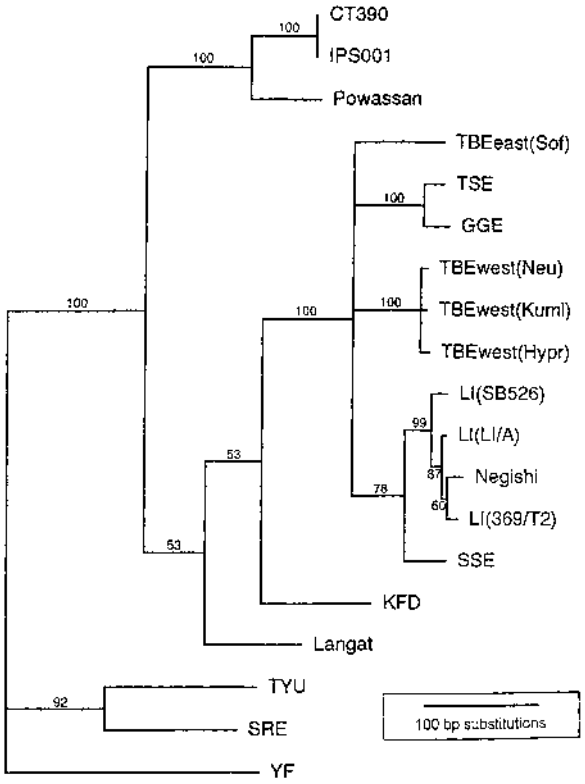

FIGURE 1 Maximum parsimony phylogenetic tree of tickborne encephalitis virus complex. Sequences deposited in GenBank for representative tickborne flavivirus envelope genes were aligned and compared using yellow fever virus (YF) as the outgroup. Values above the branches indicated bootstrapped confidence values for the branching pattern at that point. The branch lengths are proportional to percent similarity in sequence. Ips001 and CT390 are isolates of deer tick virus; other abbreviations as in Table 1. (Reprinted from Ref. 39.)

vole, *Clethrionomys glareolus*, are resident within circumscribed, longstanding ''natural foci'' of transmission [27,28]. Nymphal *I. persulcatus* or *I. ricinus* attach and complete feeding on a nonimmune rodent host. Within a week after the infecting tick feeds, a viremia develops in the circulating blood that, when imbibed, is sufficient to infect larval or nymphal ticks. If a larva feeds to repletion, it will eventually molt and seek a new host as a nymph; a nymph will molt and seek hosts as an adult male or female. Either stage (nymph or adult) may attach and infect humans. Although transovarial transmission of TBEV from infected

female tick to progeny (larvae) has been described, human infection appears to be rare from larval bites [29].

Other arthropods have been suggested as vectors. Although experimental evidence supports such a possibility in that TBEV may survive within various mosquitoes and indeed have been isolated from nontick arthropods collected from nature, no epidemiological association has been established. Similarly, ecological studies fully support the paradigm of tick-rodent perpetuation. ''Spillover'' from the required maintenance cycle into nonrelevant hosts seems to be axiomatic with vectorborne infections, and unless new evidence is presented, one most conclude that arthropods other than ticks are peripheral to enzootic transmission or human risk for TBE.

As with most arboviruses, vertebrate viremia is transient, with the rapid development of neutralizing antibodies. Certain insectivores, such as hedgehogs, have been reported to sustain chronic viremia, which may aid TBEV persistence [30]; more information is needed to evaluate the role of immunity in these hosts. Because a reservoir is generally thought to lose an infectious viremia rapidly by developing sterilizing immunity [31], a suitable reservoir population would require rapid turnover with virus-naive hosts being continuously generated. Rodents fulfill such a requirement, with average survival rates on the order of a few months, and reproducing two to three times during this period. A nonviremic mode of perpetuation has been suggested when uninfected ticks become infected by ''co-feeding'' in proximity to the mouthparts of an infected tick [32]. In either case, the transmission cycle depends on the nearly simultaneous temporal appearance of larvae and nymphs. This requirement poses a paradox, because in many sites of CEE transmission *I. ricinus* larvae and nymphs are usually active during different months [33,34] although this may differ with habitat type [35]. However, the prevalence of infection in the vector, usually less than 0.5% of host-seeking nymphal ticks, seems consistent with the infrequent overlap of the activity season of the larval and nymphal stages. In contrast, in western Siberian sites where *I. persulcatus* maintains RSSE, there is a 60-day season of activity for all stages of the tick (a relatively short period of permissive weather), resulting in a greater force of transmission. There, prevalence of vector infection may approach 5 to 20% in host-seeking nymphal ticks [36]. The reader is referred to Chapter 1 by Spielman and Hodgson for details on the life history traits and vectorial capacity of this group of ticks.

POW is mainly maintained in North America by *Ixodes cookei*, which focuses its feeding on woodchucks, skunks, and other medium-sized mammals, and only occasionally bites humans. This feeding preference accounts for the relative scarcity of cases for a virus that is geographically widespread and intensively transmitted; 23 to 64% of woodchucks were seropositive for POW in New York and Ontario [37]. However, the deer tick, *Ixodes dammini*, an aggressive human biter and main vector for Lyme disease in the northeastern United States, is exper-

imentally vector competent for POW [38] and indeed seems to naturally maintain a POW subtype (deer tick virus, DTV [39]) for which human infection remains to be described. Should deer ticks begin to ''bridge'' virulent POW from the *I. cookei*–woodchuck cycle, it may be that POW fever will become more prevalent. Alternatively, it may be that deer tick virus causes a fever of unknown origin–like illness that resolves without sequelae, and that transmission might intensify as has that of Lyme disease over the last decade or two.

EPIDEMIOLOGY

TBEV constitute the most important viral arthopodborne human pathogens in most of the Palearctic region. In Austria, a syndrome caused by CEE was recognized in 1927 by Schneider [40], who named it ''epidemic acute meningitis serosa.'' Although he was not able to identify the causative agent or even the mode of transmission, he suspected a viral cause and clearly differentiated the syndrome from acute poliomyelitis by clinical and laboratory findings. The causal role of CEE in this syndrome was strongly suggested in retrospective by a case-control study comparing patients who had been admitted for a CNS infection to the Neunkirchen hospital, where Schneider had made his observations, as cases and healthy controls [41]. The odds ratio for the presence of neutralizing antibodies against CEE was found to be 10.7 (95% CI 6.3–18.0) (Foppa, unpublished calculations, based on data presented in Ref. 41), demonstrating a strong association between epidemic acute meningitis serosa and CEE. A few years later, a similar syndrome was described in the Russian Far East, which led to the first isolation of the RSSE virus [1]. Isolation of the CEE virus was accomplished only after World War II [42].

Although TBE is relatively uncommon, it is an important cause of CNS disease in endemic areas. A prospective Swedish study found 108 TBE cases among 596 patients (18.1%) admitted for acute viral meningitis [43]. In healthy populations living in areas where TBE transmission occurs, seroprevalence is typically found to be under 5% [44–48]. In high-risk populations (vide infra) seroprevalence may be considerably higher. It is worth noting that the vast majority of the seroconversions found in such populations are attributable to silent or oligosymptomatic infection. The focality of TBE transmission makes the calculation of meaningful incidence rate estimates difficult. In most countries with a TBE reporting system, the yearly number of reported cases is in the range of a few hundred cases per year, but in Russia, the reported number is in the thousands [49].

In many areas, TBE incidence has drastically increased over the past few decades. In Latvia, where the number of cases ranged from 117 to 287 per year (1984–1992), 791 cases were reported in 1993, 1341 in 1994, and 736 in 1995 [50,51]. In Baden-Württemberg, southwestern Germany, 78 cases of TBE were

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reported in the years 1978 to 1984 as opposed to 234 cases in 1994 alone [48,52]. Similar trends have been reported from the Czech Republic [53], Poland [54], Estonia [55], and Switzerland [56]. A notable exception to this pattern is Austria, where a government-sponsored TBE immunization campaign attained a greater than 80% immunization coverage in the population at risk that led to a decrease of reported yearly cases from more than 500 on average from 1975 to 1982 to about 100 in the early nineties [42,57]. Furthermore, new foci of zoonotic TBE transmission are emerging [58–62]. It has not yet been established if this emergence corresponds to the spread of TBEV within tick populations, to the spread of populations of ticks and tick hosts that are circulating TBEV, to increased contact between humans and infected ticks, or to a combination of these factors. The fact that these new zoonotic transmission foci tend to appear at the fringes of established transmission areas suggests that this phenomenon may not be caused by increased human exposure alone [63].

In addition to the secular trend in tickborne encephalitis, TBE incidence fluctuates from year to year, with local incidence peaks often seen in 2- to 4-year intervals [42]. These fluctuations are likely to reflect fluctuation in tick and feeding host populations, as well as environmental factors such as temperature and humidity that may directly affect virus activity. Finally, like most arboviruses, TBE has a distinct seasonality that follows closely, but with an approximately 4-week delay, the seasonal activity of the nymphs of its vector, *I. ricinus* or *I. persulcatus*. Accordingly, incidence usually peaks in summer (mostly June or July), occasionally followed by another, smaller peak in fall [2,41,64]. TBE owes some of its names to this seasonality (Spring-Summer encephalitis, Fruehsommer Meningo-Enzephalitis [German for early summer meningoencephalitis]).

Occasionally, foodborne transmission of TBEV causes epidemics of TBE in populations consuming raw milk or dairy products from viremic goats or cows [24,65]. However, this mode of transmission only plays a subordinate role in TBE epidemiology. The course of the disease following oral infection with TBEV is usually mild.

The most important risk factor for TBEV infection is exposure to infected ticks. Traditionally, occupational (agriculture, forestry) [66] or peridomestic exposure were among the most important determinants of TBE epidemiology. Recently, however, TBE is increasingly being found among urban populations with recreational exposure to tick habitats. Indeed, TBEV-infected *I. ricinus* have been found within suburban Moscow (personal communication, Professor E. Korenberg, Gamaleya Institute). The most important known risk factor for parenchymal disease and TBE-associated mortality is age, which is positively correlated with disease severity. Mortality is generally limited to patients older than 50 years.

Experimental data comparing the pathogenicity and virulence of different TBE viruses in laboratory models, as well as insights into the molecular factors

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of virulence, suggest that there are considerable biological differences between different TBE viruses and strains. Yet, the relevance of these findings for the explanation of epidemiological observations remains to be shown, and it is not clear at this point if the widely held belief that RSSE is generally more severe than CEE is valid. Apparent differences between CEE and RSSE epidemiology with respect to severity and frequency of sequelae (few percent vs. 50 %) and mortality (1% vs. 20%) [42] may at least in part be explained by regional differences in health care utilization and quality. On the other hand, the fact that the few known human cases of POW mainly occurred among children and adolescents with a high mortality and very frequent permanent neurologic residues cannot easily be explained by such biases, and is likely to be attributable to biological properties of the virus.

POW fever is rare; of nearly 5500 sera screened from 1961 to 1985 in New York and eastern Canada, only 11 were considered to be specifically reactive [37]. However, of the 21 reported cases, virtually all were acquired between June and September. Although *I. cookei* mainly feeds on medium-sized mammals, human biting may be more frequent than previously thought, particularly in more northerly sites, with peak infestation reported during July [67]. Infection by DTV would be likely during the main season of nymphal *I. dammini* activity (May–July), when the other deer tick–transmitted zoonoses (Lyme, babesiosis, and HGE) are observed [68].

LABORATORY FINDINGS AND DIAGNOSIS

During the acute febrile phase, a leukopenia may be observed. In addition, thrombocytopenia (34,000–93,000/mm^3) and elevated liver enzymes (ALT/AST) have been described for the early illness [69]. However, cases of babesiosis and granulocytic ehrlichiosis, also transmitted by *I. ricinus*–like ticks, will also present with leukopenia, thrombocytopenia, and elevated LFTs [70,71]. With the onset of the meningeal symptoms, a lymphocytosis (6,600–15,000/mm^3) transiently appears. The erythrocyte sedimentation rate is markedly elevated (usually 40–70 mm^3/hour Westergren but as high as 100). Cerebrospinal fluid will contain 50–200 mg percent protein, with normal or only slightly elevated glucose content [72]. CSF pleocytosis may be as great as 570/mm^3 with 60% neutrophils during the first few days of the infection, progressing to mononuclear cells within the week. Of these, 60% stain with anti-CD32 monoclonal antibodies. Of the lymphoid cells in the CSF, 60% stain with anti-CD3, and 18% with anti-CD20 [73]. Although such cell typing studies seem to be preliminary, this approach may be useful in distinguishing Lyme neuroborreliosis from TBE because pleocytosis and CSF protein indices can be very similar between the two [74].

Practically, definitive diagnosis of TBE depends on seroconversion. The CSF findings, in the context of compatible clinical findings, as well as history

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of possible exposure to ticks or their habitat in a known endemic site, are excellent for presumptive diagnosis. Viral isolation might be attempted from blood or serum, but the peripheral viraemia is during the initial nonspecific febrile phase and has usually disappeared by the time samples are taken. The efficiency of isolation from CSF is variable. Autopsy samples (brain, spleen) should be minced in a buffer containing 10% heat-inactivated serum or bovine serum albumin, and then frozen. Often, autopsy samples may have virus detectable by immunohistochemistry or by polymerase chain reaction (PCR), but fail to propagate in appropriate isolation systems. Whole blood samples should be heparinized and immediately frozen without separation at −70°C, as should serum or CSF samples. Note that heparin is inhibitory to PCR, and duplicate samples without heparin should be stored frozen for nucleic acid isolation, if desired. If an ultralow freezer is unavailable, any freezer will help increase the chances of isolation or PCR.

Isolation may be attempted by a laboratory using classical virological procedures, eg, suckling mouse inoculation or cultivation in established cell lines. Suckling mice (3–7 days old) are intracranially inoculated using an insulin syringe with sterile-filtered (0.2 micron pore) serum, heparinised plasma, or clarified filtered tissue suspensions in buffer containing protein. Note that EDTA containing materials may quickly kill a suckling mouse. Virtually all arboviruses are infectious for suckling mice by this route, and most induce a lethal endpoint. Inoculated mice that die within a day or two are considered to have succumbed to trauma or sepsis. Of course, control sibling mice should simultaneously be inoculated with diluent alone. In the case of CEE, RSSE, and LI, suckling mice begin to show paralytic symptoms 7 to 14 days after inoculation, and die shortly thereafter. POW and DTV cause paralysis on the fourth or fifth day after inoculation and death within a day or two. Live virus is found in the brain even in dead suckling mice, and 20% mouse brain suspensions in buffer with protein may be stored indefinitely in the ultralow freezer as live virus stock.

Vero E6 cells, baby hamster kidney (BHK), or chick/duck primary embryonic cells (CEC, DEC) are the cells of choice for cultivation. Vero cells, in particular, are useful because plaques develop readily. Presumptive identification of a viral isolate may be made by immunostaining with reference sera, or by nucleic extraction and PCR. Laboratories attempting to directly isolate virus should do so with caution, using high containment facilities and minimizing the generation of aerosols. Vaccination (vide infra) is highly recommended for laboratory personnel working with TBEV.

PCR may obviate the need for actual isolation and the associated hazards. Samples (serum, plasma, whole blood, tissues) are placed directly into a lysis solution which solubilizes proteins and may reduce the infectivity of a sample. Note that the viral RNA itself is infectious [75], but envelope glycoproteins serve as viral cell entry determinants and their loss presumably will reduce infectivity. Our laboratory uses Trizol LS (Bethesda Research Laboratories, Gaithersburg,

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MD) according to the manufacturer's instructions for sample dissolution, but any high molarity guanidinium salt should be adequate. Tissues are homogenized in a balanced salt solution within a laminar-flow biosafety cabinet, and the homogenate added to Trizol. Blood, plasma, or sera are added directly to Trizol, and the resulting solution allowed to incubate 30 minutes to overnight at temperatures from 25 to 60°C for complete cell lysis. RNA is extracted as recommended by the manufacturer. Care should be taken to avoid contamination of reagents or materials with RNase. A number of PCR assays have been described [39,76,77], targeting conserved portions of the envelope or NS-5 (nonstructural protein) genes. Examples of primer sequences for reverse transcription, and for cDNA amplification are provided in Telford et al., 1997 [39].

Because meningoencephalitis presents at least 14 days after infection by tick bite, a specific IgM response is usually measurable [78]. The enzyme-linked immunosorbent assay (ELISA) has largely replaced the older complement fixation, neutralization, or hemagglutination inhibition tests. Neutralization, in particular, was troublesome because it required access to containment facilities where live virus could be propagated. Reliable ELISA kits by Behring AG (Germany) or ImmunoAG (Austria) contain all reagents required for assays, including positive and negative control sera. The ''Immunozym FSME'' kit uses an IgM capture procedure that minimizes false-positive reactivity attributable to rheumatoid factor and IgG blocking, where IgG competitively binds to antigen binding sites [79]. The sensitivity and specificity of this rapid assay, when performed as recommended by the manufacturer, is estimated to be 97% and 99%, respectively (package insert, Immunozym FSME IgG, 1995), although formal, peer-reviewed presentation of these calculations have not been made. Convalescent serum samples should be drawn 6 to 8 weeks after initial presentation, and analyzed on the same ELISA plate for specific IgG.

Note that individuals receiving TBE vaccine may retain specific anti-TBEV immunoglobulin, even IgM, for as long as 8 to 10 months after the final booster dose [80], and therefore eliciting precise information about vaccination when the patient's history is taken is critical to determining whether an IgM response is attributable to recent infection. Indeed, CSF may be analyzed for the presence of intrathecal antibody using these kits, and although highly suggestive of active CNS infection if specific IgM or IgG are present, false-positive results have been reported [81].

Because these kits use whole inactivated virus as antigen, some cross-reactivity to related flaviviruses (dengue, yellow fever, and West Nile) may be observed. Similarly, the antibody response to RSSE or LI should be measurable with the CEE reagents given these viruses' close relationship and extensive cross-reactivity. POW fever or presumptive DTV sera may cross-react to a lesser extent than sera from cases of RSSE or LI (authors' unpublished observations), and the value of these ELISA kits for helping to diagnose these infections is not known.

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Laboratories offering serology for POW utilize either hemagglutination inhibition assays or neutralization. Unfortunately, because of perceived fears of veterinary pathogens being introduced within the kit components, commercial TBE diagnostic kits are restricted by the U.S. Department of Agriculture and permission is required for their importation into the United States. Accordingly, American physicians confronted with possible cases of TBE in travellers, or with endemically acquired POW-like infections, must submit samples through their state health departments for analysis at the CDC or identify research laboratories that may offer testing for such viruses.

CLINICAL MANIFESTATIONS

Like many arboviruses, TBEV causes clinical illness only in a minority of infected individuals. In the majority, infection remains clinically silent, or manifests as a flu-like syndrome with fever (generally not higher than 39.5°C), fatigue, headache, aching back and limbs, catarrhal symptoms of the upper airways, and gastrointestinal symptoms [44,82]. This febrile-myalgic phase, thought to correlate with viremia [83], usually subsides within 2 to 4 days. This phase of the disease is rarely brought to the attention of a physician, and if it is, the condition is rarely correctly diagnosed because of the low specificity of the symptoms. After a symptom-free interval of 2 to 4 weeks, some patients develop overt CNS symptoms. This peculiar presentation of fever followed by an asymptomatic period, and then CNS symptoms, gives rise to the term ''biphasic meningoencephalitis.'' The proportion of patients that conform to this diphasic fever presentation has been estimated to be in the range of 20 to 30% [42], but this figure has never been empirically verified. Seroepidemiological data suggest an even lower proportion. The course of RSSE is typically monophasic and onset of neurologic symptoms is sudden within a febrile-myalgic phase [3,84].

In about half of the patients with CNS manifestations [41,43,48], TBEV causes a syndrome of viral meningitis [85], which includes malaise, nuchal stiffness, severe headache, photophobia, nausea, and drowsiness. Fever has been reported to be often higher than typically observed in other forms of viral meningitis [3]. If no parenchymal involvement ensues, the outcome of TBE meningitis is favorable.

If the CNS parenchyma becomes involved, the outcome is determined by the nervous structures affected. In general, under the age of 10, more severe forms of TBE are rare [41,86], but disease severity increases with age. Second to meningitis, meningoencephalitis is the most common manifestation of TBE [52]. Pronounced disturbances of consciousness, more often somnolence and coma than delirium, are more prevalent than in meningoencephalitides of other origins. Anamnestic functions and concentration are often impaired and found in approximately 10% of the patients 1 year after TBE meningoencephalitis [43].

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Almost pathognomonic for acute TBE meningoencephalitis is ataxia of the upper and lower limbs, which is mostly transient. The most serious form of TBE in terms of mortality and permanent sequelae is encephalomyelitis and/or radiculitis. Especially common is shoulder girdle paralysis, often unilateral, but tetraparesis and neurologic deficits associated with other spinal levels also occurs. Whereas patients with radiculitis may recover after up to 4 years after the acute illness, paresis is permanent in patients with myelitis. Myelitis and radiculitis may also develop without clear signs of encephalitis (meningoradiculitis, meningomyelitis) [52].

Even in patients with mild forms of TBE, long-lasting sequelae are relatively common. Although TBE meningitis always resolves completely, recovery takes longer on average than in meningitis of other origin. In more severe cases (involvement of the parenchyma), 10 to 20% of the patients suffer from long-lasting or permanent neuropsychological sequelae, such as headache, lack of concentration, depression, memory impairment, hearing impairment, and tinnitus [42,43,86]. Flaccid shoulder girdle paralysis is the most common residual motor symptom [3]. A chronic progressive form with epilepsy (Kozhevnikov's epilepsy) has been reported only after RSSE.

If fever with acute neurologic disease is observed during summer in patients living in or with a recent history of visiting a known endemic area, TBE should be considered a possible cause. Furthermore, as new areas of TBEV transmission continue to emerge, not only at the fringes of well-known transmission areas but also apart from such areas, it may be necessary to include TBE, along with other arboviral causes, in the differential diagnosis of any case of encephalitis occuring between May and October.

Because of the overlap of enzootic transmission cycles of different tick-borne pathogens, co-infection with at least one additional agent is relatively common [49] and should always be considered as a possibility. Concurrent Lyme borreliosis, for example, has been shown to be associated with particularly severe TBE manifestations [87]. It is not yet known if TBEV interacts with piroplasms or ehrlichiae in the human host.

TREATMENT AND PREVENTION

No specific treatment is currently available for TBE [42,84]. Despite promising results, the treatment of patients suffering from meningitic and meningoencephalitic manifestations of TBE with RNAse [88] never became established in clinical practice. Interestingly, treatment with ribavirin has not been clinically evaluated, although some efficacy in preventing mortality is observed in experimental murine infections [89]. Patient care usually is symptomatic and supportive, depending on the actual manifestations. Fever and pain management, and fluid replacement, is usually necessary in hospitalized cases. In more severe cases,

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intensive care may be required, especially if control of respiratory muscles is impaired and/or if the patient is comatose. Physical therapy is often helpful in preventing consequences of prolonged immobility and neuromuscular impairment.

Due to the limited treatment options, primary and secondary prevention is of essential importance. Primary prevention strategies aim at avoidance of tick exposure. Although interventions such as aerial spraying of DDT [90], management of populations of potential feeding hosts and/or amplification hosts, as well as environmental modifications have been proposed [30], the lack of evidence of their longterm efficacy and the potential for undesirable environmental impact renders such measures difficult to recommend. On the other hand, measures aiming at the reduction of individual tick exposure are of considerable importance. Such measures include education of the public, appropriate clothing, application of acaricides to clothes and footwear when visits to tick habitats are planned, and avoidance of tick habitats if possible. In addition, careful grooming for and removal of attached ticks after visits to likely habitats is recommended. Most of the other tick-transmitted agents require prolonged feeding by the tick before they attain infectivity [91]. However, inasmuch as transmission of TBEV is thought to begin immediately after attachment [36], grooming may be of limited use in preventing TBE.

The intervention of choice for specific primary prevention is anti-TBE immunization. A vaccine effective against all TBEV has been commercially available since 1976 [42]. The vaccine is a suspension of purified TBEV (Neudoerfl strain) cultivated in chick embryo cells, and inactivated by formaldehyde [92]. The inactivated virus is purified by gradient ultracentrifugation. The virus preparation is exhaustively tested for inactivation, as well as for adventitious viruses (by PCR). The adjuvant is aluminum hydroxide.

A full course of three doses (given intramuscularly, preferably in the deltoid muscle) induces protective antibody in about 98% of vaccinees [57]. Protective titers may last as long as 3 years without boosting. The vaccine has been extensively tested for safety and reactogenicity, with few reported problems. In the early 1900s, a report raised concern about neurologic side effects after TBE immunization [93]. However, an association between the vaccine and neurologic symptoms was not convincingly shown. Although there are few published epidemiologic studies demonstrating efficacy, estimates suggest a minimum efficacy of 94% in the field [42]. Immunization is recommended to anyone planning to visit potential tick habitats in the palearctic region, especially in central Europe and Russia. This vaccine, however, has not been approved by the U.S. Food and Drug Administration, and is not legally available in the United States.

If a nonimmune person gets bitten by a tick in a TBE-endemic area, postexposure prophylaxis with specific anti-TBE immunoglobulin may be indicated. However, there have been concerns that postexposure prophylaxis may be inef-

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fective or even aggravate the clinical course [94], especially in children and if the application follows more than 96 hours after exposure. However, postexposure prophylaxis is still widely used and further research is needed on the efficacy and potential hazards associated with passive immunization, especially in children [95].

SUMMARY

TBE burdens residents of widely distributed but circumscribed areas of Eurasia and, rarely, residents of northern North America. Morbidity and mortality attributable to infection by TBEV varies from site to site, perhaps depending on the virulence characteristics of locally circulating viral subtypes. The clinical spectrum ranges from asymptomatic seroconversion to fulminating meningomyelitis and meningoencephalitis. A biphasic fever, with neurologic signs and symptoms appearing during the second febrile phase, may be apparent. Neurologic sequelae may be frequent. Diagnosis depends on seroconversion. Treatment is largely symptomatic, although immunoprophylaxis with high-titred gamma globulin may help abort severe infection. TBE may increase in prevalence or in geographic distribution as Lyme disease has over the last decade or two because these agents share a notorious human-biting vector, ticks closely related to the deer tick.

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14

Tickborne Hemorrhagic Fever

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OVERVIEW

There are three major tickborne viral hemorrhagic fevers: Congo–Crimean hemorrhagic fever (CCHF), which is distributed throughout Europe, Asia, the Middle East, and Africa; Kyasanur Forest disease, which occurs in western India and was recently detected in Saudi Arabia; and Omsk hemorrhagic fever, which occurs in western Siberia [1,2]. These diseases are caused by RNA viruses that have zoonotic lifecycles in specific rural areas. Man is typically infected as an incidental host. Each disease has a short incubation period (less than 2 weeks), and can present with a similar clinical illness. These viruses produce vascular lesions with increased vascular permeability and, in severe cases, frank hemorrhagic symptoms. Patients typically present with acute onset of fever, headache, myalgias, nausea, and vomiting. The face is often flushed with conjunctival injection. Laboratory values will show a leukopenia and thrombocytopenia. After 3 to 5 days, the patient develops hemorrhagic symptoms with petechiae, ecchymoses, and bleeding from the gums, nose, gastrointestinal tract, uterus, or lungs.

When a case of suspected viral hemorrhagic fever is observed, the patient should be placed in isolation until a specific diagnosis can be made. The Centers for Disease Control and Prevention have developed guidelines for isolation of potential cases of viral hemorrhagic fever (see Appendix 1). Because most physicians have little experience with these diseases, consultative assistance should

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The opinions expressed herein are those of the author and do not necessarily reflect those of the Departments of the Army, Navy, or Defense.

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Table 1 Viral Hemorrhagic Fevers

Disease	Diagnostic Clues
Argentine hemorrhagic fever	Argentina, March–June, mouse exposure
Bolivian hemorrhagic fever	Bolivia, Feb–July, mouse exposure
Lassa fever	West Africa, mouse exposure
Venezuelan hemorrhagic fever	Venezuela, mouse exposure
Hemorrhagic fever with renal syndrome	Asia, Europe, Balkans; mouse exposure
Congo–Crimean hemorrhagic fever	Asia, Europe, Africa, Middle East, tick exposure, nosocomial exposure
Kyasanur Forest disease	Western India, tick exposure
Omsk hemorrhagic fever	Western Siberia, tick or muskrat exposure
Rift Valley fever	Africa, late summer, mosquito exposure
Yellow fever	Africa, Amazon; mosquito exposure
Dengue hemorrhagic fever	Asia, Caribbean, South and Central America; mosquito exposure, second course of dengue
Ebola or Marburg virus infection	Subsaharan Africa, unknown exposure

be obtained as soon as possible. The Centers for Disease Control and Prevention will provide emergency consultations, and can be reached at 404-639-1511 during the day and 404-639-2888 at night.

It is critical in cases of suspected viral hemorrhagic fever to obtain a detailed history of recent travel and exposures and to perform a careful physical exam. Viral hemorrhagic fevers usually have a limited geographic distribution and an incubation period of less than 3 weeks (Table 1). Factors that suggest a tickborne hemorrhagic fever are shown in Table 2. The most important factors are exposure to ticks within the previous 2 weeks in an area endemic for tickborne hemorrhagic fever. It should be remembered that many illnesses can mimic a viral hemorrhagic fever and most patients evaluated will ultimately be found to have another, more common illness [3]. These diseases include bacterial or rickettsial sepsis, malaria, and other disease processes that can produce disseminated

Table 2 Factors That Suggest the Presence of a Tickborne Hemorrhagic Fever

Exposure to ticks
Travel history within past 2 weeks to Western India (Kyasanur Forest disease), Siberia (Omsk hemorrhagic fever), Europe, Asia, Africa, Middle East (Congo–Crimean hemorrhagic fever)
Acute onset of fever, systemic symptoms with flushed face, and conjunctival injection
Hemorrhagic symptoms at day 3 to 5 of illness with associated petechiae/ecchymoses

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intravascular coagulation. Diseases that can produce fever and hemorrhagic symptoms are listed in Table 3.

Specific serodiagnostic assays, which include ELISA, IFA, and virus neutralization assays, have been developed for each of these viral diseases. Because most patients will be viremic at the time of presentation, a definitive diagnosis can be made by culturing the virus in vitro (a process that takes 3 to 10 days).

TABLE 3 Alternative Causes of Hemorrhagic Fever Syndromes

Bacterial Infections	**Diagnostic Clues**
Meningococcemia	Gram-negative diplococci seen on Gram stain of aspirate of skin lesion or in CSF, or grow from blood/CSF cultures
Staphylococcal sepsis	Gram-positive cocci in clusters seen on Gram stain of aspirate of skin lesion or grow from blood culture
Gram negative rod sepsis	Ecthyma gangrenosum; Gram-negative rods seen in skin biopsy of lesion or grow in blood cultures
Typhoid fever	Rose spots; travel to India, Mexico, or typhoid-endemic tropical area; exposure to carrier of *Salmonella typhi*
Plague	Rodent exposure; bubo, smears, or cultures of bubo aspirate or blood show Gram-negative bacilli; worldwide
Leptospirosis	Summer or early fall; rural exposure to soil or water contaminated with infected urine; worldwide
Rickettsial Infections	
Rocky Mountain spotted fever	Tick exposure in United States; rash begins on extremities
Epidemic typhus	Louse exposure in highlands of Africa, South and Central America; wartime or disaster conditions
Protozoan Infections	
Malaria	Travel to malaria-endemic area; mosquito exposure; positive blood smear
Trypanosomiasis	Travel to East Africa (game park or rural areas); tsetse fly exposure; positive blood smear
Psittacosis	Cough; exposure to parrots, parakeets, or similar birds; abatoir exposure
Systemic Diseases	
Acute leukemia	Blood smear showing leukemia cells (may be reported as atypical lymphocytes on automated systems)
Thrombocytopenic purpura (ITP or TTP)	Schistocytes on blood smear (TTP); no exposure history
Systemic lupus erythematosis	Family history; women of childbearing age; positive antinuclear antibody (ANA)
Snake Bites	History of snake bite/exposure to a poisonous snake
Coumarin overdose	Exposure to coumarin or rat poison

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Because these viruses are highly contagious to laboratory personnel, isolation of a viral hemorrhagic fever virus should never be attempted in a laboratory with less than a BL4 rating.

In most instances, the management of patients with viral hemorrhagic fever consists of supportive management of volume status, replacement of blood loss, and analgesia. Because there is significant vascular permeability with all of these diseases, fluid infusions should be given cautiously to avoid pulmonary edema. Colloid is preferred. Dopamine is recommended for shock. Secondary infections are common and need to be carefully monitored and aggressively treated. The exception is CCHF, where prompt initiation of ribavirin can be lifesaving. In most instances, if the patient can be supported through the acute illness, s/he will recover completely after a prolonged convalescence.

With the increasing use of global air travel and man's increasing encroachment into previously uninhabited areas, it is important for physicians to be able to recognize potential cases of viral hemorrhagic fever and initiate prompt measures for isolation and treatment of these cases.

CONGO–CRIMEAN HEMORRHAGIC FEVER

Background

A disease characterized by bleeding was described in southeast Russia as early as the twelfth century [4]. During World War II, cases of hemorrhagic fever were described in Russian soldiers in the western Crimea and were shown to be caused by a nonfilterable agent present in patients' blood [5]. The same agent was detected in ticks of the species *Hyalomma marginatum marginatum*. Chumakov isolated and maintained this virus by passage in newborn mice in 1967 [6].

In 1956, Courtois in Stanleyville, Belgian Congo, isolated a virus from a boy with fever, headache, backache, photophobia, and vomiting. Courtois himself became ill with similar symptoms, and a virus was isolated from his blood [7]. These viruses were characterized and given the name Congo virus [8]. In 1969, the viruses from the Crimea and Congo were found to be similar, and named Congo–Crimean hemorrhagic fever virus [9]. The virus is prevalent in southeast Europe, Asia, the Middle East, and Africa, where it has been isolated from many domestic animals, rabbits, and hedgehogs. Humans contract the illness as incidental hosts. Nosocomial cases are common, especially after surgery is performed on a person with unsuspected infection [10].

Virus

The CCHF virus is the type species for the genus *Nairovirus* of the family Bunyaviridae. The CCHF virus is enveloped, 70 to 100 nm with a 3-segment RNA

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genome. Viremia occurs during the first to twelfth days of illness. The virus is pathogenic in suckling mice and grows well in cell culture, producing cytopathic effects on BHK, CER, LLC-MK2, and Vero cells. Serologic diagnosis can be performed by ELISA, IFA, CF, or neutralization assays.

Ticks/Animal Host

CCHF virus is widely distributed through Europe, Asia, the Middle East, and Africa [11,12]. Although CCHF virus has been isolated from many species of ixodid ticks, it appears that *Hyalomma marginatum* is the primary species transmitting the virus to man. This tick feeds on two different hosts during its lifecycle; the larvae and nymphs feed on birds and hares, while the adults feed on large domestic animals such as cattle, sheep, goats, camels, buffalo, and, occasionally, man. Birds, especially rooks in Europe, serve to disseminate the ticks during flight. The virus can be passed in tick populations without the need for contact with infected host populations [13]. In the former USSR, the disease has a peak incidence in the months of June and July. High-risk groups include agricultural workers (especially those involved with livestock), campers, and the military [1, 14].

Clinical Syndrome

Clinical disease begins after a 2- to 12-day incubation period, with abrupt onset of fever, chills, headache, and myalgias followed by nausea, vomiting, and abdominal pain [15–18]. There is flushing of the face and neck with conjunctival injection and edema of the palate. The breath can have a foul odor. A fine petechial rash begins on the back and extends over the whole body. A hemorrhagic exanthem begins on the palate and uvula. In over 75% of patients, hemorrhagic manifestations begin on the third to seventh days with petechiae, ecchymoses, and bleeding from the nose, gums, gastrointestinal tract, lungs, or uterus. There is often heavy oozing of blood from venipuncture sites. Large areas of purpura develop in some patients. Hepatomegaly occurs in 50% of patients. Central nervous system (CNS) involvement with nuchal rigidity, excitation, depression, or coma occurs in 10 to 25% of cases and is associated with a poor prognosis. Case fatality rates of 30 to 50% have occurred in nosocomial outbreaks, usually attributable to shock with multiple organ failure, severe hemorrhage, or secondary infection. Death typically occurs on days 5 to 14 of illness.

Laboratory findings include leukopenia and severe thrombocytopenia, increased partial thromboplastin time (PTT), and fibrinogen degradation products [10,17]. Transaminase levels and creatine phosphokinase (CPK) are often markedly elevated. Proteinuria and azotemia occur. Albumin levels are depressed. Current evidence suggests that many of the disease manifestations are a result of disseminated intravascular coagulation [18]. The CCHF virus invades endothe-

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lial and reticuloendothelial cells. Autopsy studies have shown marked centrilobular liver necrosis with thrombi in the central and portal veins in severely affected patients. Severe hemorrhagic changes have been noted in the stomach, kidneys, adrenals, and intestines, with occasional pulmonary edema and hemorrhage [17].

Isolation Procedures

The blood and secretions of patients with CCHF are highly infectious. CCHF is notorious for nosocomial outbreaks among medical, nursing, and hospital staff, as well as laboratory workers [10,17]. Virus isolation should only be attempted by a laboratory with a high biocontainment level and experienced staff.

Therapy

Patients with CCHF should receive supportive medical care. Therapy consists of measures to treat pain, dehydration, hypotension, and blood loss. Ribavirin, a guanosine analog that has shown antiviral benefit for other hemorrhagic fever virus infections such as Lassa virus [19] and hemorrhagic fever with renal syndrome [20], has also shown benefit against CCHF infection in vitro, in animal studies, and anecdotal reports [10,21–23]. Ribavirin is recommended to be given intravenously as a loading dose of 33 mg/kg, followed by 16 mg/kg every 6 hours for 4 days, then 8 mg/kg every 8 hours for 3 days [20,24]. For areas where intravenous ribavirin is not available, oral ribavirin 4 g per day for 4 days then 2.4 g per day for 6 days has shown apparent clinical benefit [10]. Ribavirin consistently causes anemia, which resolves after completion of therapy [24].

Prophylaxis

There is no vaccine readily available for CCHF at this time. In Bulgaria, an inactivated mouse brain vaccine has been used to immunize high-risk individuals. In some locations, CCHF immune plasma is given to persons with high-risk exposures or early in the course of diagnosed cases. This has been superceded by ribavirin therapy [25].

KYASANUR FOREST DISEASE

Background

Kyasanur Forest virus (KFV) causes periodic epizootics in monkeys in the Kyasanur Forest in Karnataka (formerly Mysore) State in western India. The virus was originally isolated from a sick monkey (*Presbytis entellus*) in 1957 [26,27]. Most human cases occur in persons working in forested areas during the dry season.

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There is an incidence of 400 to 500 virologically confirmed cases per year in India with higher rates during epidemic years. The largest outbreak occurred in 1983 with 1142 cases and 104 deaths. The case fatality rate is usually 3 to 10%. Most episodes of Kyasanur Forest disease have occurred in a localized region of western India, but recently a related virus (provisionally named ''Fakeeh virus'') has been detected in Saudi Arabia [28].

Virus

KFV is a flavivirus that belongs to the tickborne encephalitis (TBE) complex. KFV causes lethal infection in infant and suckling mice and can be propagated with cytopathic effects (CPE) in chick embryo, hamster kidney (BHK), monkey kidney (Vero, LLCMK2), and HeLa cell cultures. The virus propagates without CPE in *Haemaphysalis spinigera* tick cell lines.

The disease is usually diagnosed by serology (ELISA) or neutralization assays. Virus can be isolated from the patient's blood from 2 days before onset of illness until days 10 to 12 of illness with peak levels seen between days 3 and 6.

Ticks/Animal Host

KFV is transmitted by ixodid ticks, primarily *Haemaphsalis spinigera* [29]. KFV is passed by both transtadial and transovarial mechanisms in the tick vectors. Animal hosts include wild rodents, insectivores, and domestic livestock (goats, cows, and sheep). Monkeys develop lethal hemorrhagic symptoms when infected and epizootics can decimate monkey populations in affected areas [30]. Human disease has been associated with the clearing of forest areas and introduction of cattle close to the forests. The incidence of human disease is related to the density of tick vectors.

Clinical Syndrome

Clinical illness occurs in humans 2 to 9 days after tick exposure. Patients present with the sudden onset of fever, headache, myalgias, cough, diarrhea, vomiting, and dehydration [31]. Physical findings include marked conjunctival congestion, a papulovesicular eruption on the soft palate, relative bradycardia, hypotension, and hemorrhages [32]. Generalized lymphadenopathy with splenomegaly can occur. Some patients develop pneumonia. Illness can follow a biphasic course, with 6 to 11 days of the symptoms just described, followed by an afebrile period of 9 to 21 days and the reappearance of fever with evidence of meningoencephalitis. The CNS symptoms are possibly mediated by immune complexes. Although convalescence can be prolonged, no sequelae have been reported in patients who survive acute infection.

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During the acute illness, patients frequently have leukopenia, thrombocytopenia, and elevated transaminase levels [33]. Hemorrhages are felt to be caused by disseminated intravascular coagulation.

Pathology studies from humans have shown parenchymal degeneration of the liver and kidneys, hemorrhagic pneumonitis, and increased reticuloendothelial tissue in the liver and spleen with erythrophagocytosis [34].

Isolation Procedures

No cases of human-to-human transmission or nosocomial spread have been described. Clinical isolation is not required once KFV disease is confirmed. Laboratory-acquired infections from cultivation of the virus are common, and more than 100 cases of laboratory-acquired KFV disease have been documented [1]. Most laboratory infections are acquired by inhalation of aerosols [35].

Therapy

There is no specific therapy for KFV disease. Therapy consists of supportive measures to treat pain, dehydration, hypotension, and blood loss.

Prophylaxis

A killed vaccine of KFV has been developed, and has shown to be protective in field trials despite weak serologic responses [29]. The vaccine, which is formalin inactivated and produced in chick embryo fibroblasts, is used in western India [36].

OMSK HEMORRHAGIC FEVER

Background

The Omsk hemorrhagic fever virus (OHFV) was first isolated during an epidemic in Omsk and Novosibirsk Oblasts, in the former USSR in 1947. There were nearly 1500 cases reported in the Omsk region between 1945 and 1958 with a 0.5 to 3.0% case fatality rate. The disease has predominantly affected rural populations of field workers in western Siberia during the spring and summer months. Periodic cases of Omsk hemorrhagic fever were reported in the 1960s. The disease has not recently been reported.

Virus

OHFV is a flavivirus that belongs to the TBE virus complex. The virus is pathogenic in infant and suckling mice and Guinea pigs. OHFV causes hemorrhagic

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disease when experimentally inoculated in muskrats and narrow skulled voles (*Microtus gregalis*). It can be propagated under BL4 conditions in pig kidney, BHK, HeLa, and chick embryo cells.

Laboratory diagnosis is usually made by serology although virus isolation can be performed at specialized laboratories.

Ticks/Animal Host

OHFV is spread by ixodid tick vectors, principally *Dermacentor reticulatus* and possibly *Ixodes apronophorus* [37]. Transovarial virus transmission occurs in ixodid ticks. Several species of mosquitoes can be infected with OHFV, but their role in the transmission cycle for this disease remains unknown [38]. Water voles (*Arvicola terrestris*) and other rodents serve as the principal animal hosts in nature [39]. Muskrats, which were imported into the lake district of Western Siberia during the period 1929 to 1936 from North America to develop the fur industry, are incidental hosts. There have been periodic epizootic die-offs of muskrats with transmission to humans by direct contact with infected blood, tissues, urine, or feces. The source of transmission to muskrat populations is uncertain. Muskrat hunters in western Siberia have been at highest risk for infection.

Clinical Syndrome

Clinical illness occurs in humans 2 to 9 days after tick exposure. Patients present with the sudden onset of fever, headache, myalgias, cough, diarrhea, vomiting, and dehydration. Physical findings include marked conjunctival congestion, a papulovesicular eruption on the soft palate, relative bradycardia, hypotension, and hemorrhages. A marked hyperemia of the face and upper trunk without rash has been noted. Generalized lymphadenopathy with splenomegaly can occur. Some patients develop pneumonia. CNS symptoms are rare. Convalescence can be prolonged. Sequelae of Omsk hemorrhagic fever include hearing loss, hair loss, and neuropsychologic complaints.

During the acute illness, patients frequently have leukopenia, thrombocytopenia, and elevated transaminase levels. Hemorrhages appear to be caused by disseminated intravascular coagulation.

Isolation Procedures

No cases of human-to-human transmission or nosocomial spread have been described. No isolation procedures are required once the diagnosis of OHF has been confirmed. Laboratory-acquired cases are common with virus cultures probably by way of aerosol spread.

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Therapy

Patients with Omsk hemorrhagic fever should receive supportive medical care. Therapy consists of measures to treat pain, dehydration, hypotension, and blood loss. No specific antiviral therapy is available at this time.

Prophylaxis

There is no specific vaccine for OHFV. Tickborne encephalitis vaccine appears to provide some cross-protective immunity and has been used in some high-risk populations.

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APPENDIX 1

Notice to Readers

Update: Management of Patients with Suspected Viral Hemorrhagic Fever—United States

In 1988, CDC published guidelines for managing patients with suspected viral hemorrhagic fever (VHF) (*1*). Pending a comprehensive review of the 1988 guidelines, this notice provides interim recommendations that update the 1988 guidelines for health-care settings in the United States. This update applies to four viruses that cause syndromes of VHF: Lassa, Marburg, Ebola, and Congo-Crimean hemorrhagic fever viruses; although the risk and/or mode of nosocomial transmission differs for each of these viruses, the limited data do not permit clear distinctions.

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Background

In Africa, transmission of VHF has been associated with reuse of unsterile needles and syringes and with provision of patient care without appropriate barrier precautions to prevent exposure to virus-containing blood and other body fluids (including vomitus, urine, and stool). The risks associated with various body fluids have not been well defined as most caregivers who acquired infection had multiple contacts with multiple fluids. Epidemiologic studies of VHF in humans indicate that infection is not readily transmitted from person to person by the airborne route (*1*,*2*). Airborne transmission involving humans has never been documented and is considered a possibility only in rare instances from persons with advanced stages of disease (e.g., one patient with Lassa fever who had extensive pulmonary involvement may have transmitted infection by the airborne route) (*3*). In contrast, investigation of VHF in nonhuman primates (i.e., monkeys) has suggested possible airborne spread among these species (*4–7*). Despite uncertainties regarding the applicability to humans of data regarding airborne transmission in nonhuman primates, such information must be considered in the development of infection-control precautions because information regarding exposure and transmission in humans is limited.

The risk for person-to-person transmission of hemorrhagic fever viruses is highest during the latter stages of illness, which are characterized by vomiting, diarrhea, shock, and often hemorrhage. VHF infection has not been reported in persons whose contact with an infected patient occurred only during the incubation period (i.e., before the patient became febrile; the incubation period ranges from 2 days to 3 weeks, depending on the etiology of the VHF [*1*]). In the 1995 Zaire outbreak, some instances of Ebola virus transmission within a few days after onset of fever were reported; however, other symptoms in the source patients and the level of exposure to body fluids among these secondary cases were unknown (CDC, unpublished data, 1995). In studies involving three monkeys experimentally infected with Ebola virus (Reston strain), fever and other systemic signs of illness preceded detection of infectious virus in the pharynx by 2–4 days, in the nares by 5–10 days, in the conjunctivae by 5–6 days, and on anal swabs by 5–6 days (P. Jahrling, U.S. Army Medical Research Institute of Infectious Diseases, unpublished data, 1995).

Reporting

All suspected cases of infection with Ebola virus and other hemorrhagic fever viruses should be reported immediately to local and state health departments and to CDC (telephone [404] 639–1511; from 4:30 p.m. to 8 a.m., telephone [404] 639-2888). Specimens for virus-specific diagnostic tests should be sent to CDC as rapidly as possible according to instructions provided when contact is made.

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General information regarding Ebola virus infection is available through the CDC Ebola Hotline (telephone [800]900-0681).

Recommendations

The following recommendations apply to patients who, within 3 weeks before onset of fever, have either 1) traveled in the specific local area of a country where VHF has recently occurred; 2) had direct contact with blood, other body fluids, secretions, or excretions of a person or animal with VHF; or 3) worked in a laboratory or animal facility that handles hemorrhagic fever viruses. **The likelihood of acquiring VHF is considered extremely low in persons who do not meet any of these criteria**. The cause of fever in persons who have traveled in areas where VHF is endemic is more likely to be a different infectious disease (e.g., malaria or typhoid fever); evaluation for and treatment of these other potentially serious infections should not be delayed.

1. Because most ill persons undergoing prehospital evaluation and transport are in the early stages of disease and would not be expected to have symptoms that increase the likelihood of contact with infectious body fluids (e.g., vomiting, diarrhea, or hemorrhage), universal precautions are generally sufficient (*8*). If a patient has respiratory symptoms (e.g., cough or rhinitis), face shields or surgical masks and eye protection (e.g., goggles or eyeglasses with side shields) should be worn by caregivers to prevent droplet contact (*8*). Blood, urine, feces, or vomitus, if present, should be handled as described in the following reocmmendations for hospitalized patients.
2. Patients in a hospital outpatient or inpatient setting should be placed in a private room. A negative pressure room is not required during the early stages of illness, but should be considered at the time of hospitalization to avoid the need for subsequent transfer of the patient. Nonessential staff and visitors should be restricted from entering the room. Caretakers should use barrier precautions to prevent skin or mucous membrane exposure to blood and other body fluids, secretions, and excretions. All persons entering the patient's room should wear gloves and gowns to prevent contact with items or environmental surfaces that may be soiled. In addition, face shields or surgical masks and eye protection (e.g., goggles or eyeglasses with side shields) should be worn by persons coming within 3 feet of the patient to prevent contact with blood, other body fluids, secretions (including respiratory droplets), or excretions. The need for additional barriers depends on the potential for fluid contact, as determined by the procedure performed and the presence of clinical symptoms that increase the likelihood of contact with body fluids from the patient (*8*). For

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example, if copious amounts of blood, other body fluids, vomit, or feces are present in the environment, leg and shoe coverings also may be needed. Before entering the hallway, all protective barriers should be removed and shoes that are soiled with body fluids should be cleaned and disinfected as described below (see recommendation 6). An anteroom for putting on and removing protective barriers and for storing supplies would be useful, if available (*1*).

3. For patients with suspected VHF who have a prominent cough, vomiting, diarrhea, or hemorrhage, additional precautions are indicated to prevent possible exposure to airborne particles that may contain virus. Patients with these symptoms should be placed in a negative-pressure room (*9*). Persons entering the room should wear personal protective respirators as recommended for care of patients with active tuberculosis (high efficiency particulate air [HEPA] respirators or more protective respirators) (*9*).
4. Measures to prevent percutaneous injuries associated with the use and disposal of needles and other sharp instruments should be undertaken as outlined in recommendations for universal precautions (*8*). If surgical or obstetric procedures are necessary, the state health department and CDC's National Center for Infectious Diseases, Hospital Infections Program (telephone [404] 639-6425) and Division of Viral and Rickettsial Diseases (telephone [404] 639-1511; from 4:30 p.m. to 8 a.m., telephone [404] 639-2888) should be consulted regarding appropriate precautions for these procedures.
5. Because of the potential risks associated with handling infectious materials, laboratory testing should be the minimum necessary for diagnostic evaluation and patient care. Clinical laboratory specimens should be obtained using precautions outlined above (see recommendations 1–4 above), placed in plastic bags that are sealed, then transported in clearly labeled, durable, leakproof containers directly to the specimen handling area of the laboratory. Care should be taken not to contaminate the external surfaces of the container. Laboratory staff should be alerted to the nature of the specimens, which should remain in the custody of a designated person until testing is done. Specimens in clinical laboratories should be handled in a class II biological safety cabinet following biosafety level 3 practices (*10*). Serum used in laboratory tests should be pretreated with polyethylene glycol p-tert-octylphenyl ether (Triton® X-100)*; treatment with 10 μL of 10% Triton®

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X-100 per 1 mL of serum for 1 hour reduces the titer of hemorrhagic fever viruses in serum, although 100% efficacy in inactivating these viruses should not be assumed. Blood smears (e.g., for malaria) are not infectious after fixation in solvents. Routine procedures can be used for automated analyzers; analyzers should be disinfected as recommended by the manufacturer or with a 500 parts per million solution of sodium hypochlorite (1:100 dilution of household bleach: 1/4 cup to 1 gallon water) after use. Virus isolation or cultivation must be done at biosafety level 4 (*10*). The CDC mobile isolation laboratory is no longer available (*1*).

6. Environmental surfaces or inanimate objects contaminated with blood, other body fluids, secretions, or excretions should be cleaned and disinfected using standard procedures (*8*). Disinfection can be accomplished using a U.S. Environmental Protection Agency (EPA)-registered hospital disinfectant or a 1:100 dilution of household bleach.
7. Soiled linens should be placed in clearly labeled leak-proof bags at the site of use and transported directly to the decontamination area. Linens can be decontaminated in a gravity displacement autoclave or incinerated. Alternatively, linens can be laundered using a normal hot water cycle with bleach if universal precautions to prevent exposures are precisely followed (*8*) and linens are placed directly into washing machines without sorting.
8. There is no evidence for transmission of hemorrhagic fever viruses to humans or animals through exposure to contaminated sewage; the risk of such transmisison would be expected to be extremely low with sewage treatment procedures in use in the United States. As an added precaution, however, measures should be taken to eliminate or reduce the infectivity of bulk blood, suctioned fluids, secretions, and excretions before disposal. These fluids should be either autoclaved, processed in a chemical toilet, or treated with several ounces of household bleach for $\geq$5 minutes (e.g., in a bedpan or commode) before flushing or disposal in a drain connected to a sanitary sewer. Care should be taken to avoid splashing when disposing of these materials. Potentially infectious solid medical waste (e.g., contaminated needles, syringes, and tubing) should either be incinerated or be decontaminated by autoclaving or immersion in a suitable chemical germicide (i.e., an EPA-registered hospital disinfectant or a 1:100 dilution of household bleach), then handled according to existing local and state regulations for waste management.
9. If the patient dies, handling of the body should be minimal. The corpse should be wrapped in a sealed leakproof material, not em-

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balmed, and cremated or buried promptly in a sealed casket. If an autopsy is necessary, the state health department and CDC should be consulted regarding appropriate precautions (*1*).

10. Persons with percutaneous or mucocutaneous exposures to blood, body fluids, secretions, or excretions from a patient with suspected VHF should immediately wash the affected skin surfaces with soap and water. Application of an antiseptic solution or handwashing product may be considered also, although the efficacy of this supplemental measure is unknown. Mucous membranes (e.g., conjunctiva) should be irrigated with copious amounts of water or eyewash solution. Exposed persons should receive medical evaluation and follow-up management (*1*).

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Reported by: Hospital Infections Program, Div of Vital and Rickettsial Diseases, and Div of Quarantine, National Center for Infectious Diseases; Office of the Director, National Institute for Occupational Safety and Health; Office of Health and Safety, CDC.

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15

New Tick-Transmitted Rickettsial Diseases

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INTRODUCTION

Rickettsioses are caused by obligate intracellular bacteria belonging to the genus *Rickettsia*. These bacteria are associated with arthropods that may act as vectors. They represent some of the oldest recognized infectious diseases. Epidemic typhus is suspected to be the cause of the Athens plague during the fifth century BC, and was differentiated from typhoid in the sixteenth century AD [1]. At the beginning of the twentieth century, ticks were implicated as reservoirs and vectors of rickettsiae. Ricketts proved that the wood tick, *Dermacentor andersoni*, was involved in the transmission of *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever [2]. Furthermore, he demonstrated that ticks were infective during all feeding stages and that rickettsia was maintained in ticks by transovarial transmission [2]. In 1910, the first cases of Mediterranean spotted fever were reported in Tunis by Conor and Brush [3]. The role of *Rhipicephalus sanguineus*, the brown dog tick, in the transmission of the disease was established in 1930. Rickettsioses are also some of the most recently recognized infectious diseases. Prior to 1984, only eight rickettsioses were clinically recognized (Table 1) [4] and in the subsequent 13 years a further seven new rickettsial diseases have been described [4, 5]. The recent discoveries of new rickettsioses have not been confined to countries with relatively low levels of medical research; for example, Japanese spotted fever was described in Japan in 1984 (Fig. 1) [6]. The main clinical symptoms of rickettsioses include fever, headache, rash that sometimes includes an inoculation eschar, and local lymphadenopathy (Table 2). Careful clinical ex-

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TABLE 1 Old and New Rickettsial Diseases

Rickettsia	Disease	Vector	Year of isolation
Rickettsia prowazekii	Epidemic typhus	*Pediculus humanus corporis*	1916
Rickettsia rickettsii	Rocky Mountain spotted fever	*Dermacentor andersoni, Dermacentor variabilis*	1919
Rickettsia typhi	Murine typhus	*Xenopsylla cheopis*	1920
Rickettsia conorii	Mediterranean spotted fever	*Rhipicephalus sanguineus*	1932
Rickettsia akari	Rickettsial pox	*Allodermanyssus sanguineus*	1946
Rickettsia sibircia	Siberian tick typhus North Asian tick typhus	*Dermacentor nuttali, Dermacentor marginatus, Haemophysalis concinna*	1949
Rickettsia australis	Queensland tick typhus	*Ixodes holocyclus*	1950
Israeli tick typhus rickettsia	Israeli spotted fever	*Rhipicephalus sanguineus*	1974
Rickettsia honei	Flinders Island spotted fever	Unknown	1991
Astrakhan fever rickettsia	Astrakhan fever	*Rhipicephalus pumilio*	1991
Rickettsia africae	African tick bite fever	*Amblyomma hebraeum, Amblyomma variegatum*	1992
Rickettsia japonica	Japanese or Oriental spotted fever	*Dermacentor taiwanensis, Haemaphysalis flava, Haemaphysalis formosensis, Haemaphysalis hystricis, Haemaphysalis longicornis, Ixodes ovatus*	1992
Rickettsia felis	Pseudotyphus of California	*Ctenophtalides felis*	1994
Rickettsia mongolotimonae	Spotted fever	*Haemaphysalis asiaticum*	1996
Rickettsia slovaca	Fever	*Dermacentor marginatus*	1997

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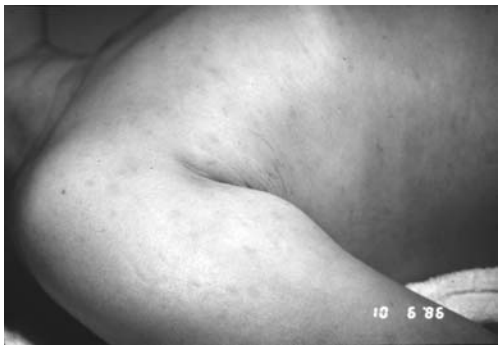

FIGURE 1 Patient with Japanese spotted fever. (Courtesy of Dr. Mahara).

aminations of patients on initial presentation and new diagnostic tools have been critical in the description of these emerging rickettsial diseases. Furthermore, some rickettsiae, previously isolated from ticks only, have recently been shown to be pathogenic in humans. New rickettsial strains continue to be isolated from arthropods, ticks in particular, around the world (Table 2) [4] and their roles as human pathogens have yet to be determined. Using animal models to predict the pathogenicity of rickettsial strains in humans is not reliable. For example, *Rickettsia rickettsii* T-type strain is highly pathogenic in humans but is responsible for only a mild illness in guinea pigs. One of the most important factors determining human pathogenicity of a rickettsial strain is the ability of the arthropod to feed on humans and inoculate the strain into the blood. For example, a new rickettsia has been found in the ladybird beetle [7]. This strain has never been implicated in human disease, probably because its host does not feed on people. On the other hand, all the rickettsial strains isolated to date from ticks only (including highly anthropophilic ticks), have to be considered as potential pathogens.

BACTERIOLOGY

Bacteria of the order *Rickettsiales* were first described as short (0.8 to 2 μm long and 0.3 to 0.5 μm in diameter), Gram-negative rods that retained basic fuchsin when stained by the method of Gimenez [8] and grew in association with eukariotic cells. Rickettsiae belong to the *Rickettsiae* tribe, which is included in the *Rickettsiaceae* family, and has long been divided into three genera: *Rickettsia, Coxiella*, and *Rochalimaea* [9]. In recent years, the advent of molecular taxonomic methods, particularly 16S rRNA sequence analysis, has enabled a reclassification of several bacterial species, including the rickettsiae. *Coxiella* and *Rochalimaea*, which had been united with the genus *Bartonella*, have now been removed from the order *Rickettsiales* [10,11]. Thus, the *Rickettsieae* have now

TABLE 2 Rickettsiae of Unknown Pathogenicity Isolated from Ticks

Rickettsia	Tick reservoir	Geographic location
Rickettsia massiliae	*Rhipicephalus sanguineus, Rhipicephalus sp.*	France, Greece, Spain, Portugal, Central Africa
JC880	*Rhipicephalus sanguineus*	Pakistan
Bar 29	*Rhipicephalus sanguineus*	Spain
Strain S	*Rhipicephalus sanguineus*	Armenia
Rickettsia rhipicephali	*Rhipicephalus sanguineus, Dermacentor andersoni*	United States, France, Portugal, Central Africa
Rickettsia montana	*Dermacentor andersoni, Dermacentor variabilis*	United States
Rickettsia peacockii	*Dermacentor andersoni*	United States
Rickettsia bellii	*Dermacentor sp., Ornithodoros concanensis, Argas cooleyi, Haemophysalis leporispalustris*	United States
Thai tick typhus rickettsia	Pool *Rhipicephalus sp., Ixodes ssp.*	Thailand
Rickettsia helvetica	*Ixodes ricinus*	France, Switzerland
''Rickettsia aeschlimannii''	*Hyalomma marginatum*	Morocco
''Rickettsia amblyommi''	*Amblyomma americanum*	United States
Rickettsia parkeri	*Amblyomma maculatum*	United States
Rickettsia texiana	*Amblyomma americanum*	United States
Rickettsia canada	*Haemophysalis leporispalustris*	Canada
HL-93	*Haemophysalis concinna*	China
Unnamed rickettsia	*Dermacentor occidentalis, Dermacentor parumapertus Ixodes pacificus Amblyomma americanum*	United States

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been reduced to the genus *Rickettsia* only, which has classically been divided into three subgroups: the spotted fever group, the typhus group, and the scrub typhus group which includes *Rickettsia tsutsugamushi*. Recent phylogenetic studies have shown the evolutionary unity of the typhus group and the spotted fever group rickettsiae, and the position of *R. tsutsugamushi* has been shown to be sufficiently distinct to justify the creation of a new genus, *Orientia* [12].

Rickettsiae are strict intracellular bacteria, and their growth in the laboratory requires living host cells (animal models, embryonated eggs) or cell cultures (Vero, L929, HEL, or MRC5 cells). In the past few years, the development of a new cell culture isolation technique, the shell vial technique [13], has allowed the isolation and characterization of many rickettsial strains from humans and arthropods. In the cytoplasm, rickettsiae are not enclosed by a vacuole and spotted fever group rickettsiae can be observed in the nuclei of the host cells. This may be explained by their ability to move within the cell by means of actin polymerization [14]. Rickettsiae multiply by binary fission and have both synthetic and energy-producing enzyme systems. Among the rickettsial protein antigens, two high surface proteins (rOmpA and rOmpB) contain species-specific epitopes [15] while their lipopolysaccharide layer contains highly immunogenic antigens that are strongly cross-reactive with all members of the subgroup and other bacteria. The Weil Felix test, one of the first serological diagnostic tests for rickettsiae, was based on detection of antibodies to various *Proteus* species containing antigens with cross-reacting epitopes to rickettsial antigens [16]. The genome of rickettsiae is small (1–1.6 Mb) and consists of a single circular chromosome [17]. Rickettsiae are associated with arthropods. Most of the spotted fever group rickettsiae are associated with *Ixodid* ticks, which may act as vectors for human and animal infections. Rickettsiae infect and multiply in almost all the organs of ticks. They are maintained in these arthropods through transstadial and transovarial transmissions [18,19]. The bacteria infecting the salivary glands of the ticks are able to be transmitted to verterbrate hosts, including humans, while feeding. Many rickettsiae are pathogenic for humans (Table 1) where the target cells of the organisms are the endothelial cells. Multiplication of the bacteria in these cells results in vasculitis. The main symptoms of rickettsioses include fever, headache, rash, and, sometimes, an inoculation eschar (Table 3). The role of humans in the natural cycle of spotted fever group rickettsiae is, however, secondary.

Biological Criteria for the Identification of Rickettsiae

For many years the identification of a rickettsial strain was based solely on immunological methods; initially the toxin neutralization test [20] was used, followed by the complement fixation test [21] and later comparative microimmunofluorescence (MIF). To date, the MIF remains the reference method for identification of rickettsiae. Two high molecular weight outer membrane protein of the bacteria

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TABLE 3 Cutaneous Particularities of Old and New Tickborne Rickettsiosis

Disease	Rickettsia	Rash	Rash specificity	Eschar	Multiple eschars	Enlarged local nodes
Rocky Mountain spotted fever	*Rickettsia rickettsii*	90%	45% purpuric	Very rare	No	No
Mediterranean spotted fever	*Rickettsia conorii*	97%	10% purpuric	72%	No	Rare
Siberian tick typhus North Asian tick typhus	*Rickettsia sibirica*	100%	None	77%	No	Yes
Queensland tick typhus	*Rickettsia australis*	100%	Vesicular	65%	No	Yes
Israeli spotted fever	Israelii tick typhus rickettsia	100%	Rarely purpuric	No	No	No
Flinders Island spotted fever	*Rickettsia honei*	85%	8% purpuric	28%	No	Yes
Astrakhan fever	Astrakahan fever rickettsia	100%	None	23%	No	No
African tick-bite fever	*Rickettsia africae*	30%	Vesicular	100%	Yes	Yes
Japanese or Oriental spotted fever	*Rickettsia japonica*	100%	None	90%	No	No
Infection attributable to *''Rickettsia mongolotimonae''*	*''Rickettsia mongolotimonae''*	Yes	None	Yes	No	No
Infection attributable to *Rickettsia slovaca*	*Rickettsia slovaca*	No	—	Yes, surrounded by a redish halo	No	Yes

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(rOmpA and rOmpB) are species specific and provide the basis for rickettsial serotyping [15]. The main problem with serological identification is the need for reference sera. Each time a new isolate is tested, it and all previous isolates have to be screened against all antisera. Recently, species-specific monoclonal antibodies against *R. conorii* [22], *R. rickettsii* [23], *R. japonica* [24], and *R. africae* [25] have been developed. Although these are useful, an exhaustive collection of monoclonal antibodies is required. Protein analysis by SDS-PAGE has also been used to identify rickettsial species [26] because the precise sizes of the rOmpA and rOmpB proteins are specific for each rickettsial species. This method is laborious and time consuming. More recently, the advent of molecular methods has enabled the development of useful, sensitive, and rapid methods for the detection and identification of rickettsial strains. To date, only few rickettsial genes have been studied. The first molecular method used to characterize rickettsiae was based on polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) analysis of the rOmpA-encoding gene and the citrate-synthase encoding gene [27]. Subsequently, PCR-RFLP analysis of the rOmpB-encoding gene [28,29] and of a 17 kD protein [30] have also been used. These methods have been shown to be sensitive and reproducible. The species-specific PCR–RFLP profiles are stored in databases, and reference to these simplifies identification of newly detected organisms. Currently, sequence analysis of PCR product is a rapid, convenient, and sensitive technique for the identification of rickettsiae. The sequence of a new isolate can be compared with those previously obtained and stored in data banks. Detection and identification strategies based on recognition of sequences within the 16S rRNA gene [31], the citrate synthase–encoding gene [32], the rOmpA [33], and the rOmpB [34] encoding genes or a 17 kD protein encoding gene [35] have been described. With these techniques, numerous samples, including blood, skin biopsies, and ticks [28,36–37], can be used for the detection and identification of rickettsial strains. Thus, any laboratory with facilities for molecular methods and access to sequence data bases is able to detect and identify all species of the genus *Rickettsia*. Moreover, the development of these molecular methods has greatly facilitated collaborative research between rickettsial reference laboratories and those in countries with less-developed facilities for research.

NEW TICK-TRANSMITTED RICKETTSIAL DISEASES

African Tick-Bite Fever

Although African tick-bite fever has been recognized since the beginning of the century, the first case that could reliably be attributed to infection with *Rickettsia africae* was reported from Zimbabwe in 1992 [38]. In the 1930s, Pijper described a tickborne disease in South Africa that was very mild and not associated with

skin rash or complications [39,40]. This disease was usually contracted in rural areas after contact with ticks of cattle and wild animals, particularly, *Amblyomma spp*. On the basis of this clinical and epidemiological data, Pijper considered African tick-bite fever to be distinct from Boutonneuse or Mediterranean spotted fever, a disease caused by *R. conorii* and transmitted by the dog brown tick *Rhipicephalus sanguineus*, which had been described in North Africa in 1910. Furthermore, Pijper failed to demonstrate cross-protection between the agent of African tick-bite fever and *R. conorii* in guinea pig infection studies. Subsequently, however, *R. conorii* was isolated in South Africa [41,42] and was eventually considered to be the causal agent of all cases of tick-bite fever in Africa. In 1990, Kelly et al. isolated rickettsial strains from *Amblyomma hebraeum* ticks in Zimbabwe [43] and showed them to be distinct from *R. conorii* and indistinguishable from an isolate obtained from *A. variegatum* ticks in Ethiopia [44]. This rickettsia was shown to have a high prevalence in *Amblyomma* ticks [45], which are known to readily feed on humans. Also, high prevalences (up to 80%) of antibodies reactive with the *Amblyomma* isolates were found in people from Zimbabwe [46]. In 1992, Kelly et al. reported the first human tickborne infection attributable to a rickettsial strain indistinguishable from those isolated from *Amblyomma hebraeum* ticks [38]. The strain was subsequently characterized as a distinct species of the spotted fever group rickettsiae and was named *R. africae* [47,48]. Subsequently, several cases have been reported among travellers returning from either Zimbabwe or South Africa, allowing for the determination of a typical clinical picture of the disease [49]. The clinical features of the patients correspond to those described by Pijper. The incubation period is similar to that of Mediterranean spotted fever (about 6 days). The disease is mild, with signs including headache, fever, eschar at the tick-bite site, and regional lymphadenopathy. Because the immature stages of *Amblyomma* ticks readily feed on man, multiple eschars are not uncommon in patients with African tick-bite fever. Also the rash is frequently absent or very transient and may be vesicular. The prevalence of this newly described rickettsiosis is probably high in sub-Saharan Africa where most tick bites in people are attributable to *Amblyomma spp*. The seroprevalence against spotted fever group ricketsiosis is higher in sub-Saharan Africa than elsewhere in the world, and parallels the geographic distribution of *Amblyomma* ticks [50]. These are prevalent in rural areas as their main hosts are cattle and wild ungulates. As tourism and travel to sub-Saharan Africa increase, it is expected that more cases of African tick-bite fever will be reported in the future. Recently, monoclonal antibodies against *R. africae* have been developed to distinguish *R. conorii* and *R. africae* in tissue culture isolates and skin biopsies [25].

Japanese or Oriental Spotted Fever

The first clinical cases of this rickettsiosis were reported in 1984 by Mahara [6]. During the summer of 1984, three patients presented with high fever and a rash.

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They lived in the countryside and had collected shoots from bamboo plantations on the same mountain. In two patients, an eschar was observed. The patient's sera tested positive in the Weil Felix Test [6] and then by indirect immunofluorescence using antigens of a spotted fever group rickettsia [51]. In 1986, the causative agent was isolated from patients [52], characterized as a new spotted fever group rickettsia, and named *Rickettsia japonica* [24]. This rickettsia has been detected in six species of ticks: *Dermacentor taiwanensis, Haemaphysalis flava, Haemaphysalis formosensis, Haemaphysalis hystricis, Haemaphysalis longicornis*, and *Ixodes ovatus* [53]. Of these, *H. flava, H. longicornis*, and *I. ovatus* commonly feed on humans in Japan and may act as vectors [54]. Since 1984, more than 140 cases have been reported, mainly in the southwestern and central areas of Japan [53]. Most of the cases are observed from April to October [53]. The disease has an abrupt onset with headache, high fever (39–40°C), and shaking chills, followed by the occurrence of a macular rash, all over the body, including the palms and soles. The rash becomes petechial after 3 or 4 days and disappears in 2 weeks. An eschar is frequently observed. Defervescence seems to occur about 10 days after the onset of the disease even after inappropriate treatment. No fatal cases of Japanese spotted fever have been reported [53].

Astrakhan Fever

In Astrakhan on the Caspian Sea, an eruptive summer disease has been reported since 1983 [55]. This disease was apparently unknown before this time and has been named Astrakhan fever by Tarasevitch et al. [56]. Dog ticks are suspected to act as vectors [55,57]. The causative agent has been isolated from patients [58] and from *Rhipicephalus pumilio* ticks [59]. This as-yet unnamed spotted fever group rickettsia is closely related to, but distinct from, *Rickettsia conorii* [59]. The disease is more frequently diagnosed in males (61%) than females. The onset of the disease includes high fever, headache, and rash that seldom develops into a petechial form (14%). An eschar is observed in about 20% of the patients. To date, although more than 1000 cases have been recognized, severe or fatal cases have not been reported [56].

Flinders Island Spotted Fever

This disease was described in 1991 by Stewart, the only physician on Flinders Island of Tasmania [60]. He reported 26 cases over 12 years of a febrile eruptive disease that occurred in summer. The rash was erythematous in most patients and purpuric in two severe cases with thrombocytopenia. An eschar was noted in 25% of cases and enlarged local lymph nodes in 55% of cases. The patient's sera tested positive in the Weil-Felix Test and subsequently by indirect immunofluorescence using antigens of a spotted fever group rickettsia. The causative

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agent was isolated in 1992 [61] and named *Rickettsia honei* [62]. The vector has yet to be identified.

Spotted Fever Attributable to *Rickettsia mongolotimonae*

In 1991, a spotted fever group rickettsia was isolated from a *Hyaloma asiaticum* tick collected in Inner Mongolia, China. It was subsequently characterized and described as a distinct species of the spotted fever group rickettsiae [63]. In March 1996 [64], an indistinguishable isolate was obtained from the blood and skin of a 63-year-old woman from Marseille who presented with fever, a discrete rash (about 20 maculo-papular lesions, all over the body), and an eschar in her left groin. The woman had no previous travel history in Mongolia or contact with individuals from Mongolia. The patient presented in March, which is not a typical month for Mediterranean spotted fever, the most prevalent rickettsiosis in the South of France. She had, however, collected compost from a garden where migratory birds were resting. Such birds are known to carry ticks, in particular *Hyalomma spp.,* and to travel from the Artic to Africa by way of Mongolia and France [65]. It is suspected, then, that the patient was bitten by a tick carried by a migratory bird to the compost she collected. The name *Rickettsia mongolotimonae* has been proposed to acknowledge the sites (Mongolia and La Timone Hospital, Marseille) where the organism has been isolated. The prevalence of the disease caused by the organism is unknown. If the theory of migratory birds playing a role in the distribution of spotted fever group rickettsioses is correct, it may explain sporadic cases of infections in nonendemic areas, particularly if the seasonal occurence is inappropriate [66]. Moreover, it is possible that as-yet unrecognized spotted fever group rickettsiae occur in Inner Mongolia and other areas, and may be transported in ticks on migratory birds to nonendemic areas where clinical infections of people might occur.

Infection Attributable to *Rickettsia slovaca*

The first documented case of infection attributable to *Rickettsia slovaca* was reported in 1997 by Raoult et al. [5]. A 39-year-old woman had been visiting the Pyrenes mountains in autumn, walking in the woods of the region. One week later, she presented with fever, arthralgias, and fatigue. Prior to the onset of symptoms she had removed a tick from her hair and kept it. She was hospitalized in Marseille on suspicion of having Lyme disease. Clinical features included fever, headache, a necrotic eschar at the site of tick attachment on the head surrounded by an erythematous halo 8 cm in diameter, and four enlarged cervical lymph nodes. Two days after 200 mg/day doxycycline treatment, she became afebrile. She continued to complain of severe headache and general weakness and fatigue for 2 months. Serology for Lyme disease was negative. The tick was identified as *Dermacentor marginatus* and the patient was seroconverted against *Rickettsia*

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slovaca, which was isolated from the tick she had removed from her hair. *R. slovaca* DNA was also detected in a biopsy taken from the escar on her scalp. *R. slovaca* was first isolated in 1968 from *D. marginatus* ticks in Czechoslovakia [67]. Subsequently it has been detected or isolated from this tick species in other European countries, including France [68], Switzerland [36], Armenia [69], and Portugal [70]. A suspected case of *R. slovaca* infection was reported in 1980 [51]. The patient presented with meningo-encephalitis and erythema. Although sera from the patient showed seroconversion against *R. slovaca* (0 to 1/32) and *R. conorii* (1 to 1/16) in complement fixation tests, no direct evidence of a rickettsial origin was provided. In 1981, low levels of anti-*R. conorii* antibodies were detected in 26 sera collected in central France from patients who had developed cutaneous lesions and neurologic signs after a tick bite [71]. An infection by a spotted fever group rickettsia distinct from *R. conorii*, which is not endemic in this area, was suspected. Also, Giroud reported several cases of illness in people after *D. marginatus* bites in central France [72]. These patients presented with similar symptoms to that of the first documented patient with *R. slovaca* infection and seroconverted to *R. conorii*. Giroud reported he had isolated rickettsial strains from the ticks but they were not identified and are now lost. The prevalence of *R. slovaca* infection has yet to be described. These rickettsia may follow the distribution of their host, *D. marginatus*, which is found throughout Europe.

CONCLUSION

High states of awareness, careful history taking, and thorough physical and laboratory examinations by primary physicians have been the major factors leading to the discovery of new tickborne rickettsial diseases. In addition, the study of new rickettsial strains in highly anthropophilic ticks has played an important role in the subsequent description of new human rickettsioses. Although there are numerous rickettsiae that are known to occur in ticks, their roles in human disease have yet to be determined. Further investigations into these organisms will no doubt depend on the new diagnostic tools available, particularly methods based on molecular biology techniques, which have been shown to greatly facilitate the description and investigation of the epidemiology of emerging human rickettsioses all over the world.

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Tularemia

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HISTORY

Soken, a Japanese physician, first described ulceroglandular tularemia in rabbits in 1837. The first cases of ulceroglandular tularemia in the United States were described in 1907 by Martin in Arizona, in patients also having acquired the infection from rabbits. McCoy and Chapin, investigating potential cases of plague, described a ''plague-like disease of rodents'' in ground squirrels in Tulare, California, and named the organism Bacterium tularense. Deer fly–transmitted tularemia was recognized by Pearse and colleagues in Utah in 1911, and was termed ''deer fly fever.'' After investigating the cause of ''deer fly fever'' in Utah between 1919–1921, Francis renamed the disease tularemia because of its frequent isolation from blood specimens in patients with tularemia. Tick-transmitted tularemia was described by Parker, Spenser, and Francis in 1924. In 1925, O'Hare and Francis confirmed that the disease originally described in Japan by Soken was tularemia. Francis described the protean clinical manifestations of tularemia in 1925. Subsequently, the genus of the organism was renamed *Francisella tularensis* in recognition of his pioneering work [1–4].

EPIDEMIOLOGY

F. tularensis occurs in northern temperate climates and is worldwide in distribution. Most cases in the United States are from the western, central, and southern states. The most common sources of tularemia in the United States are infected

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animals and carcasses. Tick and deer fly bites are the most common insect vectors [5–9]. Contact with *F. tularensis*–infected tissues or ingestion of the organism also transmits the infection. Aerosol transmission has been associated with handling contaminated hay, and, in the laboratory, associated cases. Mosquitoborne tularemia is the most common mode of transmission in Scandinavia. Most cases in the United States are rabbit or deer associated. Cats, squirrels, and muskrats have also been implicated in tularemia [10–16]. Domestic pet rabbits have not been associated with tularemia. Over a dozen tick species are known to transmit *F. tularensis*. Ticks may harbor the organism in their saliva/gut for as long as 2 decades. The primary tick vectors in the United States are *Dermacentor variabilis* (dog tick), *D. andersoni* (wood tick), and *Amblyomma americanum* (Lone Star tick). Entry of *F. tularensis* by way of contaminated saliva or feces into the tick-bite wound is the usual mechanism of transmission. Most cases of tickborne tularemia occur in the summer months, whereas winter cases are usually associated with rabbit hunting. Deer fly–transmitted tularemia peaks in late summer [8]. Cat bite–transmitted tularemia occurs through contact with infected feline saliva. Muskrats or beavers harboring *F. tularensis* may contaminate water sources, and ingestion of contaminated water has been associated with tularemia outbreaks among campers. Human to human transmission has not been reported [17,18].

Francisella are easily killed by heat, but are preserved and not killed by cold or freezing. Cooking kills *F. tularensis* in well-done meat. Viable *F. tularensis* may be cultured from biopsy specimens for nearly 2 years after they are obtained.

MICROBIOLOGY

F. tularensis is a small (1–2 μ), pleomorphic, aerobic, gram-negative coccobacillus. As *F. tularensis* is an intracellular pathogen, it does not grow well on routine laboratory culture media. *F. tularensis* grows at 37°C on glucose cysteine blood agar, and will also grow in thioglycollate broth and charcoal yeast extract (CYE) agar.

Five serologically homogenous species have been described, but *F. tularensis* is the virulent serotype. *F. tularensis* has two serotypes (biovars). *F. tularensis* (Jellison type A) is the most common and virulent variety in North America *F. tularensis biovar palearctica* (type B Jellison) is found in temperate zones worldwide and causes mild or subclinical disease. *F. tularensis biovar novicida* rarely causes disease in animals or humans. *F. tularensis biovar palearctica mediasiatica* is found in Central Asia and *F. tularensis biovar palearctica japonica* occurs in Japan. The organism is encased by multiple outer surface antigens and lipopolysaccharide. This exlains why *F. tularensis* serologically cross-reacts with *Brucella* and *Yersinia* [4,19,20].

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Francisella, like other aerobic, gram-negative bacilli, elaborate endotoxin during infection from their extracellular liposomes. Most *F. tularensis* strains produce β-lactamases, which accounts for the lack of β-lactam antibiotic activity against these organisms [21,22].

Pathology

As few as 15 *F. tularensis* organisms are needed to cause disease by aerosol transmission. In contrast, oral ingestion of 10^8 *F. tularensis* organisms are needed for enteral entry and infection. The number of organisms needed for contact transmission has not been determined. Infected granulomas in animals contain 10^9 *F. tularensis* organisms per gram.

Macrophages are capable of eliminating *F. tularensis* intracellularly. Polymorphonuclear leukocytes may phagocytose the organism in the presence of antibody. The serologic response to *F. tularensis* is biphasic, with an increase in specific IgM titers occurring within 2 weeks and followed by a sustained increase in IgG titers after 4 to 6 weeks. The cellular immune response occurs after about 2 weeks and is manifested by skin test positivity. Tularemia skin tests are not commonly available [21–24].

CLINICAL MANIFESTATIONS

The clinical presentation of tularemia is abrupt, with sudden onset of fever higher than 101°F, shaking chills, vomiting, sore throat, abdominal pain, headache, myalgias, malaise, and fatigue. Fever and lymphadenopathy can last for months. The white blood cell count and sedimentation rate may be normal or slightly elevated. Serum transaminases are usually not elevated. The only common nonspecific laboratory abnormality in tularemia is sterile pyuria (20–35%). The serum CPK may be elevated in typhoidal tularemia. If tularemia is caused by Jellison type B, the infection is usually mild, but if caused by Jellison type A, fulminant infection may result.

Six classic forms of tularemia have been described, and include ulceroglandular tularemia, which accounts for the majority of cases (70–80%). Ulceroglandular, glandular, oculoglandular, orophryngeal, typhoidal, and pulmonary tularemia (pneumonia) may be primary or complicate any of the other forms of tularemia by way of bacteremic spread [25–36].

Ulceroglandular Tularemia

Ulceroglandular tularemia was first described in 1907 and occurs after a tick or animal bite. The incubation period of tularemia is from 1 day to 3 weeks, depending on the portal of entry, inoculum size, the virulence of the strain, and host factors. In ulceroglandular tularemia, 3 to 5 days after a tick bite, *F. tular-*

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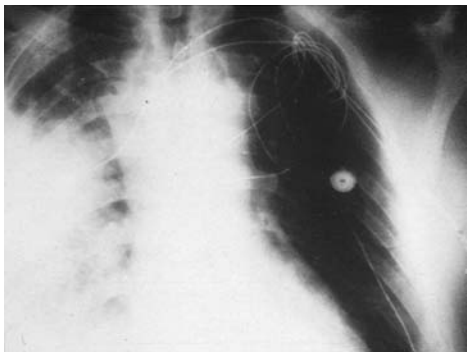

FIGURE 1 Tularemic pneumonia of the right lower lobe and right middle lobe. (Courtesy of Dr. Barney S. Graham, Vanderbilt University School of Medicine, Nashville, TN.)

ensis produces a painful, indurated, macular skin lesion. Two days later, a firm, punched-out tender ulcer with raised edges forms, which later becomes covered by a black eschar that can persist for months. From the skin, the organisms spread to the lymphatics, producing painful and sometimes suppurating regional lymphadenopathy [2,4]. Hematogenous spread to other organs follows, including the spleen, liver, lungs, and central nervous system, where *F. tularensis* causes focal necrosis, microabscesses, and caseating granulomas (Fig. 1) [25,26]. The differential diagnosis of ulceroglandular tularemia includes anthrax, sporotrichosis, and *Mycobacteium marinum* infection (Table 1) [28–36].

Glandular Tularemia

Glandular tularemia is similar to ulceroglandular tularemia with patients complaining of fever and painful lymphadenopathy, but no skin lesions are found on physical examination [2,4].

Oculoglandular Tularemia

Oculoglandular tularemia accounts for about 5% of tularemia cases. Inoculation of the eyes occurs as a result of direct contact with contaminated fingers, or by splash or aerosol transmission. Patients present with ocular pain, excessive lacrimation, mucopurulent discharge, lid edema, and photophobia. On examination there may be pinpoint conjunctival ulcers and yellowish nodules. Painful preauricular, submaxillary, and cervical adenopathy is the rule. Complications include corneal ulceration and, rarely, visual loss. The differential diagnosis of oculoglandular tularemia includes lymphogranuloma venereum (LGV), adult in-

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Table 1 Differential Diagnostic Features of Ulceroglandular Tularemia

Infectious Diseases	Ulcer Location	Ulcer	Regional Adenopathy	Fever/Systemic Symptoms	Gram Stain of Ulcer Exudate
Ulceroglandular tularemia	Rabbitborne—arms, head; Tickborne—axilla, groin, legs	Purple/blue papule—painful ulcer	Painfully enlarged, out of proportion to ulcer size	+	−
Anthrax	Neck, hands, arms	Painless ulcer, gelatinous raised edge ''satellite vesicles''	± Painless, minimal enlargement	−	Gram + bacilli
Sporotrichosis	Fingers, hands, arms	Not true ulcer, initial painless papule	Nodular lymphangitis with ''skip'' areas	−	−
Mycobacterium marinum	Fingers, hands	Verrucous nodules	± Nodular lymphangitis	−	−

Source: From Ref. 50.

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clusion conjunctivitis, herpes zoster infection (VZV), epidemic keratoconjunctivitis, and Listeria monocytogenes infection (Table 2) [30–35].

Oropharyngeal Tularemia

Oropharyngeal tularemia occurs after ingestion of contaminated food or water. Patients present with painful sore throat out of proportion to the clinical findings of exudative pharyngitis or tonsillitis. There is associated anterior cervical, preparotid, and retropharyngeal lymphadenopathy occasionally complicated by abscess formation [1–4]. The differential diagnosis of oropharyngeal tularemia includes group A streptococcal pharyngitis, EBV infectious mononucleosis, and diphtheria (Table 3) [37–40].

Typhoidal Tularemia

Typhoidal tularemia presents acutely in patients with a history of outdoor activity or contact with animals, but with no evident portal of entry, ulcers, or lymphadenopathy. Patients seem septic and complain of severe sore throat, nausea, vomiting, loose watery diarrhea, headache, high fevers, and chills. There may be prostration, delirium, stupor, coma, and septic shock. On physical examination there is abdominal tenderness and hepatosplenomegaly, but no lymphadenopathy. Blood cultures are usually positive for *F. tularensis*. Laboratory findings include elevated serum transaminases, creatinine phosphokinase, myoglobulinuria, or sterile pyuria. The cerebrospinal fluid of patients with meningitis shows a monocytic pleocytosis [1–4]. The differential diagnosis of typhoidal tularemia includes typhoid fever, brucellosis, typhoidal mononucleosis, malaria, and miliary tuberculosis (Table 4) [2,4]. The mortality rate of typhoidal tularemia is 30 to 60%.

Pleuropulmonary involvement in tularemia was described in 1924 by Verbryke. Pleuropulmonary involvement is seen in about 45% of typhoidal and 30% of ulceroglandular tularemia. The presentation is usually that of a zoonotic pneumonia, as a mild upper respiratory infection, or severe bronchopneumonia with complications of adult respiratory distress syndrome and death [10–16]. The differential diagnosis includes the other zoonotic and nonzoonotic atypical pneumonias: *Mycoplasma, Legionella, Chlamydia pneumoniae*, Q fever, and psittacosis (Tables 5 and 6) [41–46].

Tularemia Pneumonia

Primary lung involvement occurs after direct inhalation of infected aerosols and is most common in persons in high-risk occupations, especially laboratory workers. There are no skin ulcers and lymphadenopathy. The onset of symptoms is abrupt, with high fevers up to 104°F and severe chills without relative bradycardia, dyspnea, nonproductive cough, and pleuritic chest pain accompanied by profuse

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TABLE 2 Differential Diagnostic Features of Oculoglandular Tularemia

Infectious Diseases	Preauricular Adenopathy	Periorbital Edema	Conjunctivitis	Cornea
Oculoglandular tularemia	+ submaxillary, anterior cervical nodes	+	Unilateral, follicular, purulent ulcer	Clear
LGV (L_{1-3} serotypes)	± inguinal, generalized adenopathy	−	−	Clear
Adult inclusion conjunctivitis (Chlamydia trachomatis serotypes D–K)	±	−	Hemorrhagic, mucopurulent, lower lid predisposition	
Herpes zoster	±	Ptosis/ophthalmoplegia	−	± Dendritic ulcers
Epidemic keratoconjunctivitis (Adenovirus types 8 and 19)	±	−	Hemorrhagic	Cloudy corneal infiltrates
Listeria	+	−	Occasionally hemorrhagic	± Ulcer

Abbreviation: LGV, lymphogranuloma venereum.
Source: From Ref. 50.

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TABLE 3 Differential Diagnostic Features of Oropharyngeal Tularemia

Infectious Diseases	Exudative Pharyngitis	Cervical Adenopathy	WBC	Peripheral Eosinophila	↑ Serum Transaminases	Group A Strep + Throat Culture
Oropharyngeal Tularemia*	Painful ulcers	Anterior/Δ/submandibular (N)	N	−	±	−
Group A Streptococci	Very painful	Anterior Δ	(N)/↑	+	−	+
Infectious mononucleosis (EBV)	Painful tonsillar enlargement	Posterior Δ	(N)/↓	−	+	±
Diphtheria	Adherent bluish white membrane, relatively painless	Anterior Δ/submandibular	(N)/↑	−	−	±

* ≤ 2/3 have associated tularemic pneumonia.
Abbrevations: WBC, white blood count; N, normal.
Source: From Ref. 50.

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TABLE 4 Differential Diagnostic Features of Typhoidal Tularemia

Infectious Diseases	Onset	Severe Headache/Myalgias	Relative Bradycardia	WBC	Platelets	ESR	Bone Marrow
Typhoidal tularemia	Acute	+	±	N	N	N/+	Necrotizing granulomas
Typhoid fever	Subacute	+	+	+	N	N	Salmonella in smears
Brucellosis	Subacute	+	−	N/↓	N	N/↓	Brucella/granulomas
Typhoidal infectious mononucleosis	Subacute	−	−	↓	N/↓	↑	−
Malaria	Acute	+	−**	N/↓	↓	↑	−
Miliary tuberculosis	Subacute	−*	−**	↓	↓	↑	AFB/granulomas

* Headache only if central nervous system involvement.
** May demonstrate double quotidian fever pattern.
Abbreviations: WBC, white blood count; ESR, erythrocyte sedimentation rate; N, normal; AFB, acid-fast bacilli.
Source: From Ref. 50.

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TABLE 5 Differential Diagnostic Features of Tularemic Pneumonia*

Infectious Diseases	Pleuritic Chest Pain	Severe Headache	Relative Bradycardia	↑ Serum Transaminases	Bilateral Hilar Adenopathy	Consolidation	Pleural Effusion	CXR
Tularemic Pneumonia	+	+	−	−	+†	±	Unilateral/bilateral bloody	''avoid densities''
Q Fever	−	+	+	+	−	±	−	Lower lobes infiltrates
Psittacosis	−	+	+	+	−	+	−	Lower lobes infiltrates
Mycoplasma	−	−	−	−	±	−	Small bilateral effusions	Unilateral lower lobe infiltrates
Legionella	±	−	+	+	−	±	±	Asymmetrical, rapidly progressive multilobar infiltrates

* Usually associated with typhoidal or ulceroglandular tularemia.
† May be unilateral.
Abbreviation: CXR, chest x-ray.
Source: From Ref. 50.

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sweating. Some patients develop mucopurulent sputum or mild hemoptysis. Signs and symptoms may be mild and nonspecific, last for a few days, or persist for several weeks when occurring as a complication of ulceroglandular, glandular, or oculoglandular tularemia. In these cases, patients also complain of cachexia and severe fatigue. Lymph node suppuration may complicate the aglandular variations of tularemia. Physical findings in the lungs are the same as with other zoonotic atypical pneumonias, with crackles varying with the location of lung involvement. Pleural rubs are not uncommon. The chest radiograph appearance is variable, and there may be extensive infiltrates in 25 to 30% without pulmonary physical findings. Secondary pneumonias presenting as new bilateral lower lobe infiltrates may occur late in the illness [47–52]. Bilateral pleural effusions occur in 60 to 80% of patients as early as 3 days after the onset of symptoms. The pleural fluid in tularemic pneumonia is an exudate with greater than 3 g elevated protein more than 1,000 leukocytes/mm^3, and a lymphocytic predominance. Granulomas on pleural biopsy may be confused with pulmonary tuberculosis. Empyemas and bronchopleural fistulas can occur.

Chest radiographic findings vary widely and are nonspecific. These include apical or miliary infiltrates resembling tuberculosis, single or multiple lobar infiltrates and consolidation, hilar adenopathy with or without infiltrates, mediastinal adenopathy, and abscesses with cavitation. Residual calcific and fibrotic lesions, pneumothorax, ARDS, and characteristic ''ovoid densities'' described in tularemia literature are rare (Fig. 2) (Table 7) [53,54].

DIAGNOSIS

Although *F. tularensis* may be cultured in the laboratory, serologic diagnosis is the method of choice because laboratory-associated tularemia is potentially dangerous [21,22].

The tube agglutination test is usually used for serologic diagnosis. Tube agglutinins are usually present in the infected patient after 2 weeks and titers peak in 4 to 6 weeks. Low tularemia IgG titers (1:10–1:80) are common in the general population. In acute tularemia, a titer of at least 1 to 160 is diagnostic, as is a fourfold or greater rise between acute and convalescent titers. Serologic cross-reactivity may be differentiated from actual infection by the magnitude of the tularemia IgG titer elevation compared with the lower titers of *Brucella* or *Yersinia* [21–24].

ANTIBIOTIC THERAPY

The mortality of untreated tularemia is about 5%. With early diagnosis or treatment, mortality is reduced to about 1%. Mortality is highest with typhoidal tularemia, which may reflect the difficulty in diagnosing this form of tularemia and

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TABLE 6 Diagnostic Features of the Atypical Pneumonias

	Zoonotic Atypical Pneumonias			Nonzoonotic Atypical Pneumonias		
Key Characteristics	Psittacosis	Q fever	Tularemia	*Mycoplasma pneumoniae*	Legionnaires' Disease	*Chlamydia pneumoniae*
Symptoms						
Mental confusion	−	−	−	±	+	−
Prominent headache	+	+	+	−	−	−
Myalgias	+	+	−	±	+	±
Ear pain	−	−	−	+	−	−
Pleuritic pain	−	−	−	±	+	−
Abdominal pain	−	−	−	−	+	−
Diarrhea	−	−	−	+	+	−
Signs						
Rash	±	−	−	± *E. multiforme*	± pretibial rash	− Horder's spots
Raynaud's phenomenon	−	−	−	±	−	−
Nonexudative pharyngitis	+	−	±	+	−	+
Hemoptysis	+	−	−	−	+	−
Lobar consolidation	+	+	+	−	±	−
Cardiac involvement	± myocarditis	± myocarditis	−	± myocarditis/ heartblock	−	−
Splenomegaly	+	+	−	−	−	−
Relative bradycardia	+	+	−	−	+	−

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Chest Film						
Infiltrate	patchy/ consolidation	patchy/ consolidation	''ovoid bodies''	patchy	patchy/ consolidation	single ''circum-scribed'' funnel-shaped infiltrates
Bilateral hilar adeno-pathy	−	−	+	−	−	−
Pleural effusion	−	−	+ bloody	± small	±	±
Laboratory Abnormalities						
WBC count	↓	↑/N	↑/N	↑/N	↑	N
Hyponatremia/ hypophosphatemia	−	−	−	−	+	−
Increase in serum trans-aminases	+	+	−	−	+	−
Cold agglutinins	−	−	−	+	−	−
Microscopic hematuria	−	−	−	−	+	−
Diagnostic Tests						
Direct isolation (culture)	±	−	−	±	±	+
Serology (specific)	CF	CF	TA	CF	IFA	CF
Psittacosis CF titers	↑	−	−	−	↑	↑
Legionella IFA titers	−	−	↑	−	↑	−

Abbreviations: WBC, white blood count; N, normal; CF, complement fixation; TA, tube agglutination; IFA, indirect fluorescent antibody.
Source: From Ref. 50

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TABLE 7 Chest Radiograph Findings in Tularemia

Alveolar opacities	74%
Consolidation	14%–18%
Ovoid densities	7%– 8%
Bilateral hilar adenopathy	14%–32%
Cavitation	16%
Apical infiltrates	4%–14%
Miliary pattern	2%– 3%
Bronchopleural fistula	3%– 4%
Lung abscess	3%
Residual calcifications	2%– 3%
Residual fibrosis	6%

Source: From Ref. 45.

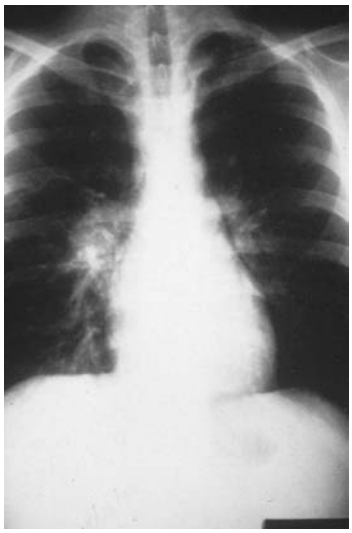

FIGURE 2 Tularemia pneumonia with bilateral hilar adenopathy. (Courtesy of Dr. Barney S. Graham, Vanderbilt University School of Medicine, Nashville, TN.)

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TABLE 8 Antimicrobial Therapy of Tularemia

Preferred Therapy*		Alternate Therapy
Streptomycin		Chloramphenicol
Adults	500 mg (IM) q12 × 7–14 days	500 mg (IV) q6h × 7–14 days
Children	30 mg/kg (IM) q12h × 7–14 days	or
Gentamicin	3–5 mg/kg (IV) q24h or in 3 divided doses (IV) q8h × 7–14 days	Levofloxacin 500 mg (IV) q24h × 7–14 days
		or
Doxycycline	200 mg (IV) q12h × 72 hours, then 100 mg (IV) q12h, or 200 mg (IV) q24h × 7–14 days	Ciprofloxacin 400 mg (IV) q12h × 7–14 days

* Avoid erythromycin, tobramycin, rifampin, amikacin, and ceftriaxone.

thereby delay the initiation of effective antibiotic therapy. Untreated tularemia may present as a fulminant illness or as a prolonged febrile subacute illness. Nonfulminant tularemia was classically described as having three clinical phases: 31 days of fever, 31 days of bed rest, followed by 31 days of disability.

The traditional antibiotic treatment for tularemia remains streptomycin. β-lactam antibiotics are ineffective because *F. tularensis* is a β-lactamase producer. Alternate antibiotics that are effective in tularemia include gentamicin (but not other aminoglycosides), doxycycline, chloramphenicol, and fluoroquinolones (Table 8). Treatment is ordinarily continued for 7 to 14 days, although clinical improvement occurs after 3 days. With the exception of streptomycin, relapse may occur with any antibiotic. Debilitation is often prolonged [55–67].

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