Ticks and Tickborne Bacterial Diseases in Humans: An Emerging Infectious Threat

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Ticks are currently considered to be second only to mosquitoes as vectors of human infectious diseases in the world. Each tick species has preferred environmental conditions and biotopes that determine the geographic distribution of the ticks and, consequently, the risk areas for tickborne diseases. This is particularly the case when ticks are vectors and reservoirs of the pathogens. Since the identification of *Borrelia burgdorferi* as the agent of Lyme disease in 1982, 15 ixodid-borne bacterial pathogens have been described throughout the world, including 8 rickettsiae, 3 ehrlichiae, and 4 species of the *Borrelia burgdorferi* complex. This article reviews and illustrate various aspects of the biology of ticks and the tickborne bacterial diseases (rickettsioses, ehrlichioses, Lyme disease, relapsing fever borrelioses, tularemia, Q fever), particularly those regarded as emerging diseases. Methods are described for the detection and isolation of bacteria from ticks and advice is given on how tick bites may be prevented and how clinicians should deal with patients who have been bitten by ticks.

Ticks are obligate hematophagous arthropods that parasitize every class of vertebrates in almost every region of the world [1]. By 1 January 1996, 869 species or subspecies of ticks had been recorded [2]. There are 2 major tick families: the Ixodidae, or "hard ticks," so called because of their sclerotized dorsal plate, which are the most important family in numerical terms and in medical importance, and the Argasidae, or "soft ticks," so called because of their flexible cuticle [1]. A third family, the Nuttalliellidae, is represented by only a single species that is confined to southern Africa. Ticks have 3 basic life stages: the larval, nymphal, and adult (male and female). Ixodid and argasid ticks differ both anatomically and in their life cycle (table 1). Ixodids have a number of attributes that enhance their vector potential. They feed for relatively long periods (several days), during which they remain firmly attached to the host. Also, their bite is usually painless and they may go unnoticed for lengthy periods of time. Each stage of the tick feeds only once, and this feeding may involve a great variety of vertebrates that occupy very diverse habitats [1]. On the other hand, Argasids feed briefly and often, usually on a single host species. They tend to live in dry areas, and most species live in sheltered sites near their hosts [1].

Ticks have been recognized as human parasites for thousands of years and were described by ancient Greek writers, including Homer and Aristotle [1]. Although ticks have been studied for a long time, the first demonstration that they may transmit infectious diseases was made at the end of the 19th century, when Smith and Kilbourne [3] demonstrated that *Boophilus annulatus* transmitted the protozoan *Babesia bigemina,* the agent of Texas cattle fever. At the beginning of the 20th century, ticks were implicated as vectors of human bacterial diseases. Tick relapsing fever, caused by *Borrelia duttonii* and transmitted by *Ornithodoros moubata,* was described in 1905 [4], and Ricketts [5] proved that the wood tick, *Dermacentor andersoni,* was involved in the transmission of *Rickettsia rickettsii,* the agent of the Rocky Mountain spotted fever. In 1910, the first cases of Mediterranean spotted fever were reported in Tunis

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Table 1. Comparison of 2 major families of ticks, Ixodidae and Argasidae.

Characteristic	Ixodidae (hard ticks)	Argasidae (soft ticks)
Morphological features		
Capitulum	Visible from dorsal aspect[a]	Not visible dorsal aspect[b]
Scutum	Present[a]	Absent[b]
Coxal pores	Absent	Present in adults and nymphs
Sexual dimorphism	Well-marked[a]	Slight
Ecology		
Habitats	Open environments (except in certain nidicolous *Ixodes* species, particularly immatures)	Sheltered environments (nest, burrow, cave, man-made primitive shelter)
Seasonal activity	Yes	No
Host-seeking behavior	Mostly non-nidicolous parasiting free-ranging hosts (except certain *Ixodes* species, particularly immatures)	Mostly nidicolous in the habitat of their hosts
Host	Usually 3 hosts (1 per stage), often different species	Usually multi-host feeding pattern
Life span	Range from several months to 3 years; less resistant to starvation and desiccation	Long-lived (up to 10 years); highly resistant to starvation
Biological features		
Nymphal instars	1	Many
Feeding	Each stage feeds slowly (several days), firmly attached and once only	Nymphs and adults feed briefly (minutes to a few hours) and several times
Weight gain while feeding	High (≤100 times unfed weight)[c]	Low (≤12 times unfed weight)
Osmoregulation	Salivary glands	Coxal fluid
Bacterial diseases transmitted to humans	Spotted fever rickettsioses, ehrlichioses, Lyme borreliosis, tularemia, Q fever[d]	Relapsing fever borrelioses, Q fever[d]

[a] See figure 3.
[b] See figure 9.
[c] See figure 5.
[d] Role of ticks in transmission to humans is probably minimal.

by Conor and Bruch [6], and the role of *Rhipicephalus sanguineus,* the brown dog tick, in the transmission of the disease was established in the 1930s [7]. In 1929, Francis [8] described the epidemiology of tularemia and the role of blood-sucking arthropods, including ticks. After World War II, a number of viral, protozoan, and bacterial tickborne diseases were described in animals and in humans [9]. In the 1980s, Lyme borreliosis due to *Borrelia burgdorferi* [10] was described, and the disease is currently considered the most important vectorborne disease in Europe and the United States. More recently, a number of emerging tickborne rickettsioses have been reported from around the world [11], and bacteria of the genus *Ehrlichia* have become recognized as tickborne human pathogens in the United States and Europe [12]. Ticks may act not only as vectors, but also as reservoirs of tick-transmitted bacteria, including spotted fever group rickettsiae, recurrent fever borreliae, and *Francisella tularensis.* In these cases, the bacteria are transmitted transstadially (from stage to stage—from larvae to nymphs and adults) and also transovarially (from one generation to the next via the female ovaries). There are optimal environmental conditions and biotopes for each tick species, and these determine the geographic distribution of the ticks and, thus, the risk areas for tickborne diseases, particularly when ticks are both vectors and reservoirs of pathogens.

Despite the increasing information available on ticks, there is no recent review on ticks and tickborne bacterial diseases. Here, we focus particularly on emerging tickborne diseases of humans and describe the ticks that are vectors of the diseases, their biology, and the clinical and epidemiological features of the diseases they cause.

TICKS

Taxonomy, Systematics, and Evolution

Ticks and mites are currently grouped with members of the subclass Acari, which is the largest subclass in the class Arachnida of the suborder Ixodida within the order Parasitiformes. It has been suggested, however, that ticks should be classed as a distinct order [1, 2, 13]. There are 3 generally recognized

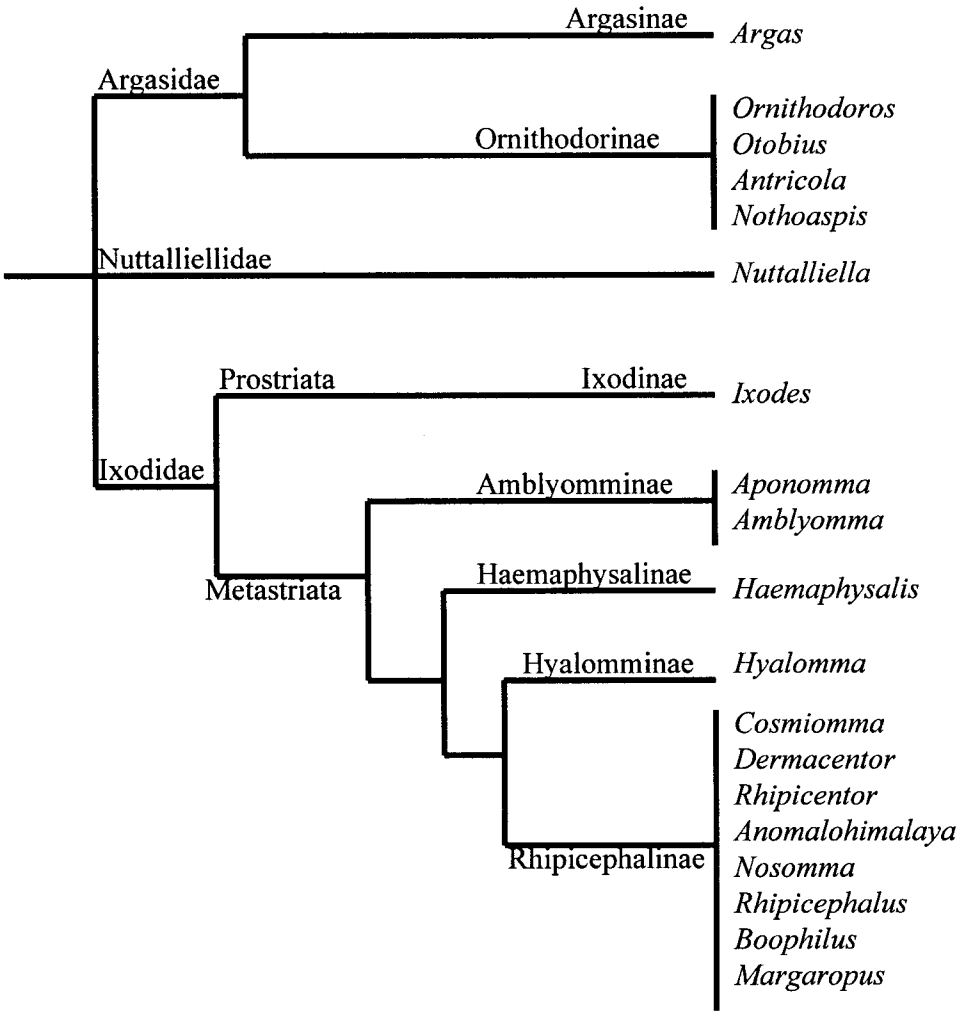

Figure 1. The traditional Hoogstraal classification for ticks

families of ticks: the Ixodidae, or "hard ticks" (694 species), the Argasidae, or "soft ticks" (177 species), and the Nuttalliellidae, represented by a single species confined to southern Africa [1, 14]. In the most commonly used classification of ticks [15], the family Ixodidae comprises 2 major groups, the Prostriata and the Metastriata (figure 1). Two subfamilies are currently recognized in the Argasidae: the Argasinae and the Ornithodorinae [16].

The classic concept of the long-term evolution of ticks is based on hypotheses suggesting that structural, physiological, and biological modifications of ticks reflect adaptations associated with specialization for particular hosts, host specificity, and broad cospeciation [13]. Ticks may have appeared in the late Paleozoic or early Mesozoic eras ~225 millions years ago, when they parasitized reptiles. A number of dates have been suggested for the appearance of the different taxa [13]. Fossil records have not been useful in confirming these dates, because there are none from earlier than the Eocene era in the early Tertiary period, ~50 million years ago [13]. Recently, gene sequence analysis of mitochondrial or nuclear rDNA has been used to determine phylogenic relationships among ticks. Studies with partial sequences of the 16S rDNA mitochondrial gene gave results that were inconsistent with traditional phylogeny; the studies maintain that the Argasinae are not monophyletic, but are a sister group of the hard ticks [17]. Other studies that used partial sequences of nuclear rDNA genes have subsequently reported similar results [18]. The major conflicts with morphologically based phylogeny studies have been resolved by means of analyses of total sequences of the 18S rDNA genes [19, 20] (figure 2). Analyses of these sequences, however, often reveal no differences between closely related species and even those belonging to different genera, for example *Rhipicephalus pusillus* and *B. annulatus.* Comparisons of sequences of the mitochondrial 16S rRNA [21] and, more recently, the 12S rRNA [22] genes have been described as good methods to study the phylogeny of closely related species. Although these methods enabled some relationships within the Rhipicephalinae and the closely related species of the genus *Rhipicephalus* to be resolved

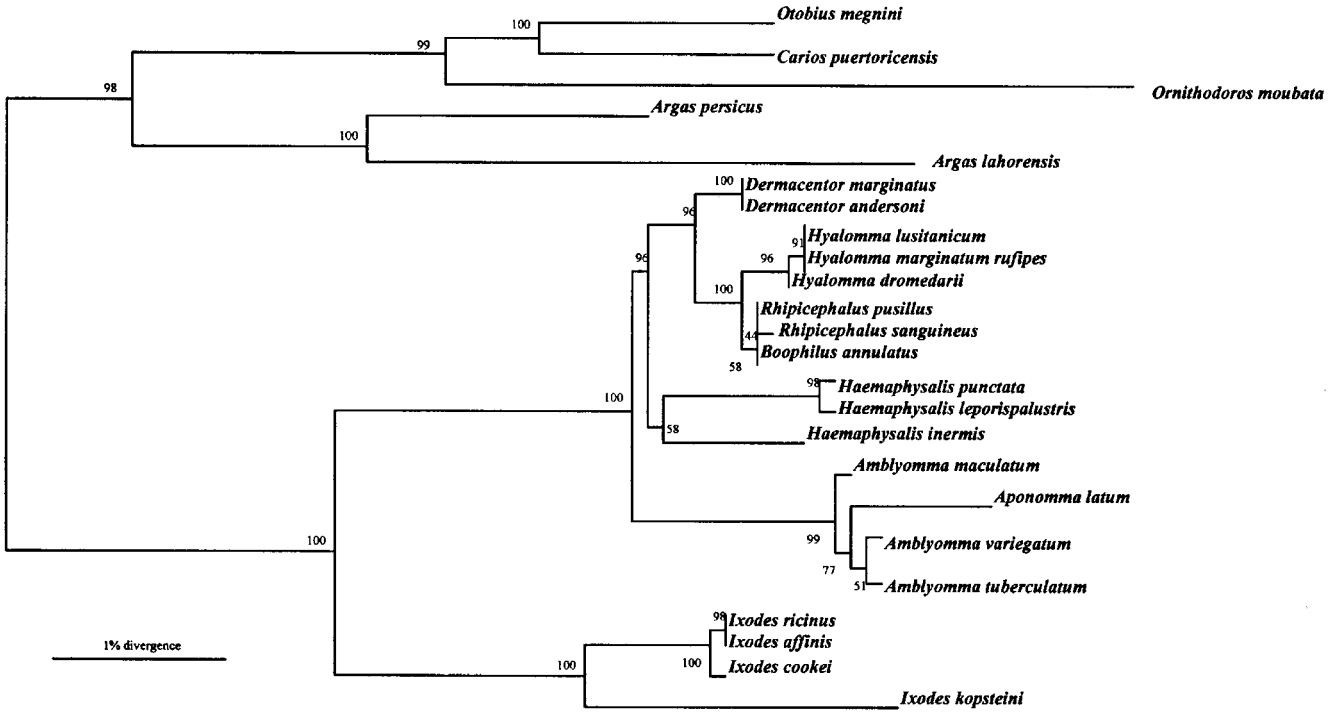

Figure 2. Phylogenic trees for ticks derived from 18S rRNA gene sequences that were deposited in GenBank by Mangold et al. [20] and Black et al. [19]. Evolutionary distance values were determined by use of the method of Kimura, and the tree was constructed by use of the neighbor-joining method. Numbers at nodes are proportions of 100 bootstrap resamplings that support the topology that is shown.

[22], the phylogeny of members of the Metastriata still remains unclear, and studies of additional genes are required.

Ixodidae or Hard Ticks

Anatomy. Ticks are large-body-size (2–30 mm) acarines. Adults and nymphs have 4 pairs of walking legs, and larvae have 3 (figure 3). All stages have no antennae, and unlike insects, their bodies are not divided into a distinct head, thorax, and abdomen [1, 23]. The anterior part of the body, the capitulum, bears the mouthparts, including sensory organs, cutting organs, and a median immobile organ (the hypostome) with numerous recurved teeth that anchor the tick to the host's skin (figure 4). The ixodids are characterized by the presence of a sclerotized plate (the scutum) on the dorsal surface of the body, and the remainder of the body is able to expand during feeding (figure 5) [1, 23]. Ticks posses a circulatory system, and all organs and tissues are bathed by a circulating fluid, the hemolymph [1, 23]. Many ticks lack eyes, and even when eyes are present, it is doubtful that they enable a detailed perception of the environment. However, ticks have a variety of peripheral sensory organs. These include hair-like structures on the body, legs, and mouthparts and a sensory complex located on the dorsal surface of the tarsus of leg I, which contains a cluster of olfactory and gustatory receptors (Haller's organ). These sensory organs are evidently important in enabling ticks to locate their hosts and also to communication with other ticks (see below).

Life cycles and ecology. Typically, ixodid ticks have a 3-host life cycle, with each feeding stage of the tick (larva, nymph, and adult) having a single host [1, 9, 23, 24]. Each stage of the tick seeks out a host, attaches, and then feeds over a period of several days. Once replete, the tick detaches and, after dropping from the host, finds a resting place where it can digest its blood meal and molt to the next feeding stage, or enter diapause, a state characterized by reduced metabolism and delayed development (figure 6). In a few species, the immature forms may remain on the host during molting. Generally, adult males feed only briefly and sparingly and some do not feed at all.

Mating generally occurs on the host. Thereafter, the females detach and drop off the host to digest their blood meal. They then lay their eggs, from 400 to >20,000 depending on the species (~5000 for the American dog tick, *Dermacentor variabilis*), in a sheltered environment and die [1, 9]. Pheromones play an important role in the behavior of ticks and facilitate ticks' finding their hosts and their mates. They include assembly pheromones, which bring ticks together, and sex pheromones, which attract males to females and stimulate mounting.

The life cycle of ixodid ticks is usually completed in 2–3 years, but it may take from 6 months to 6 years, depending on environmental conditions, including temperature, relative humidity, and photoperiod. The ixodid ticks are relatively sen-

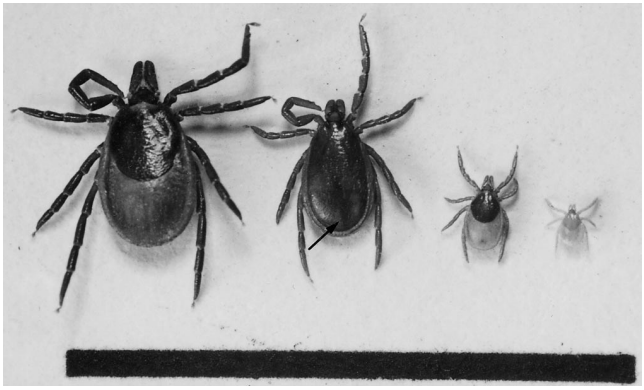

Figure 3. Four stages of unfed *Ixodes ricinus.* Left to right: female, male, nymph, and larva (bar, 1 cm). Scutum *(arrow)* covers entire dorsal surface in male, whereas it is confined to anterior part of body in other stages. Note the 3 pairs of walking legs in larva. (Provided by Claudine Perez, Institut Pasteur, Paris).

sitive to desiccation and are especially common in grasslands and woodlands, with each species having its own particular optimal environmental conditions and biotopes that determine the geographic distribution of the ticks (table 2). For example, in Europe, the dog brown tick *R. sanguineus* is well adapted to Mediterranean vegetation and climatic conditions and is thus endemic in the Mediterranean area but is absent in the north of Europe except in human homes, where conditions enable it to survive. On the other hand, *Ixodes scapularis* in the United States and *Ixodes ricinus* in Europe favor woods and forests with high relative humidity and are absent from dry places.

Host seeking. Ixodid ticks spend >90% of their life unattached from the host [25], and most of them are exophilic: they live in open environments, meadows, or forests. Here they are usually seasonally active, seeking their hosts when environmental conditions are most suitable. They are highly responsive to stimuli that indicate the presence of hosts. These include chemical stimuli (such as CO_2, NH_3) phenols, humidity, and aromatic chemicals, and airborne vibrations and body temperatures associated with warm-blooded animals. For example, ticks are attracted by feet hitting the ground or by the CO_2 emitted by a car stopped in the bush (JL Camicas, personal communication). Two typical host-seeking behavior patterns occur among exophilic ticks. In the ambush strategy, ticks climb up vegetation and wait for passing hosts, with their front legs held out in the same manner as are insect antenna (e.g., *R. sanguineus,* the brown dog tick, and *I. ricinus* adults in Europe; *I. scapularis* and *D. variabilis* in the United States; figure 7). In the hunter strategy, ticks attack hosts. They emerge from their habitat and run toward their hosts when these animals appear nearby (e.g., adult and nymph *Amblyomma hebraeum* and *Amblyomma variegatum* in Africa) [1, 9]. Some species (for example, the lone star tick, *Amblyomma americanum,* in the United States) use both strategies [1, 9]. Other species of ticks (those of the genus *Ixodes,* for example) are endophilic and exhibit a third host-seeking behavior: they remain hidden in hosts' nests and burrows awaiting their arrival.

A number of tick species are host-specific, feeding on only a limited variety of animals. Other ticks have different hosts for each feeding stage, and host specificity may vary between the different stages in the same species (tables 2 and 3). Each feeding stage of *R. sanguineus,* for example, has high specificity and feeds readily on dogs. On the other hand *I. scapularis* and the American lone star tick, *A. americanum,* and adults of the European sheep tick, *I. ricinus,* usually feed on different host species, particularly large mammals but also small mammals and birds [1, 9, 23]. Generally, habitat distribution also influences host selection, because ticks that are adapted to a habitat or a vegetation type, for example woods for *I. ricinus* and *I. scapularis,* will encounter vertebrates that are adapted to the same habitat. Different species of ticks also have different affinities for people (table 2) [26]. Although *R. sanguineus* will feed on humans only if there are no dogs or other hosts avail-

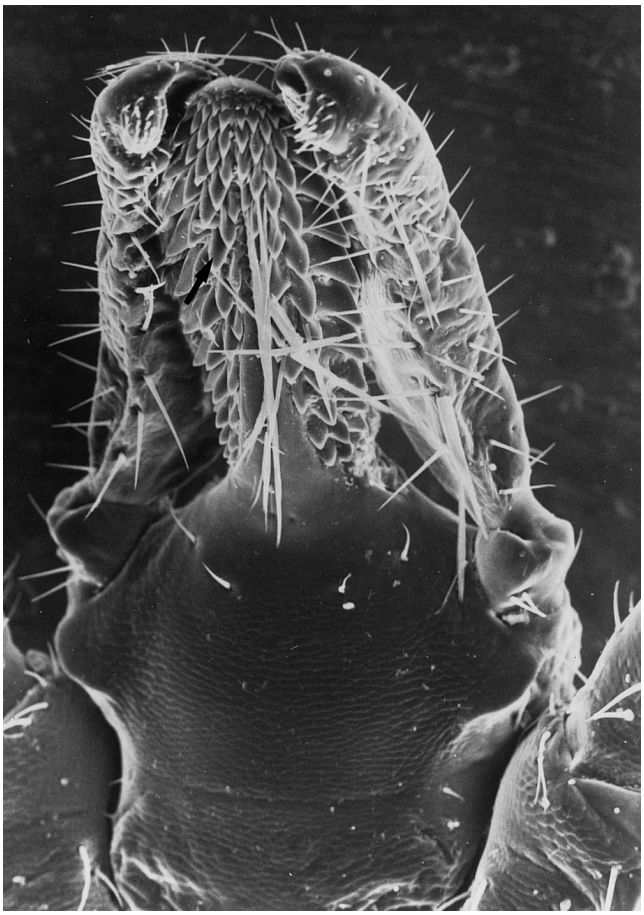

Figure 4. Scanning electron micrograph of mouthparts (ventral aspect) of female *Ixodes ricinus.* Note numerous recurved teeth on hypostome *(arrow).*

Figure 5. Engorged and unfed female *Dermacentor marginatus* (bar, 1 cm).

able, *I. ricinus* in Europe, *I. scapularis* in the United States, and *A. hebraeum* in Africa feed readily on humans that enter their biotopes [1, 9, 23].

Attachment and feeding. Before feeding, a tick may wander around on its host for several hours. It inserts only its hypostome into the skin (figure 8), and various substances produced by the salivary glands enter the host during this penetration, creating a feeding pool [1, 23]. During the first 24–36 h of attachment, there is no or little ingestion of blood, and penetration and attachment are the predominant activity. The salivary secretions produced by ixodid ticks include a cement to anchor the mouthparts to the skin of the host; enzymes; vasodilators; and anti-inflammatory, antihemostatic, and immunosuppressive substances. These facilitate successful blood feeding, and an anesthetic in the saliva makes the bite of ixodid ticks usually painless. There may also be toxins in the saliva of some species that may cause paralysis of the host (see below) [1, 9]. Ixodid ticks feed for long periods, and 2–15 days are required for a complete blood meal to be ingested, depending on the feeding stage, species of tick, type of host, and site of attachment. An initial slow feeding period (3–4 days) is followed by a period of rapid engorgement (1–3 days) when ticks, particularly females, may increase their body weight up to 120-fold (figure 5). While feeding, there are alternating periods of sucking blood and salivation, with regurgitation occurring frequently, particularly at the end of the rapid engorgement phase. During the initial slow feeding period, there is continuous digestion of the blood meal in the midgut, and defecation may occur. In the period of rapid engorgement, there is reduced digestion, but this becomes continuous again after the tick is replete and detaches from the host. Ticks rapidly concentrate the blood meal by eliminating water and electrolytes in the feces, during transpiration, and in salivary gland secretions. Undigested residues from the midgut and wastes from the excretory body are eliminated through the anus.

Argasidae or Soft Ticks

The argasids, or "soft ticks," are quite different from the ixodids (table 1; figure 9). The salivary glands of argasids do not produce cement and contain anticoagulant and cytolytic substances, because feeding only takes a brief time [1, 23]. Apart from the larval stages, argasids may feed up to 10 times, during which they become replete in a few hours. The coxal organs concentrate the blood meal, and the coxal fluid is secreted during and after the meal. The time spent on the host is relatively short, and after each meal these ticks are typically found in cracks and crevices in their habitats or just below the soil surface (figure 10).

Tick Paralysis: A Noninfectious Tickborne Disease

In addition to transmitting many pathogens, including the bacteria reviewed below, prolonged attachment (5–7 days) of certain species of ticks may result in paralysis of the host [9, 27, 28]. This is caused by neurotoxic substances produced by the salivary glands of attached engorged ticks (particularly females). Tick paralysis was first recognized in Australia in 1824 and is now known to occur in many countries worldwide. More than 40 species of ticks from both families have been implicated in the condition, but *D. andersoni* and *D. variabilis* in North America, *I. ricinus* in Europe, *Ixodes holocyclus* in Australia, and *Rhipicephalus evertsi evertsi* in Africa are most commonly involved [27, 28]. Tick paralysis occurs more often in children, although adults may also be affected. Clinical signs include weakness in the lower extremities, which ascends within hours or days to involve the trunk musculature, upper extremities, and head. Patients may present with ataxia or respiratory distress, and mortality rates of up to 10% have been reported [27, 28]. Analysis of CSF samples usually reveals no abnormalities, and the diagnosis of the condition depends on a history of tick bite or finding a tick on the body of the patient. Removal of the tick leads to a rapid recovery within 24 h [27, 28].

Control

Reducing and controlling tick populations is difficult [29, 30]. Habitat modifications, including vegetation management by cutting, burning, and herbicide treatment, and drainage of wet areas are one strategy for tick control, but their effects are often short-lived and they can cause severe ecological damage. In some areas, host exclusion or depopulation may result in a reduction in the density of ticks, but this is mostly impractical and is also not ecologically sound. The use of organophosphates or pyrethroids, which may be combined with pheromones to control ticks, may cause environmental contamination and toxicity for animals and humans, even when applied only to selected habitats. Acaricides,

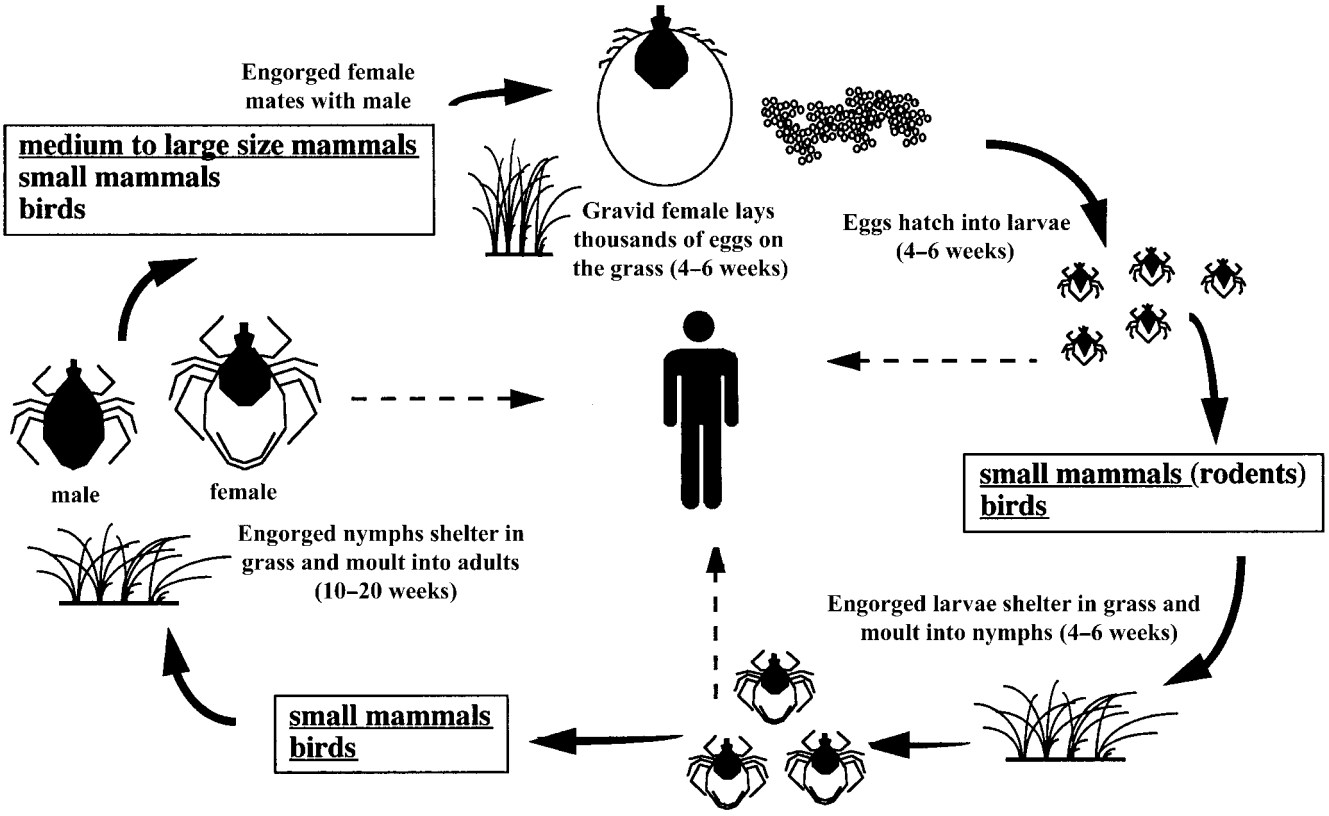

Figure 6. Life cycle of *Ixodes ricinus*. Hosts are listed in boxes; humans are potential hosts.

however, can be applied directly to wild or domestic hosts to kill attached ticks and disrupt tick feeding.

Biological control methods for ticks are also available, and these include the promotion of natural predators (including beetles, spiders, and ants), parasites (insects, mites, and nematodes), and bacterial pathogens of ticks; the mass release of males sterilized by irradiation or hybridization; and the immunization of hosts against ticks [29, 30, 31]. At the present time, tick control is best based on the concept of integrated pest management, in which different control methods are adapted to one area or against one tick species with due consideration to their environmental effects.

EPIDEMIOLOGY OF TICKBORNE DISEASES

Bacteria and ticks. Ticks may become infected with bacteria by feeding on bacteremic animals or by transstadial and transovarial transmission. All forms of transmission may occur for some bacteria; for example, the spotted fever group rickettsiae can be transmitted via all routes [11]. Three points are essential to understanding the ways in which bacteria are transmitted by ticks and the consequences for tickborne bacterial diseases. First, rickettsiae multiply in almost all organs and fluids of ticks, in particular the salivary glands and ovaries, which enables transmission of organisms during feeding and transovarially, respectively. Other bacteria may be transmitted transovarially but do not infect the salivary glands of their tick hosts and cannot then be transmitted to susceptible vertebrate hosts where they might cause disease [32].

Second, each stage of ixodid tick feeds only once, and bacteria acquired by a tick during feeding can then be transmitted to another host only when the tick has molted to its next developmental stage. Not all species in a genus are capable of transmitting bacteria transstadially; for example, not all *Ixodes* species that acquire *B. burgdorferi*, the agent of Lyme disease, pass the agent transstadially and, therefore, act as vectors.

Finally, if bacteria such as the rickettsiae are transmitted both transstadially and transovarially in a tick species, this tick will also be the reservoir of the bacteria, and the distribution of the disease caused by the bacteria will be identical to that of its tick host (table 3). It is very rare, but ticks may become infected with bacteria by cofeeding—that is, several ticks feeding in close proximity on the host—and, thus, direct spread of bacteria from an infected tick to an uninfected one might occur [11]. In only some rickettsiae and some species of relapsing fever borreliae has sexual transmission of bacteria from infected male to female ticks been described [11].

Little is known about the consequences of bacterial infections

Table 2. Some characteristics of the ticks that commonly act as vectors in human bacterial diseases.

Tick	Distribution	Ecology	Feeding hosts	Affinity for humans
Ixodes species				
I. scapularis (black legged tick)	Eastern and southeastern United States, Canada	Wooded coastal areas with maritime influence; deciduous forest, thickets	Small mammals, reptiles, birds (l); large mammals (deer, dogs) (A)	High
I. pacificus	Canadian Pacific coast south through California to Mexico	Shrub, desert, coniferous forest areas	Small mammals, reptiles, birds (l); large mammals (A)	Yes
I. dentatus	Eastern United States	Wooded coastal areas, forest	Rabbits and other small mammals (I, A); birds (l)	Low
I. ricinus (European sheep tick)	From western Europe to central Asia; North Africa	Grassy woodlands, heathlands, forest, pastures; humid microhabitat	Small mammals, birds, reptiles (l); medium to large mammals (I, A)	High
I. persulcatus	Eastern Europe, Russia, Asia, northern Japan	Woods, coniferous and deciduous forest areas	Small mammals, birds (l); medium to large mammals (I, A)	Yes
I. uriae	Circumpolar in both hemispheres, Europe, Asia	Host's nest or burrows	Seabirds (which may transport ticks over great distances)	Yes
I. trianguliceps	Europe, Russia, Asia	Coniferous and deciduous forest; host's nest or burrows	Small mammals (rodents and insectivores)	Yes
I. hexagonus	Western and eastern Europe, Russia, North Africa, southern Asia	Forest and caves; host's nest or burrows; also in urban and suburban areas	Wide range of mammals (foxes, polecats, dogs, rabbits, badgers, rodents) but rarely sheep, cattle, and horses	Yes
I. nipponensis	Japan, Korea	Forest	Reptiles, rodents (l); ungulates (A)	Yes
I. ovatus	Asia, including Nepal, China, Thailand, Vietnam, Japan	Forest, open field	Rodents (l); wild and domestic large mammals (A)	High
I. holocyclus	Queensland, East Australia	Rainforest, coastal area	Small mammals, birds, carnivores (l); birds, small and large domestic mammals (A)	Yes
Rhipicephalus species				
R. sanguineus (brown dog tick)	Africa, Israel, Mediterranean littoral, Black Sea, India, United States	Mediterranean climate; survives with dogs in kennels and houses, even in colder country	Dogs principally	Low
R. pumilio	Central Asia, including former USSR, China	Desert and semidesert steppe	Hedgehogs, hares, birds (l); hedgehogs, hares, large rodents, domestic and wild mammals (A)	Yes
Amblyomma species				
A. americanum (Lone Star tick)	South central, southeastern United States; parts of Central and South America	Brushy or wooded habitats, especially young second-growth forest with dense understory	Wide variety of small to large mammals and birds	High
A. canjennense (cayenne ticks)	Southern United States; Central and South America	Wide spectrum of habitats in different climatic zones	Birds, small to large mammals, including ungulates, ruminants, and carnivores	High
A. hebraeum; A. variegatum	Southeastern Africa; sub-Saharan Africa, West Indies, respectively	Wide spectrum of habitats in different climatic zones	Wide spectrum of hosts, particularly wild and domestic mammals (cattle, sheep)	High
Dermacentor species				
D. variabilis (American dog tick)	Throughout United States, Canada, Mexico	Woodland, old field-forest ecotones, brushy habitats, deciduous forest, clearings around homes; high relative atmospheric humidity	Small mammals, mice, voles (l); dogs and large mammals (A)	High
D. andersoni (Rocky mountain wood tick)	Western United States, southwestern Canada	Mountainous areas, sagebrush country near streams	Wild rodents and other small mammals (l); large mammals (A)	Yes
D. marginatus (ornate sheep tick)	From Morocco, through Spain, France, central Europe, and central Asia	Scrub steppe, temperate forest, grassland, pastures	Small mammals and birds (l); large mammals (A)	Yes
D. reticulatus	From Europe to central Asia	Woodlands, grassland, pastures	Small mammals (l); domestic and wild mammals (cattle, dogs, horse deer) (A)	Yes
D. taiwanensis	Japan	Forests, wooden areas	Small mammals (l); large mammals (A)	Yes
D. nuttalli	Central Asia, including former USSR, China, Mongolia	Steppe, high grasslands	Rodents (l); large mammals (cattle, horse, dog, sheep) (A)	Yes

(*continued*)

Table 2. Continued

Tick	Distribution	Ecology	Feeding hosts	Affinity for humans
Hyalomma species				
H. asiaticum	Central Asia, southern former USSR, Iran	Desert and semidesert areas	Hedgehogs, hares, rodents, and carnivores (I); herbivores (A)	Yes
Haemaphysalis species				
H. flava	Japan, Caucasia, India	Forest, pasture	Rodents (I); ungulates, carnivores (A)	Yes
H. longicornis	Japan, far eastern former USSR, China, Korea, southeastern Australia	Forest, pasture	Rodents, birds (I); large mammals (cattle, sheep, dogs, horse) (A)	Yes
Ornithodoros species				
O. moubata	Africa (central, eastern, southern)	Mud or grass human huts	Humans principally	High
O. erraticus sonrai	Morocco, Libya, Egypt, Iran, Turkey, Senegal, Kenya, Chad	Burrows in semiarid and Sahelian regions	Rodents	Yes
O. graingeri	East Africa	Burrows and caves	Rodents	Yes
O. erraticus erraticus	Iberian Peninsula, North Africa, Greece, Cyprus, Syria	Burrows	Rodents, small mammals	Yes
O. verrucosus (O. aspersus)	Caucasia, Armenia, Iraq, Azerbaijan, Georgia	Burrows and caves in semidesert areas	Rodents	Yes
O. tartakovski	Central Asian, former USSR, Iran	Burrows and nests	Rodents, tortoises	Yes
O. tholozani	Former USSR, western China and Kashmir to Iran, Iraq, Egypt, Syria, Israel, India	Man-made shelters (primitive barns, buildings used to house domestic stocks), caves (burrows into the dry sand or dusty ground surface near cave entrance)	Rodents, jackals, and all mammals using the shelters	Yes
O. hermsi	Western United States, Canada	Forested mountainous habitats; cavities in dead trees, fallen logs, log cabins	Rodents	Yes
O. talaje	Southern United States, Mexico, Guatemala, Central and South America	Burrows, nests, and caves	Bats, birds (chicken), small mammals (rodents)	Yes
O. parkeri	Western United States	Identical to *O. hermsi* but at much lower altitudes	Rodents	Yes
O. turicata	Southwestern, northern United States, Canada, northern Mexico	Semiarid plains; in caves, rodent burrows, wood rat nests, primitive man-made buildings	Rodents and all mammals found in those habitats	Yes
O. rudis	Central and South America	Burrows, nests and caves; primitive man-made habitats	Rodents, humans	High

NOTE. A, adults; I, immature ticks (nymphs and larvae); USSR, Union of Soviet Socialist Republics.

on the host ticks themselves, but lowered fertility and high mortality have been reported in ticks infected with *R. rickettsii* [33]. Also, it has yet to be established if the properties of bacteria, for example virulence, change in the tick hosts. It is known, however, that *B. duttonii* lose pathogenicity after repeated transovarial passage in *O. moubata* [34], and *R. rickettsii* loses its virulence in guinea pigs when its host ticks are subjected to physiological stress [11].

Tickborne zoonoses. The tick-transmitted bacterial diseases of humans that are currently recognized are zoonoses, with the bacteria being maintained in natural cycles involving ticks and wild and/or domestic animal hosts. The ticks may, however, occasionally feed on people and thereby cause infections. For each bacterial disease, one or several tick vectors and one or several reservoirs may exist [34, 35]. Animal hosts are susceptible to the bacteria and need to develop a relatively long duration of bacteremia to be effective reservoirs of infection. The infectivity of the reservoir hosts, the tick infestation rate, and the host density are major variables determining the epidemiology of tick-transmitted diseases. These are influenced by several physiological and ecological factors, which have been reviewed recently [34, 35], and which include the preference for the host of the different stages of ticks, degree of tick-host contact, seasonal activity of both ticks and host, susceptibility of preferred hosts to the bacteria, environmental conditions, and host immunity. The proportion of ticks that acquire bacterial infections may increase with the duration of their attachment on the reservoir host while feeding, as is the case with *B. burgdorferi* infections [36].

Transmission from ticks. Ticks transmit bacteria to hu-

Figure 7. Ixodid (or "hard tick") on vegetation waiting for passing hosts with its front legs held out in an ambush strategy. (Provided by Pr. A. Aeschlimann, Institut de Zoologie, Neuchâtel, France).

mans when their feeding sites are contaminated with infected salivary secretions (e.g., spotted fever group rickettsiae, *B. burgdorferi,* relapsing fever borreliae), regurgitated midgut contents (e.g., *B. burgdorferi*), feces (e.g., *Coxiella burnetii*), or coxal fluid in the case of argasid ticks (some species of relapsing fever borreliae). The increased risk of disease transmission with increased attachment time has been clearly demonstrated for spotted fever group rickettsiae and *B. burgdorferi* [9, 11]. Indirect routes of transmission are also possible, such as contamination of abraded skin or the eyes following crushing of ticks with the fingers.

Ticks may attach on people at numerous sites but are most frequently found around the head and neck and in the groin. Recently, the attachment sites for several tick species in the United States were reported: *D. variabilis,* a vector of the Rocky mountain spotted fever, favored the head and neck (59%); *A. americanum,* a vector of human ehrlichiosis, attached mainly on the lower extremities and buttocks and in the groin (54%); and *I. scapularis,* which transmits *B. burgdorferi,* attached at a wide variety of sites [37]. In Europe, *Dermacentor* species prefer to attach on the head [38], and it has been reported that the preferred site of attachment of the brown dog tick, *R. sanguineus,* is the head of children and the remainder of the body of adults [39]. Possible reasons for these preferential feeding sites have not been given. The height of clumps formed by ticks on vegetation and host-seeking behaviors may contribute to explaining these clinical findings [40].

It is important to note that ixodids usually do not cause pain while feeding, and immature stages are frequently not detected on people because of their small size. The highest incidence of Mediterranean spotted fever occurs in August, the period when the immature forms of the vector are most prevalent [11]. A history of tick bite is then often not reported by persons who have tickborne disease diagnosed [11]. From 1981 through 1992, the Center for Disease Control reported 9223 cases of Rocky Mountain spotted fever. Only 59.6% of patients reported a history of tick bite in the 2 weeks prior to illness [41].

Tick-related factors that influence the rates of human infections with tickborne bacterial diseases include the prevalence of vector ticks and their infection rate, their readiness to feed on people, and the prevalence of their usual hosts. Human factors, such as the likelihood of people entering the tick's biotope, the act of crushing ticks between fingers, and susceptibility to the bacteria, also play a role. For example, the brown dog tick, *R. sanguineus,* is well adapted to a human urban environment, where it is relatively host-specific and rarely feeds on people. Although ~5% (up to 12%) of these ticks are infected with *Rickettsia conorii* in southern France, the incidence of Mediterranean spotted fever and the prevalence of seropos-

Figure 8. Hypostome of an ixodid tick inserted in skin. (Provided by Pr. A. Aeschlimann, Institut de Zoologie, Neuchâtel, France).

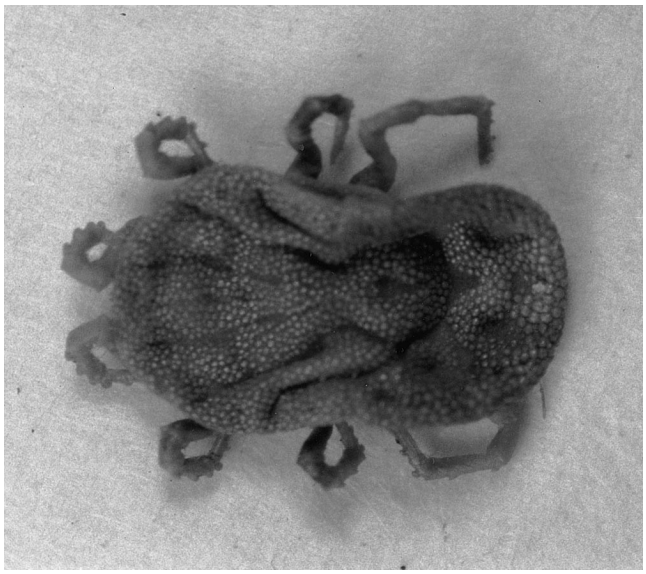

Figure 9. *Ornithodoros moubata,* a soft tick. The leathery integument can expand 3- to 5-fold during rapid feeding.

itive persons are relatively low in that area [11]. In North Carolina, *R. rickettsii* has been found to infect only 0.5% of *D. variabilis,* but these ticks feed readily on humans and Rocky Mountain spotted fever is common in the area [41, 42]. In sub-Saharan Africa, *Amblyomma* species are the vectors of *Rickettsia africae,* which is the agent of African tick bite fever. These ticks are commonly infected with the organism (>50% in certain areas) and feed readily on people who enter their biotopes (the bush). The seroprevalence in people from these areas is consequently very high; it is often >80% [11].

Epidemiology of tickborne zoonoses. The origin and dissemination of tickborne zoonoses has been the subject of numerous hypotheses and includes the concept of coevolution of the microorganisms, the ticks, and the animal hosts [43]. Tickborne diseases are geographically localized (figures 11–15) and occur only in foci with optimal conditions for the ticks and animals involved in the circulation of the bacterial pathogens. In this specific setting, the vectors and hosts are subjected to selective pressure that leads to coevolution. This is supported by the continental distribution of most tickborne diseases, including the rickettsial diseases caused by *R. rickettsii* in America; *R. conorii* from southern Europe to southwest Asia, India, and Africa; *R. africae* in sub-Saharan Africa; *Rickettsia sibirica* in north central Asia, China, and northeastern Asia; *Rickettsia australis* in Australia; and the Thai tick typhus agent in southeast Asia and Australia [11]. A number of events can disturb these associations, however, including macroclimatic changes, urbanization, and deforestation.

Molecular methods have been found to be useful tools in the study of the spread of arthropod-borne diseases and have been particularly useful in studies of Lyme disease [44–46]. Lyme disease is principally caused by 3 of the described species of the *B. burgdorferi* sensu lato complex [47], mainly *B. burgdorferi* sensu stricto, *Borrelia garinii,* and *Borrelia afzelii. B. garinii* and *B. afzelii* occur in Eurasia and are absent from America, whereas *B. burgdorferi* sensu stricto is the only species known to occur in both the United States and Europe. A number of molecular studies have shown that strains from Europe and North America are very closely related and probably share a recent common ancestor. More recent studies of the sequences of a very variable gene (*osp*C) of *B. burgdorferi* sensu stricto have confirmed the above finding and have shown a larger genetic polymorphism among North American strains [44–46]. European isolates, then, probably represent a subset of the North American population of *B. burgdorferi* sensu stricto that have been recently introduced into Europe [44–46].

The dissemination of tickborne diseases requires the dispersal of the tick vectors and/or the reservoir hosts [43]. To enable the maintenance of infections in new areas, the vector ticks or reservoir hosts must find hosts or ticks, respectively, that are susceptible to infections and that can maintain the pathogenic organism. Ticks may disperse by walking, but this occurs only over short distances that seldomly exceed 50 m (e.g., 5 m for *I. ricinus*). Ticks may also become dispersed while attached to hosts that might travel over larger distances, particularly in the case of migrating birds or mammals [1, 9].

Humans may also influence tick dispersal by agricultural practices or modification of tick habitats or by the shipment of livestock with ticks over very large distances. African tick bite fever due to *R. africae* and transmitted by *Amblyomma* ticks is an emerging tickborne disease that has been spread from sub-Saharan Africa, where the disease is endemic, to the West Indies by the movement of livestock [48, 49]. One potential vector and reservoir of the disease, *A. variegatum,* was introduced into the West Indies during the 18th or 19th century

Figure 10. Argasids (or "soft ticks") hide in cracks and crevices of barns in Africa.

Table 3. The ticks that commonly act as vectors in human bacterial diseases.

Tick	Transmitted bacteria			
		Mode of acquisition by ticks		
	Bacteria	Transstadially	Transovarially	Mode of transmission to humans
Ixodes species				
I. scapularis (Black legged tick)	*Borrelia burgdorferi* sensu stricto	+	+/−	Salivary secretions, regurgitations
	HGE agent	+	−	Salivary secretions
I. pacificus	*B. burgdorferi* sensu stricto	+	+/−	Salivary secretions, regurgitations
	HGE agent	+	−	Salivary secretions
I. dentatus	*B. burgdorferi* sensu stricto	+	?	Salivary secretions, regurgitations
	Francisella tularensis	+	+	Salivary secretions, feces
	Coxiella burnetii	+	+	Salivary secretions, feces
I. ricinus (European sheep tick)	*B. burgdorferi* sensu stricto	+	+/−	Salivary secretions, regurgitations
	Borrelia afzelii	+	+/−	Salivary secretions
	Borrelia garinii	+	+/−	Salivary secretions, feces
	HGE agent	+	+	Salivary secretions
	Francisella tularensis	+	+	Salivary secretions, feces
	Rickettsia helvetica	+	+	Salivary secretions
I. persulcatus	*B. afzelii*	+	?	Salivary secretions, regurgitations
	B. garinii	+	?	Salivary secretions, regurgitations
I. uriae	*B. garinii*	+	?	Salivary secretions, regurgitations
I. trianguliceps	*B. garinii*	+	?	Salivary secretions, regurgitations
	C. burnetii	+	+	Salivary secretions, feces
I. hexagonus	*B. garinii*	+	-	Salivary secretions, regurgitations
I. nipponensis	*B. afzelii*	+	?	Salivary secretions, regurgitations
I. ovatus	*Rickettsia japonica*	+	+[a]	Salivary secretions
I. holocyclus	*Rickettsia australis*	+	+[a]	Salivary secretions
Rhipicephalus species				
R. sanguineus (Brown dog tick)	*Rickettsia conorii*	+	+[a]	Salivary secretions
	R conorii Israel	+	+[a]	Salivary secretions
	C. burnetii	+	+	Salivary secretions, feces
R. pumilio	*R. conorii* Astrakhan	+	+[a]	Salivary secretions
Amblyomma species				
A. americanum (Lone Star tick)	*Ehrlichia chaffeensis*	+	−	Salivary secretions
	Ehrlichia ewingii	+	−	Salivary secretions
	F. tularensis	+	+	Salivary secretions, feces
	C. burnetii	+	+	Salivary secretions, feces
A. canjennense (Cayenne tick)	*Rickettsia rickettsii*	+	+	Salivary secretions
A. hebraeum	*Rickettsia africae*	+	+	Salivary secretions
A. variegatum	*Rickettsia africae*	+	+	Salivary secretions
Dermacentor species				
D. variabilis (American dog tick)	*F. tularensis*	+	+	Salivary secretions, feces
	R. rickettsii	+	+	Salivary secretions
D. andersoni (Rocky Mountain wood tick)	*F. tularensis*	+	+	Salivary secretions, feces
	R. rickettsii	+	+	Salivary secretions
	C. burnetii	+	+	Salivary secretions, feces
D. marginatus (Ornate sheep tick)	*F. tularensis*	+	+	Salivary secretions, feces
	Rickettsia slovaca	+	+	Salivary secretions
D. reticulatus	*F. tularensis*	+	+	Salivary secretions, feces
	C. burnetii	+	+	Salivary secretions, feces
D. taiwanensis	*Rickettsia japonica*	+	+[a]	Salivary secretions
D. nuttalli	*Rickettsia sibirica*	+	+	Salivary secretions

(*continued*)

Table 3. Continued

Tick	Transmitted bacteria			
		Mode of acquisition by ticks		
	Bacteria	Transstadially	Transovarially	Mode of transmission to humans
Hyalomma species				
H. asiaticum	*"Rickettsia mongolotimonae"*[b]	+	+[a]	Salivary secretions
Haemaphysalis species				
H. flava	*R. japonica*	+	+[a]	Salivary secretions
H. longicornis	*R. japonica*	+	+[a]	Salivary secretions
Ornithodoros species				
O. moubata	*Borrelia duttonii*	+	+	Salivary secretions, coxal fluid
O. erraticus sonrai	*Borrelia crocidurae*	+	+	Salivary secretions, coxal fluid
O. graingeri	*Borrelia graingeri*	+	+	Salivary secretions, coxal fluid
O. erraticus erraticus	*Borrelia hispanica*	+	+	Salivary secretions, coxal fluid
O. verrucosus (O. aspersus)	*Borrelia caucasica*	+	+	Salivary secretions, coxal fluid
O. tartakovski	*Borrelia latyschewii*	+	+	Salivary secretions, coxal fluid
O. tholozani	*Borrelia persica*	+	+	Salivary secretions, coxal fluid
O. hermsi	*Borrelia hermsii*	+	+	Salivary secretions, coxal fluid
O. talaje	*Borrelia mazzottii*	+	?	Salivary secretions, coxal fluid
O. parkeri	*Borrelia parkeri*	+	?	Salivary secretions, coxal fluid
O. turicata	*Borrelia turicatae*	+	+	Salivary secretions
O. rudis	*Borrelia venezuelensis*	+	?	Salivary secretions, coxal fluid

NOTE. HGE, human granulocytic ehrlichiosis.

[a] Although transovarial transmission is usually admitted for spotted fever group rickettsiae, it has not been definitively demonstrated in this case.

[b] Disease has been reported in France, where tick vector is unknown.

on cattle shipped from Senegal to Guadeloupe. The tick became established in the Caribbean and even spread among the islands by movement of livestock and migrating birds. Because *A. variegatum* readily feeds on people and contact with humans is frequent, seroprevalences of antibodies to *R. africae* are high in people from Guadeloupe (~50%), and African tick-bite fever may occur commonly in the Caribbean islands.

The reasons for the distribution of some tickborne diseases are yet to be determined, however. For example, the brown dog tick, *R. sanguineus*, is a vector and potential reservoir of *R. conorii,* the agent of Mediterranean spotted fever in Europe and Africa. However, although this species of tick is distributed worldwide, including in the United States, infection with *R. conorii* has never been reported there.

Coinfection in ticks and humans. Some ticks are able to transmit several pathogenic bacteria (table 3). *I. scapularis* in the United States is a vector of *B. burgdorferi* sensu stricto and the agent of human granulocytic ehrlichiosis (HGE) and may also transmit babesiosis, a protozoan disease due to *Babesia microti* [9]; furthermore, 1 tick may be infected with all 3 organisms. Both *B. burgdorferi* sensu stricto and the HGE agent have been detected in 2% of *I. ricinus* ticks in Switzerland [50] and in 5.5% of nymphal *I. scapularis* at a Westchester County site in New York [51]. Humans may then be infected with multiple organisms if fed upon by such ticks, and patients with antibodies against *B. burgdorferi* sensu stricto, the HGE agent, and/or *B. microti* have been reported [52]. Cases of multiple infections have been described [53–55]. Such multiple infections may result in atypical clinical manifestations of tickborne diseases [56].

Bacteria detected only in ticks. Numerous bacteria have been found to be regularly associated with arthropods, including ticks, and these have been called symbionts (literally "living together"), microsymbionts, or endosymbionts (living in endocellular symbiosis) by entomologists, ecologists, or endocytobiologists [57]. The bacteria may have negative, positive, or no effects on their arthropod hosts, with such effects including sex-ratio distortion and alteration in the sex determination of their hosts. Members of the genera *Wolbachia, Rickettsia,* and *Francisella* [11, 32] include symbionts that are maintained in ticks through transovarial and transstadial transmission. It is not yet possible to predict if symbionts found in arthropods may be human pathogens. If the organisms are restricted to the ovarial tissue of their arthropod hosts, however, it would appear unlikely that they would be transmitted to people during feeding [11]. Although animal models have been used to predict the pathogenicity of symbionts found in arthropods in humans, this technique is unreliable with the rickettsiae. Although *R. rickettsii* T-type strain causes only a mild illness in guinea pigs, it is highly pathogenic in humans [11]. The pathogenic role of a tickborne bacteria in humans cannot be established by inoculation of volunteers, because this is unethical. It can be

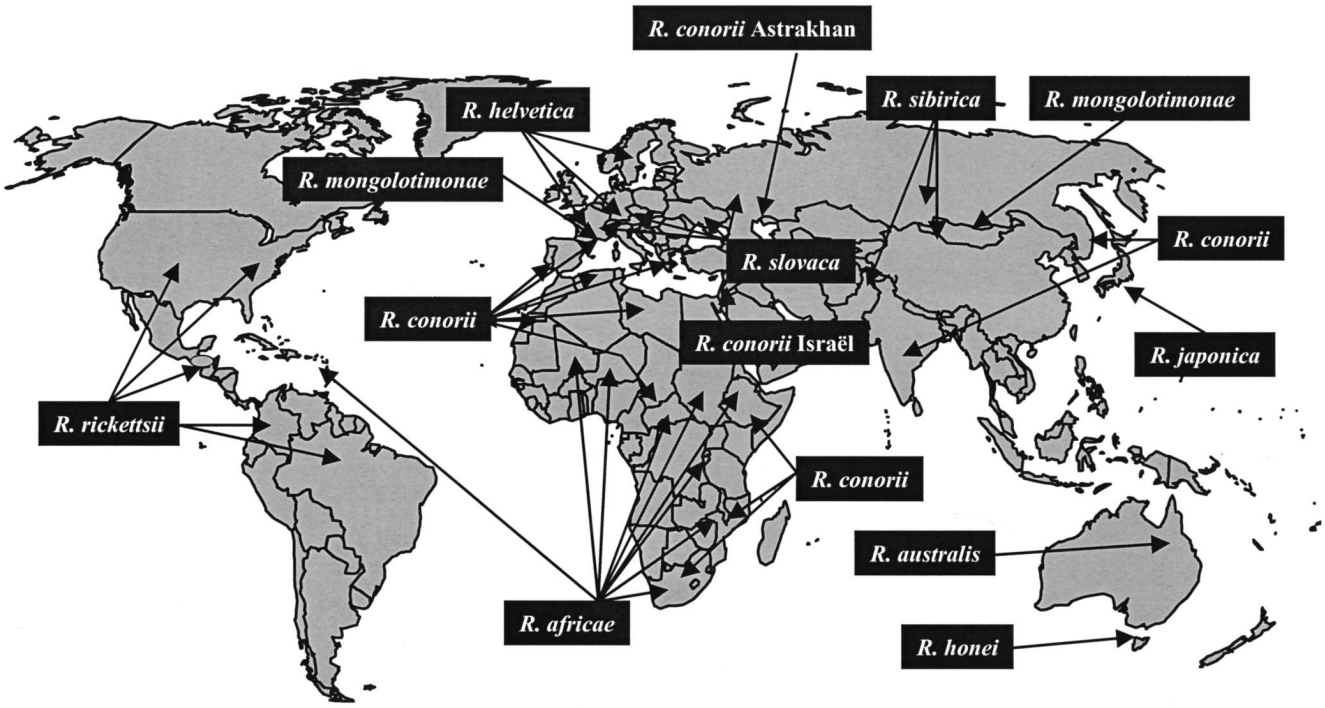

Figure 11. Geographic distribution of tickborne pathogenic rickettsiae

determined only by isolating the organisms from patients with signs of disease. There are as yet no ways to determine if a bacteria is not pathogenic in humans.

Of the 10 known tickborne-associated bacteria of the *B. burgdorferi* sensu lato complex, the 3 species implicated in human diseases (*B. burgdorferi* sensu stricto, *B. garinii,* and *B. afzelii*) are the organisms most frequently isolated from ticks. These different species may be found in a large variety of animal hosts over large areas [47]. In the United States, *R. rickettsii,* which is one of the more pathogenic spotted fever group rickettsiae, has also been associated with a large number of tick species from several genera *(Dermacentor, Amblyomma,* and possibly *Rhipicephalus, Haemaphysalis,* and *Ixodes)* and may cause infections in a large number of vertebrate hosts [11]. The pathogenicity of arthropod-borne bacteria may then be associated with an increased diffusion of the organisms and the resultant increased risk of exposure to them. From an evolutionary aspect, one may suppose that a bacterium may be a pathogen for humans if it is pathogenic for a number of other distantly related mammals—if it is pathogenic for mice, guinea pigs, sheep, and monkeys, it will be probably pathogenic for humans. Furthermore, if it is pathogenic for mammals that are closely related to humans (e.g., monkeys), one may hypothesize it is also pathogenic for humans. If the pathogenicity of a bacterium has been studied only in mammals that are distantly related to humans, such studies should not be used to predict the pathogenicity of the organism in humans.

The pathogenicity in humans of most bacteria, particularly the rickettsiae, that have only been isolated from ticks around the world has yet to be determined [11]. It should be remembered, however, that bacteria such as *C. burnetii, Rickettsia slovaca,* and *R. africae* were first isolated from ticks and considered "nonpathogenic rickettsiae" until they were found to be pathogenic in humans [11]. All of the bacteria that have been isolated to date from ticks only, particularly those from highly anthropophilic species, should be considered as potential human pathogens.

COLLECTION, IDENTIFICATION, AND PRESERVATION OF TICKS

Ticks can be collected from their microhabitats and in temperate countries, "flagging" or "dragging" is the standard method for collecting active ticks on vegetation [1, 23]. A cloth or blanket is drawn over the vegetation. Ticks become attached to it and can be removed periodically (figure 16). As stated above, carbon dioxide emitted by animals is an important stimulus that guides ticks to their hosts, and carbon dioxide traps then can be used to both attract and collect ticks. Dry ice is the most convenient source of CO_2, and a 1-kg block is usually placed in a perforated container in the middle of a square of cloth that is placed in the area to be sampled. Ticks are attracted to the CO_2 and become trapped in the cloth as they approach the perforated container [23]. Finally, ticks can be collected

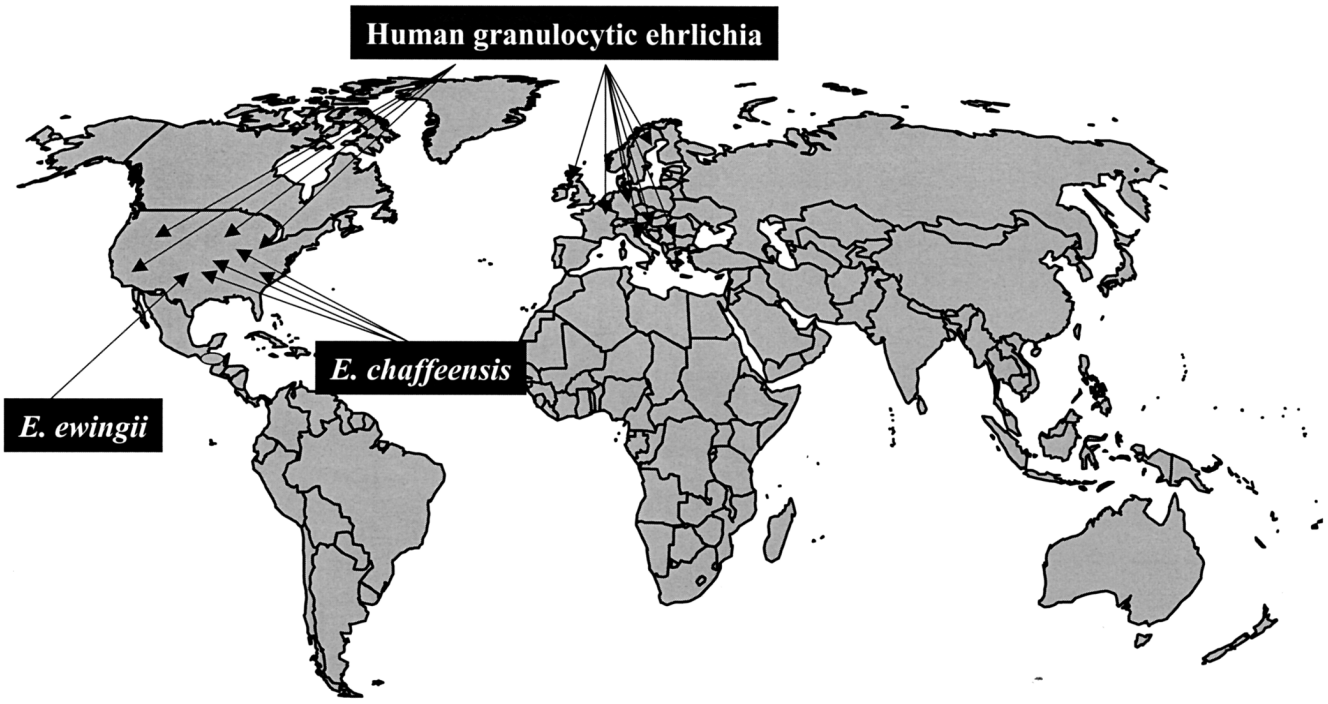

Figure 12. Geographic distribution of tickborne ehrlichiae that are pathogenic for humans

from the nests and burrows of their hosts or from the hosts themselves. Each species has a preferred feeding site; on mammals, these are generally the ears, head, legs, and/or anogenital region. Ticks may be pulled from their hosts with forceps that are used to grasp the capitulum firmly and close to the skin.

Ticks can be identified according to the family, genus, and species level by use of the numerous taxonomic keys available for ticks from different regions of the world (e.g., [23, 58–60]). Immature stages are often difficult to identify, and it may be necessary to allow ticks to molt to the adult stage before definitive identification is possible. Molecular methods are currently being developed to identify ticks, and in the future it is expected that such methods will be used more widely and particularly for the differentiation of closely related species [61]. Depending on the studies to be done, ticks can be kept in the laboratory, preserved in 70%–80% ethanol or 10% formalin, frozen at −20 or −80°C or kept alive at 20–25°C and 85% relative humidity, which are the optimal conditions for molting and oviposition. Ticks may be kept for up to 3 months at 0 to 5°C, in the dark, and at 95% relative humidity before they require feeding [23].

DETECTION AND ISOLATION OF BACTERIA FROM TICKS

Bacteria may invade and multiply in all of the organs and fluids of ticks, and their detection in the hemolymph or salivary glands is relatively simple. The hemolymph test [62] is done on live ticks when the distal portion of a leg is amputated. Hemolymph that appears at the site can be smeared onto a microscope slide, stained, and examined for the presence of bacteria (figure 17). Ticks from which leg segments have been amputated can be kept alive and used for subsequent hemolymph tests or for other experiments. Impression smears may be made of salivary glands or ovaries removed from dissected ticks, or the organs may be used for histologic testing. Although the spotted fever group rickettsiae, *C. burnetii,* and ehrlichiae stain poorly, if at all, with Gram's staining, they are readily observed as short rods with Gimenez or Giemsa staining [63]. Borreliae may be seen by use of darkfield microscopy, are gram-negative, and stain well with various aniline dyes. Immunodetection methods may also be used to detect organisms in hemolymph or organ smears. Slides are air-dried and fixed in acetone before being treated with polyclonal or monoclonal antibodies conjugated with immunofluorescent labels, as has been described for the rickettsiae [64, 65]. An antigen-capture EIA has also been used for the detection of these bacteria in ticks [66]. Immunodetection methods are also available for the detection of *C. burnetii* [67], ehrlichiae [68, 69], *F. tularensis* [70], and borreliae [71].

At the present time, rickettsiae are most commonly isolated by means of cell culture systems, and the centrifugation shell-vial technique with human embryonic lung fibroblasts is the method of choice in our laboratory [11, 72]. A drop of hemolymph from a surface-sterilized tick is inoculated onto confluent monolayers in the shell vials, and ticks are kept alive in case the

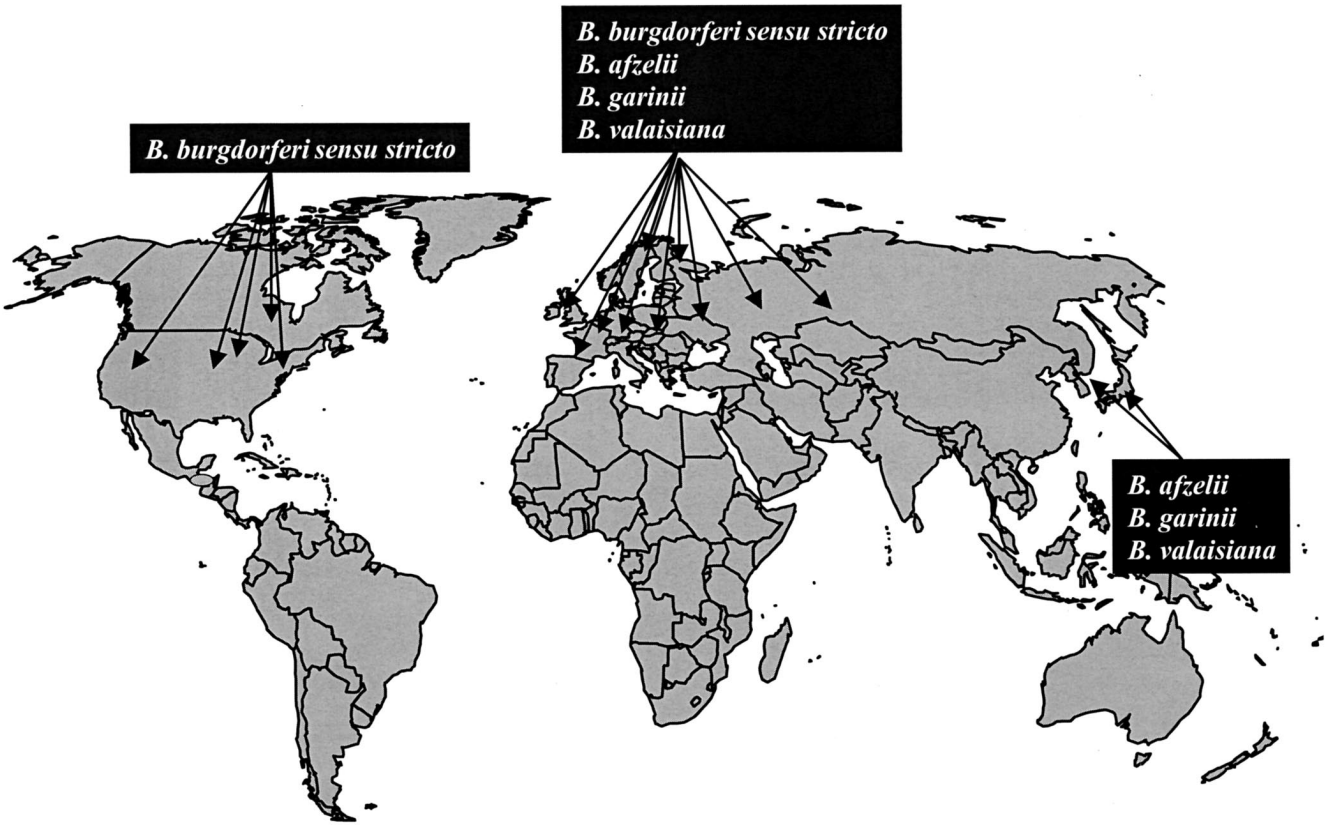

Figure 13. Geographic distribution of pathogenic bacteria of *Borrelia burgdorferi* complex (pathogenic role of *Borrelia valaisiana* is highly suspected)

procedure needs to be repeated. If only dead ticks are available or if the ticks are frozen, they are disinfected with iodinated alcohol, rinsed in sterile water, and dried on sterile paper in a laminar flow cabinet. The tick is macerated in 1 mL of cell culture medium and inoculated into shell vials. Cotrimoxazole, an antimicrobial agent that is not effective against rickettsiae, and amphotericin B are added to the media to prevent contamination of the cultures with gut bacteria and fungi of the crushed ticks [49, 73]. With these drugs, however, contamination of the tissue cultures with *Mycoplasma* species is still possible. *C. burnetii,* ehrlichiae, and *F. tularensis* can also be isolated by use of the shell vial technique [67–69, 74, 75], whereas borreliae are best isolated and grown on the specialized growth medium BSK II, which is a complex broth that contains long-chain fatty acids, bovine serum albumin, and rabbit serum [76].

The advent of molecular methods, such as sequence analysis of PCR products, has enabled the rapid detection and identification of tickborne pathogens [77, 78]. Such techniques may be used to screen large numbers of ticks during surveys or single ticks that have been collected from patients suspected of having tickborne diseases. Identification strategies based on the DNA sequences of a number of genes have been described for rickettsiae [11, 72, 79, 80], ehrlichiae [81–86], borreliae [45, 86–90], *F. tularensis* [91–94], and *C. burnetii* [95]. Also, species-, group-, and genus-specific primers have been described for the above organisms (table 4), and it is relatively simple for a laboratory to detect and identify bacteria in ticks if there are facilities for molecular methods and access to sequence data. With the arsenal of techniques now available to detect and/or isolate bacteria from ticks, we propose the strategy presented in table 5 for the investigation of tickborne bacterial pathogens.

TICKBORNE BACTERIAL DISEASES IN HUMANS

Ticks have been described as vectors of human bacterial diseases (e.g., spotted fever rickettsioses, recurrent fever borrelioses, Q fever, tularemia) since the beginning of the 20th century. Their major impact on public health in the United States and in Europe was recognized with the emergence of *B. burgdorferi* as the etiologic agent of Lyme disease in 1982. Since then, 8 newly recognized tickborne rickettsioses have been described [11, 96, 97]; 3 species of ehrlichiae, which are bacteria previously thought to be only animal pathogens, have been implicated in human diseases [12, 98]; 3 pathogenic species of the *B. burgdorferi* complex have been well described, another has been recently reported, and yet another is suspected (table 6) [99, 100].

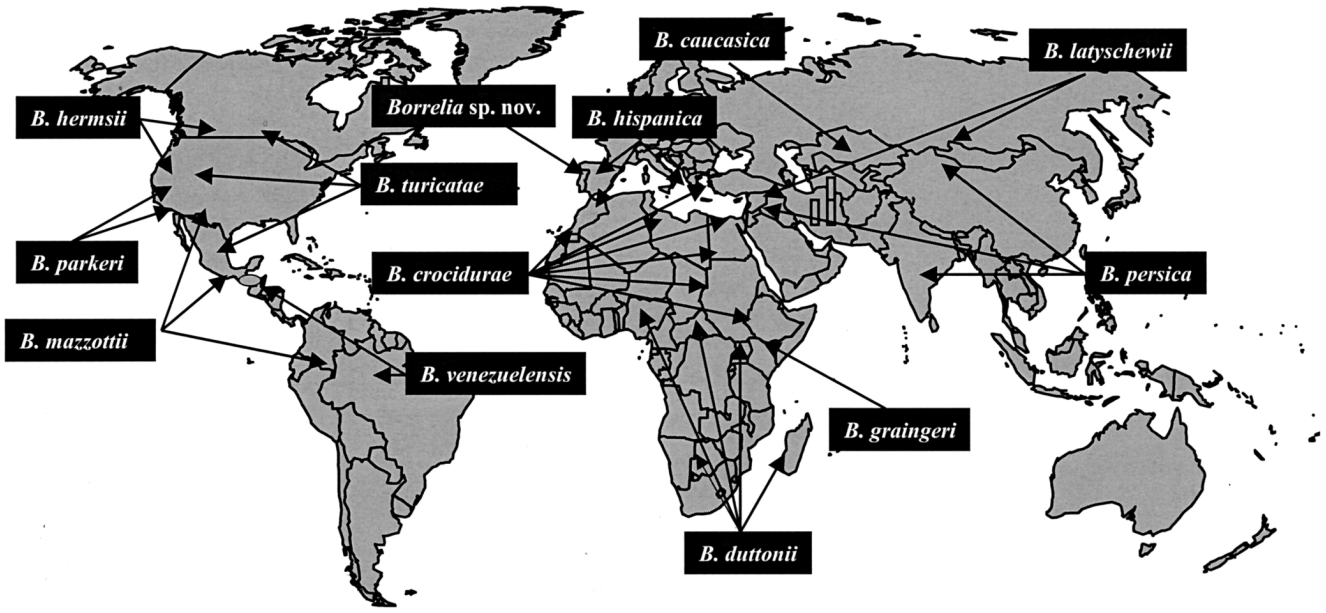

Figure 14. Geographic distribution of tickborne relapsing fever borreliae

Currently, ticks are considered to be second only to mosquitoes in importance as vectors of human infectious diseases in the world, but the first in importance in North America. As a result, a number of centers for tickborne diseases have been opened in the United States and in Europe. An example is the center for tickborne diseases in Hungary, which is visited by thousands of patients each year [101]. Moreover, some tickborne disease syndromes remain unexplained, such as tickborne lymphadenopathy [101] and Southern tick-associated rash illness, currently a topic of interest in the United States [102].

Rickettsioses

Rickettsioses are caused by obligate intracellular bacteria belonging to the genus *Rickettsia* and are one of the oldest known arthropod-borne diseases [11]. Rocky Mountain spotted fever due to *R. rickettsii* was first described as a new clinical entity by Maxey in 1899, and Ricketts showed the Rocky Mountain wood tick, *D. andersoni,* to be a vector of the organism in 1910 [5]. Mediterranean spotted fever, also known as boutonneuse fever, was described in 1910 in Tunis [6]. The inoculation eschar at the site of the tick bite was reported in Marseille in 1925, and the causative agent, *R. conorii,* was described by Brumpt in 1932 [11]. The tickborne rickettsioses are a model of emerging diseases, because before 1974, only 4 tickborne rickettsioses were recognized, with only 1 pathogenic tickborne spotted fever group rickettsia identified in the Americas *(R. rickettsii);* Europe, southwest Asia, and Africa *(R. conorii);* Siberia and western Russia *(R. sibirica);* and Australia *(R. australis)* [11].

More recently, the development of new techniques, including the shell vial assay for isolation of organisms and molecular methods for their characterization, has led to a further 9 pathogenic tickborne rickettsiae being described, 7 since 1991 [11, 96, 97] (figure 11). Some of these organisms were found in areas where spotted fever group rickettsiae were not known to occur—for example, in Japan, Tasmania, and Astrakhan (former USSR) [11]. Other organisms were reported from areas where *R. conorii* was thought to be the only pathogenic rickettsia, mainly *R. africae* in Africa [103, 104] and "*Rickettsia mongolotimonae*" in the south of France [105, 106].

Finally, the study of atypical cases in areas of endemicity led to the description of new clinical syndromes caused by organisms that include *R. slovaca* [96] and *Rickettsia helvetica* [97, 107] in Europe. Numerous rickettsiae of unknown pathogenicity have been isolated or detected in ticks in recent years [11]. Their role in human disease has yet to be determined, however, and they may represent agents of new rickettsial diseases to be described in the future.

Bacteriology. Bacteria of the genus *Rickettsia* are strict, intracellular, gram-negative, short rods that retain basic fuchsin when stained by the method of Gimenez [11]. Their growth in the laboratory requires living host cells (animal models, embryonated eggs) or cell cultures (Vero, L929, human embryonic lung, or MRC5 cells). Among the rickettsial protein antigens, 2 high-molecular-mass surface proteins (rOmpA and rOmpB) contain species-specific epitopes [11]. The lipopolysaccharide layer of the organisms contains highly immunogenic antigens that are strongly cross-reactive with all members of the subgroup and other bacteria.

Comparative microimmunofluorescence remains the reference method for identification of rickettsiae, and the rOmpA

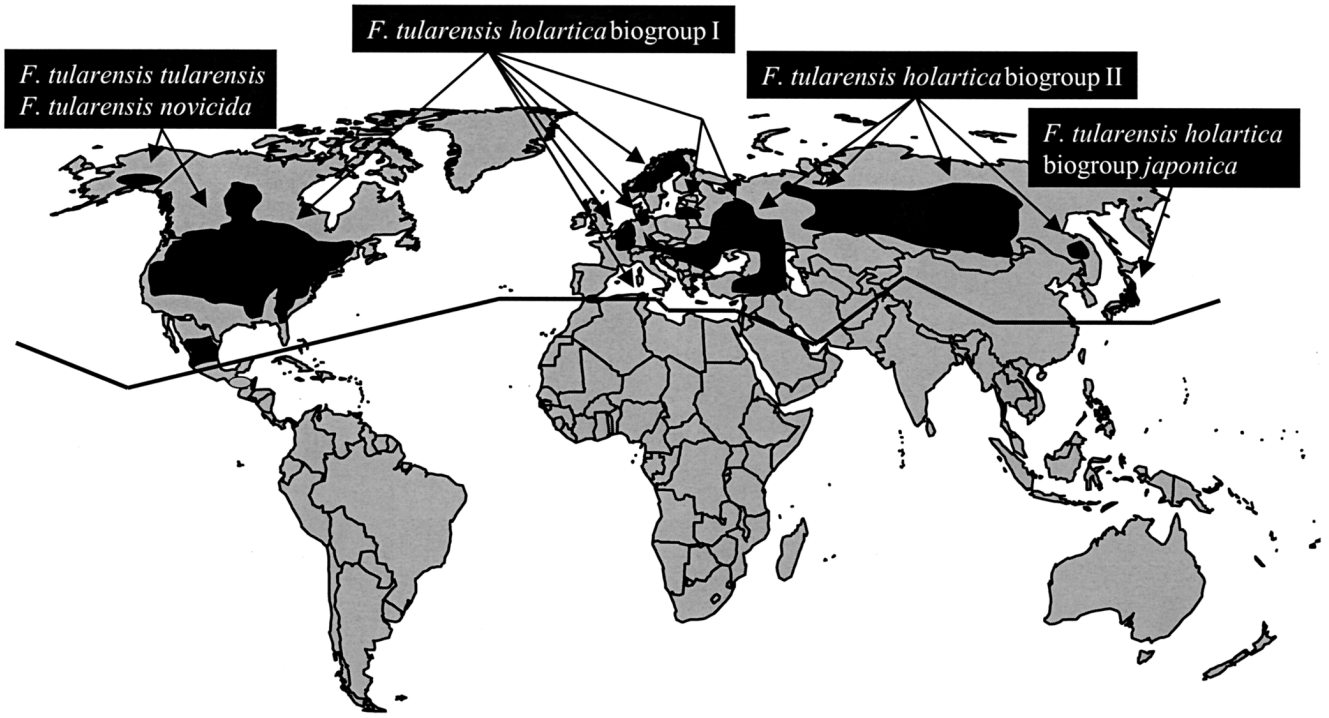

Figure 15. Southern limit and geographic distribution of tularemia. *F., Francisella*

and rOmpB proteins provide the basis for rickettsial serotyping [11, 79]. Although protein analyses by means of SDS-PAGE or species-specific monoclonal antibodies have also been used to identify rickettsial species, sequence analysis of PCR product is currently the most rapid, convenient, and sensitive technique for the identification of rickettsiae [11, 79]. To date, sequences of several rickettsial genes have been used to detect and identify rickettsiae in various samples, including blood samples, skin biopsies, and ticks [11].

Vectors and reservoirs. Rickettsiae infect and multiply in almost all organs of ticks, in particular the salivary glands, which enables the rickettsiae to be transmitted to vertebrate hosts during feeding. Only ixodid ticks have been implicated as diseases vectors (table 3), and rickettsiae are known to be maintained in these ticks by transstadial and transovarial transmission [11]. Because ticks are the main reservoirs of spotted fever group rickettsiae, the distribution of the spotted fever group rickettsioses are determined by the distribution of the ticks. Although many animals (mammals and birds) are susceptible to rickettsial infections and may develop rickettsemia and clinical signs, the role of vertebrates as reservoirs in maintaining zoonotic foci has yet to be determined [11]. Humans do not appear to be reservoirs for tickborne rickettsiae, because they are only occasionally parasitized by ticks and they are rickettsemic for only short periods [11].

Clinical characteristics, diagnosis, and treatment. The main clinical signs vary depending on the rickettsial species that are involved (table 7), but they generally begin 6–10 days after a tick bite and include fever, headache, muscle pain, rash, local lymphadenopathy, and 1 or several inoculation eschars [11]. Thrombocytopenia and leukocyte count abnormalities are common, and hepatic enzyme levels are often elevated. The specific laboratory methods for the diagnosis of infections have been reviewed recently [79]. They include serological tests (in particular immunofluorescence), immunodetection of organisms in blood samples and tissues, isolation of organisms in cell culture, and identification of organisms by molecular-based methods. Empirical treatment is usually started before laboratory confirmation diagnosis, with the treatment of choice being doxycycline, 200 mg/day, given for 1–7 days, depending on the severity of the disease. Other antibiotics have also been found to be effective [108].

Ehrlichioses

Although ehrlichiae were known for a long time as veterinary pathogens, they have recently been recognized as emerging tickborne pathogens in humans [68, 69]. Currently, 3 ehrlichiae that are pathogenic for humans have been reported (figure 12). The first human case of monocytic ehrlichiosis was described in 1987 and was assumed to be due to *Ehrlichia canis,* the agent of canine ehrlichiosis [109]. The actual agent, *Ehrlichia chaffeensis,* was isolated in 1991 in the United States [110], and to date the organism has yet to be isolated elsewhere [12]. HGE

Figure 16. Collection of ticks by means of blanket dragging

was first described in the United States in 1994 [82] and has subsequently been shown to occur in Europe [111–114]. The causative organism is currently known as the HGE agent; it is very closely related to *Ehrlichia equi* and *Ehrlichia phagocytophila* (pathogens of horses and ruminants, respectively). In 1999, it was found that *Ehrlichia ewingii,* the agent of canine granulocytic ehrlichiosis, could also cause disease in humans [98]. The development of molecular tools is greatly increasing our understanding of ehrlichiae and their association with ticks and their hosts, and it is likely that additional tickborne ehrlichioses will be discovered in the future.

Bacteriology. Ehrlichiae are obligate intracellular small gram-negative cocci that stain dark blue to purple with use of Romanovsky's stains, including Wright's and Giemsa stains [115]. In vivo, ehrlichiae mainly infect cells of bone marrow origin, in particular leukocytes, where they occur within membrane-bound vacuoles. The intraphagosomal ehrlichiae divide by binary fission to produce a cluster of organisms called a morula. Both the HGE agent and *E. equi* have recently been cultivated by use of promyelocyte cell lines and tick cell lines [115–117]. Sequence analyses of the 16S rRNA gene and the groESL heat-shock operon indicate that there are 4 genogroups of ehrlichiae [68, 69]. The species that have been implicated in human diseases in the *E. canis* genogroup are *E. ewingii* and *E. chaffeensis.* In the *E. phagocytophila* genogroup, the HGE agent, an ehrlichia very closely related or identical to *E. equi* and *E. phagocytophila,* is known to be a human pathogen.

Vectors and reservoirs. The tick vectors (which are not reservoirs) of human ehrlichioses belong to the genus *Ixodes* and are shown in table 3. The suspected reservoir host of *E. chaffeensis,* the agent of human monocytic ehrlichiosis, is the white-tailed deer *Odocoileus virginianus* [118], and although domestic dogs may become infected with *E. chaffeensis,* their precise role as reservoirs of infection has yet to be determined [119]. Small mammals, in particular white-footed mice *(Peromyscus leucopus)* have been implicated as reservoir hosts of the HGE agent in the eastern and midwestern United States, and a role for deer as reservoirs has been suggested [120]. In Europe the reservoirs of the HGE agent are unknown.

Clinical characteristics, diagnosis, and treatment. Human ehrlichioses present most commonly as undifferentiated illnesses with fever that occur in summer or spring [121]. Other clinical signs include headache, myalgia, anorexia, and digestive, respiratory, and neurological disturbances. Rashes are observed rarely [68, 69, 121], and more than half of patients require hospitalization during the disease, which often lasts >3 weeks. Ehrlichioses may be severe and even fatal, particularly in patients with underlying immunosuppression. Leukopenia (lymphopenia followed by neutropenia), thrombocytopenia, and elevated liver enzymes are frequent laboratory abnormalities. Most patients report exposure to ticks or tick bites up to 3 weeks before the onset of the disease [121, 122].

A specific diagnosis of ehrlichiosis may be made by observing morulae in leukocytes after Wright's or Giemsa staining of blood smears. Specific diagnoses may also be made by means of serological testing (immunofluorescence assays being the reference method, in spite of cross reactions [69]) on paired blood samples, by isolation of organisms in cell culture systems, or by molecular methods [74, 123, 124] (table 4). Tetracyclines appear to be very effective in treating ehrlichioses, and *E. chaffeensis* has been found to also be susceptible to rifampin but

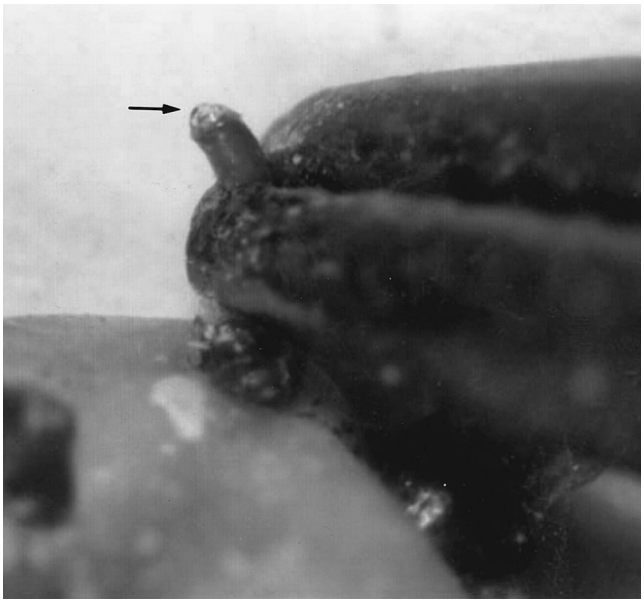

Figure 17. The hemolymph test, which involves severing the leg and collecting a drop of hemolymph, which can be spread onto a slide, stained, and examined for the presence of bacteria.

Table 4. Oligonucleotide primers useful to amplify DNA of human pathogenic bacteria from ticks by use of PCR.

Bacteria species, primer pair sequence (5′ to 3′)	Target gene	Product size, bp	Reference
Rickettsia			
All			
RpCS.877p:GGGGGCCTGCTCACGGCGG	*gltA* (citrate synthase)	381	[72]
RpCS.1258n: ATTGCAAAAAGTACAGTGAACA			
All except *R. helvetica, R. australis, R. bellii,*[a] *R. canadensis*[a]			
Rr190.70p: ATGGCGAATATTTCTCCAAAA	*ompA*	532	
Rr190.602n: AGTGCAGCATTCGCTCCCCCT			
Rr190.70p: ATGGCGAATATTTCTCCAAAA		632	[49]
Rr190.701n: GTTCCGTTAATGGCAGCATCT			
All except *R. helvetica, R. bellii,*[a] *R. massiliae*[a]			
BG1-21: GGCAATTAATATCGCTGACGG	*ompB*	650	[80]
BG2−20: GCATCTGCACTAGCACTTTC			
Ehrlichia			
E. chaffeensis			
HE1:CAATTGCTTATAACCTTTTGGTTATAAAT	rRNA 16S	389	[84]
HE3: ATAGGGAAGATAATGACGGTACCTATA			
HGE agent (*E. phagocytophila* genogroup)			
GE9f: AACGGATTATTCTTTATAGCTTGCT	rRNA 16S	919	[81–83]
GE10r: GGAGATTAGATCCTTCTTAACGGAA			
LA 1: CGTTCAGCCATCATTGTGAC	*epank1*	153	[85]
LA6: GAGAGATGCTTATGGTAAGAC			
All known ehrlichiae (broad-spectrum primers)			
EHR16SD: GGTACCYACAGAAGAAGTCC	16S rRNA	345	[163]
EHR16SR: TAGCACTCATCGTTTACAGC			
16F8FE:GGAATTCAGAGTTGGATCMTGGYTCAG	16S rRNA	448	[86]
B-GA1B:CGGGATCCCGAGTTTGCCGGGACTTCTTCT			
Borrelia			
B. burgdorferi			
OspA2: GTTTTGTAATTTCAACTGCTGACC	*ospA*	158	[87]
OspA4: CTGCAGCTTGGAATTCAGGCACTTC			
fla outer 1:AAGTAGAAAAAGTCTTAGTAAGAATGAAGGA	*fla*	611	[88]
fla outer 2:AATTGCATACTCAGTACTATTCTTTATAGAT			
fla inner 1:CACATATTCAGATGCAGACAGAGGTTCTA	*fla*	390	[88]
fla inner 2:GAAGGTGCTGTAGCAGGTGCTGGCTGT			
5SCB: GAGAGTAGGTTATTGCCAGGG	23S-5S rRNA spacer	226	[86]
23SN2: ACCATAGACTCTTATTACTTTGACCA			
OspC3: AAGTGC(AG)ATATTAATGACTTTA	*ospC*	614	[45]
OspC4: TTTTTTGGACTTTCTGCCACA			
16S rRNA LDF: ATGCACACTTGGTGTTAACTA	16S rRNA	357	[89]
16S rRNA LDR: GACTTATCACCGGCAGTCTTA			
Relapsing fever borreliae			
BBRNA8: ACGCTGGCAGTGAGTCTTA	*rrs*	669	90
BBRNA14: ATATCAACAGATTCCACCC			
Francisella tularensis			
FT393: 5′-ATGGCGAGTGATACTGCTTG	TUL4	250	[93]
FT642: 5′-GCATCATCAGAGCCACCTAA			

(*continued*)

Table 4. Continued

Bacteria species, primer pair sequence (5′ to 3′)	Target gene	Product size, bp	Reference
TUL4-435: 5′-GCTGTATCATCATTTAATAAACTGCTG	TUL4	400	[94]
TUL4-863: 5′-TTGGGAAGCTTGTATCATGGCACT			
P1: 5′-TGGCGAGTGATACTGCTTG	TUL4	211	[91]
P2: 5′-TAGGATCCCATTAGCTGTCCACTTACC			
P2: 5′-TAGGATCCCATTAGCTGTCCACTTACC	TUL4	347	[91]
P3: 5′-GGAATTCGTTAGGTGGCTCTGATGAT			
P1: 5′-TGGCGAGTGATACTGCTTG	TUL4	568	[91]
P4: 5′-CGCTAAACCTGCGATTGAT			
Coxiella burnetii	htpAB-associated repetitive element	257	[95]
CB-1: 5′-ACTCAACGCACTGGAACCGC			
CB-2: 5′-TAGCTGAAGCCAATTCGCC			

NOTE. HGE, human granulocytic ehrlichiosis.

[a] Rickettsiae of unknown pathogenicity.

resistant to erythromycin, cotrimoxazole, penicillin, and quinolones [125]. The optimal duration of treatment has yet to be determined, but it is recommended that tetracycline treatment be continued for at least 7 days or for 3–5 days after defervescence.

Lyme Borreliosis

Lyme borreliosis was first recognized in 1975, when there was a cluster of cases of juvenile arthritis with an unusually high incidence of erythematous rash in the town of Old Lyme, Connecticut [9, 10, 126]. A disease with similar signs had been observed in Europe ~100 years previously, with the rash being named "erythema chronicum migrans" (currently "erythema migrans"). In 1982, the etiologic agent of the disease was found by Burgdorfer and colleagues and named *B. burgdorferi* in 1984 [9, 10]. The tick *I. scapularis* (formerly *Ixodes dammini*) was subsequently incriminated as a vector of the organism, and Lyme borreliosis is now the most common vectorborne disease in the United States, with >16,000 cases reported in 1996 [127]. The disease also occurs widely in Europe, the former Soviet Union, China, Japan, Australia, and perhaps in North Africa [128].

Bacteriology. *Borrelia* species are gram-negative microaerophilic mobile spirochetes. Among borreliae, *B. burgdorferi* is the longest and narrowest (20–30 μm × 0.2– 0.3 μm) and has the least flagellae. The organism contains several membrane lipoproteins, including OspA through OspF. Biosafety level P2 facilities are required for in vitro culture of the organisms [126]. Growth is optimal at 33°C in a complex liquid medium called BSK II medium but also occurs on various solid media. Lyme borreliosis is caused by 3 of the 10 described species in the *B. burgdorferi* sensu lato complex: *B. burgdorferi* sensu stricto, *B. garinii,* and *B. afzelii* [47, 71, 126]. A novel isolate (A14S) of the *B. burgdorferi* sensu lato complex has recently been made from a skin biopsy of a patient with erythema chronicum migrans in the Netherlands [100]. A pathogenic role for *Borrelia valaisiana,* which occurs in Europe and Asia, is suspected [99, 129].

Vectors and reservoirs. Several ixodid ticks have been reported to be vectors of Lyme borreliosis (table 3). Competent tick vectors acquire *B. burgdorferi* while feeding on infected reservoir hosts. The organisms remain inactive in the midguts of the ticks while they molt, and in the next developmental stage the spirochetes disseminate rapidly (within 3 days) to all other body organs, including the salivary glands [9]. Regurgitations during feeding may also result in transmission of the organisms to susceptible hosts [9]. Transovarial transmission in ticks is rare, and ticks are thus not considered to be reservoirs of *B. burgdorferi.*

A number of mammals, in particular rodents, have been demonstrated to be reservoir hosts for *B. burgdorferi.* The white-footed mouse *(P. leucopus)* is regarded to be the most important reservoir of *B. burgdorferi* sensu stricto in northeastern America. Although the white-tailed deer *(O. virginianus)* is an important host for adult *I. scapularis,* it has not been found to be a reservoir of *B. burgdorferi* [9, 126]. In Europe, *Apodemus* species are considered to be the most important reservoirs of the bacteria [130]. Various species of birds, especially ground-foraging birds (e.g., thrushes, blackbirds, robins, and pheasants), have also been incriminated as reservoirs. Although each pathogenic species of *Borrelia* has been found to be associated with birds in Europe, *B. afzelii* is thought to be perpetuated in rodents and *B. garinii* in avian reservoirs host [47].

Clinical characteristics, diagnosis, and treatment. The course of Lyme borreliosis is thought to proceed in 3 stages [9, 71, 126]. In the first stage, in which the infection is localized, erythema migrans occurs, which is the hallmark of the disease, and appears initially as a red macule or papule at the site of a

Table 5. Strategy of investigation to detect and/or isolate bacteria from ticks.

Step	Procedure
1	Identification of ticks to species level
2	Detection of bacteria in ticks by use of staining tests (hemolymph test for alive ticks, salivary glands if ticks were frozen) or PCR-based methods (using one-half of tick, other half being kept frozen); PCR may also be used on ticks positive by staining
3	Sequencing of PCR-amplified fragment and comparison with sequences available in sequence database
4	If there is 100% similarity between obtained and corresponding sequence of known bacterium, identification is obtained
5	If obtained sequence appears different from all corresponding sequences available, bacterium is probably new strain and has to be isolated and characterized by use of second half of tick that had been frozen

tick bite 7–10 days earlier. It is recognized in ~90% of patients with objective evidence of infection with *B. burgdorferi* [71]. Although a history of tick bite is reported in <30% of the cases in the United States, it is common in cases from Eurasia (64%) [71]. The erythema migrans lesion later expands over days or weeks, and central clearing occurs frequently.

In stage 2, when the infection becomes disseminated, there are multiple secondary annular lesions elsewhere in the skin, which are generally smaller and which expand to a lesser degree. The erythema migrans and secondary skin lesions usually resolve within 3–4 weeks, but neurological, cardiac, ocular, and rheumatological manifestations may also occur several weeks to months after the onset of the disease, during the disseminated stage of infection (table 8). In the third stage of the disease, the late or persistent stage of infection, which occurs months to years after the onset of the illness, there may be skin abnormalities (acrodermatitis chronica atrophicans), intermittent attacks of joint swelling and pain, fatigue, and ocular and neurological signs (including chronic axonal neuropathy, encephalopathy, or encephalomyelitis) [71].

Differences in clinical manifestations of the disease in patients from the United States and those from Eurasia have been suggested to be due to differences in the organotropism of the *Borrelia* species in these areas. *B. afzelii* is associated with erythema migrans and a milder disease than that caused by *B. burgdorferi* sensu stricto. It is also the principal cause of acrodermatitis chronica atrophicans and a cause of borrelial lymphocytoma. Infections with *B. garinii* are more frequently associated with neurological abnormalities, whereas those with *B. burgdorferi* sensu stricto are more often associated with rheumatological disorders [44, 71, 99, 126].

The diagnosis of Lyme borreliosis is made on the basis of clinical and epidemiological findings and serological testing. Specific antibodies to *Borrelia* can be detected by use of ELISA or immunofluorescence tests, although positive and equivocal test results must be confirmed by means of Western blotting [71, 131, 132]. In the early stages of infection, serological testing lacks sensitivity and specificity and can be used only to support a clinical suspicion of the disease [131, 132]. Bacteria may be visualized by direct examination of blood or CSF by means of darkfield microscopy, but this diagnostic technique is not specific or sensitive. The bacteria may also be demonstrated in histological sections of lesions by use of Warthin-Starry strain. By use of BSK II specialized media, *B. burgdorferi* may be isolated from skin biopsy specimens from 57%–86% of patients who present with erythema migrans and also from heparinized blood, joint fluid, or CSF [133].

However, diagnosis by isolation of organisms is time-consuming. Recently, PCR has been used to amplify *B. burgdorferi* DNA (*osp*A and 16S rRNA genes) in blood, CSF, skin, synovial fluid, and urine samples from infected patients [71, 126, 134] (table 4). In the early stages of Lyme disease, doxycycline (200 mg/day, given for 20–30 days) is the recommended treatment. *B. burgdorferi* is, however, also susceptible to β-lactams, including ceftriaxone, which is used to treat neurological forms of the disease [71]. The vaccine against Lyme disease is now available [135, 136]. It is derived from a lipidated outer surface protein of *B. burgdorferi* and works inside the tick itself (antibodies ingested during the blood meal by the tick can neutralize the bacteria in the tick gut), thus preventing transmission of the spirochete [136].

Tickborne Relapsing Fever

Relapsing fever has been recognized since ancient times and was recognized to be a tick-transmitted disease in 1905, when Dutton and Todd demonstrated spirochetes in *O. moubata* ticks in West Africa [137, 138]. Subsequently, it has been found that tickborne relapsing fever is caused by at least 13 *Borrelia* species that are transmitted worldwide to people by soft ticks of the genus *Ornithodoros* (tables 2 and 3; figure 14) [9]. Some species, including *B. duttonii* and *Borrelia crocidurae,* have been cultured only recently [76, 90], and a new species causing relapsing fever was recently reported in Spain in 1996 [139].

Bacteriology. Borrelia that cause relapsing fever are generally similar in morphology and physiology to *B. burgdorferi,* the agent of Lyme borreliosis. Tickborne relapsing fever borreliae are capable of antigenic variation. Recent experiments have shown that *Borrelia hermsii* reversibly changes its major outer surface protein when it is transmitted from ticks to the blood of mammals [140]. In humans, borreliae are sequestered in internal organs during the afebrile periods and reemerge antigenically modified during febrile episodes.

Vectors and reservoirs. Each *Borrelia* species that causes relapsing fever appears to be specific to its tick vector (table 3). In ticks, the borreliae invade all organs, including the sal-

Table 6. New bacterial pathogens transmitted by hard ticks and recognized since 1982.

Geographic location	Bacterial pathogen, by year recognized		Genus of principal associated tick(s)
	Before 1982	By 2000	
Americas	*Francisella tularensis*	*F. tularensis*	Diverse
	Rickettsia rickettsii	*R. rickettsii*	*Amblyomma, Dermacentor,* and possibly *Haemaphysalis, Ixodes*
	Coxiella burnetii	*C. burnetii*	Diverse[a]
		Rickettsia africae	*Amblyomma*
		Borrelia burgdorferi sensu stricto	*Ixodes*
		Ehrlichia chaffeensis	*Amblyomma*
		Human granulocytic ehrlichiosis agent	*Ixodes*
		Ehrlichia ewingii	*Amblyomma*
Europe	*F. tularensis*	*F. tularensis*	Diverse
	Rickettsia conorii	*R. conorii*	*Rhipicephalus*
	C. burnetii	*C. burnetii*	Diverse[a]
		Rickettsia slovaca	*Dermacentor*
		Rickettsia helvetica	*Ixodes*
		"Rickettsia mongolotimonae"	*Hyalomma*
		R. conorii Israel	*Rhipicephalus*
		R. conorii Astrakhan	*Rhipicephalus*
		Human granulocytic ehrlichiosis agent	*Ixodes*
		B. burgdorferi sensu stricto	*Ixodes*
		Borrelia garinii	*Ixodes*
		Borrelia afzelii	*Ixodes*
		B. burgdorferi A14S	?[c]
		Borrelia valaisiana[b]	*Ixodes*
Asia	*F. tularensis*	*F. tularensis*	Diverse
	C. burnetii	*C. burnetii*	Diverse[a]
	R. conorii	*R. conorii*	*Rhipicephalus*
	Rickettsia sibirica	*R. sibirica*	*Dermacentor, Haemaphysalis*
		R. conorii Israel	*Rhipicephalus*
		"R. mongolotimonae"	*Hyalomma*
		Rickettsia japonica	*Ixodes, Dermacentor, Haemaphysalis*
		B. burgdorferi sensu stricto	*Ixodes*
		B. afzelii	*Ixodes*
		B. garinii	*Ixodes*
		B. valaisiana[b]	*Ixodes*
Africa	*R. conorii*	*R. conorii*	*Rhipicephalus*
	C. burnetii	*C. burnetii*	Diverse[a]
		R. africae	*Amblyomma*
		Borrelia burgdorferi sensu lato (North Africa)	*Ixodes*
Australia	*Rickettsia australis*	*R. australis*	*Ixodes*
	C. burnetii	*C. burnetii*	Diverse[a]
		Rickettsia honei	?

[a] Role of ticks in transmission of *C. burnetii* to humans is low.
[b] Pathogenicity of *B. valaisiana* is suspected.
[c] Has been isolated from humans only.

Table 7. Tickborne bacteria pathogenic to humans with focus on cutaneous manifestations.

	Manifestations, presence or % of patients affected							
				Skin manifestations at the bite site				
Bacteria	Diseases	Fever	Diffuse rash	Eschar	Local rash	Enlarged local nodes	Fatality rate without treatment	Positive blood smears
Rickettsia species								
R. rickettsii	Rocky Mountain spotted fever	99%	90% (45% purpuric)	Very rare	No	No	1%-5%	No
R. conorii	Mediterranean spotted fever	100%	97% (10% purpuric)	72%	No	Rare	1%	No
R. conorii Israel	Israeli spotted fever	Yes	100%	No	No	No	<1%	No
R. conorii Astrakhan	Astrakhan fever	Yes	100%	23%	No	No	No	No
R. sibirica	Siberian tick typhus; north Asian tick typhus	Yes	100%	77%	No	Yes	Low	No
R. australis	Queensland tick typhus	Yes	100% (vesicular)	65%	No	Yes	Low	No
R. honei	Flinders Island spotted fever	Yes	85% (8% purpuric)	28%	No	Yes	Low	No
R. africae	African tick bite fever	92%	43% (20% vesicular)	98% (53% multiple)	No	Yes	Very low	No
R. japonica	Japanese or Oriental spotted fever	Yes	100%	90%	No	No	Low	No
R. mongolotimonae	Unnamed	Yes	Yes	Yes	No	No	No	No
R. slovaca	Unnamed	27%	No	Yes	Yes	44% (cervical)	No	No
R. helvetica	Unnamed	Yes	?	?	?	?	Yes	No
Ehrlichia species								
E. chaffeensis	Human monocytic ehrlichiosis	Yes	36%	No	No	No	1%-2%	Yes
E. ewingii	Human ehrlichiosis	Yes						Yes
HGE agent	Human granulocytic ehrlichiosis	Yes	2%-11%	No	No	No	Low	Yes
Borrelia species								
B. burgdorferi sensu stricto	Lyme disease[a]	16%	Possible at time of disseminated infection	No	Erythema migrans	23%	<1%	No
B. garinii	Lyme disease[b]							
B. afzelii	Lyme disease[c]							
Relapsing fever *Borreliae*	Tickborne relapsing fever	Yes	28%	No	No	Rare	2%-5%	Yes
Francisella species								
F. tularensis tularensis	Tularemia	Yes	No	Yes (inconstant)	No	Yes	1%-7%	No
Coxiella species								
C. burnetii	Acute Q fever	Inconstant	5%-21%	No	No	No	1%-2%	No

NOTE. HGE, human granulocytic ehrlichiosis.

[a] Particularly rheumatological forms.
[b] Particularly neurological forms.
[c] Particularly dermatologic forms.

Table 8. Clinical characteristics of Lyme borreliosis.

Class of symptoms	Clinical characteristics, by state of infection		
	Early		
	Localized[a]	Disseminated[b]	Persistent[c]
Dermatologic	Erythema chronicum migrans	Erythema chronicum migrans, secondary annular lesions, lymphocytoma, malar rash or urticaria (rare)	Acrodermatitis chronica atrophicans, localized scleroderma-like lesions
Neurological	—	Meningitis, cranial neuritis, radiculitis (motor or sensory), meningoradiculitis	Chronic encephalomyelitis, demyelinating-like syndrome, axonal polyneuropathy, cognitive and behavioral changes
Cardiac	—	Myocarditis, heart block, myopericarditis	—
Rheumatologic	—	Brief arthritis attacks	Prolonged arthritis attacks, chronic arthritis, peripheral enthesopathy
Ocular	—	Conjunctivitis	Keratitis
Lymphatic	Regional lymphadenopathy	Regional or general lymphadenopathy	—
Constitutional symptoms	Minor	Severe malaise and fatigue	Profound fatigue
Diverse	Influenza-like illness including arthromyalgia, headache, stiff neck, or fever (16%)	Hepatitis, splenomegaly, cough, hematuria, proteinuria	—

[a] Duration of infection is days to ~4 weeks.
[b] Duration of infection is weeks to months (range, 1–14 months).
[c] Duration of infection is months to years.

ivary glands and excretory organs [9]. People become infected when fed upon by ticks and salivary and coxal secretions contaminate the feeding site. Many rodents and small mammals serve as natural reservoirs, and borreliae also persist for many years in their long-lived tick vectors [9].

Clinical characteristics, diagnosis, and treatment. Tickborne relapsing fever begins with an acute onset of high fever with chills, headache, myalgia, arthralgia, and coughing [9]. Hemorrhage (rarely severe), iritis or iridocyclitis, hepatomegaly, or splenomegaly may also occur [141], and abdominal pain, nausea, vomiting, diarrhea, and photophobia are common in cases of infection in Africa. A rash may occur at the end of the first febrile episode, and neurological findings are frequent and may be severe, particularly in infections with *Borrelia turicatae* (United States) and *B. duttonii* (Africa) [142]. Jaundice occurs in 7% of patients, and the case-fatality rate is ~2%–5% [141]. In general, the primary episode lasts ~3 days and is followed by a second, shorter, milder episode 7 days later. Thereafter, there may be ⩾1 episodes at 4- (Africa) to 7-day (United States) intervals, each lasting ~2 days [9]. The resolution of the disease has been linked to antibody production against the different antigenic types presented by borreliae in the successive relapses.

Diagnosis is established by the demonstration of borreliae in peripheral blood of febrile patients. This test has a sensitivity of ~70% when blood smears are examined by means of darkfield microscopy or when stained with Giemsa or Wright's stain. Recently, a quantitative buffy coat analysis has been described as a very sensitive and specific technique for the detection of borreliae in blood [90]. Serological assays are not readily available, and their diagnostic value is limited because of the antigenic variation shown by the tickborne relapsing fever borreliae. Molecular methods have been described for the identification of the agents in blood samples [90]. The recommended treatment is doxycycline, 200 mg given in a single oral dose. Penicillin, erythromycin, or ceftriaxone may also be effective for treatment of severe forms of the disease [143]. In some patients, treatment may provoke a Jarisch-Herxheimer reaction [9].

Tularemia

The first report of a patient with what was probably tularemia was made in 1837 in Japan, and subsequently the disease was recognized in squirrels by McCoy in 1911 [70]. The causative agent was cultured a year later and named *Bacterium tularense.* The first documented human case was reported in 1914 [144], and the agent was renamed *Francisella tularensis* in honor of Dr. Edward Francis, who contributed greatly to our knowledge of the epidemiology and bacteriology of the disease [8]. Tularemia occurs primarily in the northern hemisphere, where it is wide-

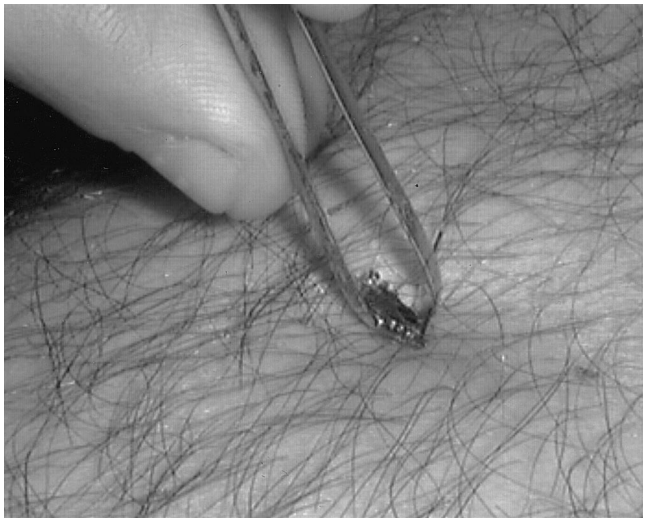

Figure 18. A method for removing a tick from skin. Forceps are used to grasp the tick's mouthparts as close as possible to the skin, and the tick is then pulled upward, perpendicular to the skin, with a continuous and steady action.

spread throughout North America, northern Asia, and Europe, although remarkably absent from the United Kingdom [9, 70] (figure 15). It is a seasonal disease, with epidemics of tick-acquired cases occurring in the United States in summer and hunting-associated cases occurring in December [9, 70, 145].

Bacteriology. *F. tularensis* is a small, aerobic, pleomorphic, gram-negative, intra- and extracellular coccobacillus. There are 3 main biovars: *F. tularensis* biogroup *tularensis* (biovar A), which is present in North America and is the most virulent species; *F. tularensis* biogroup *holartica* (biovar B), which is found predominantly in Europe and Asia, but also in North America; and *F. tularensis* biogroup *novicida* (former *Francisella novicida*), which is found in the United States and is of low virulence [70]. Isolation of *F. tularensis* requires biosafety level P3 facilities and is done by inoculation of organisms into cell culture or nonselective or selective culture media. *F. tularensis* biogroup *tularensis* and *F. tularensis* biogroup *holartica* require cysteine or cysteine (or another sulfhydril source) for growth and will not grow on most routine solid media. However, some strains lack an over-requirement for cysteine [70, 75].

Vectors and reservoirs. The epidemiology of this zoonosis is complex, with *F. tularensis* infections having been reported in >250 animal species, including mammals and invertebrates [146]. Multiple disease cycles and routes of transmission have been reported, including via aerosol droplets, contaminated water, arthropod (ticks, flies, mosquitoes) and animal bites, and direct contact with animal products [146, 147]. Rabbits, hares, and rodents seem to be the most important mammals in the ecology of the disease. In North America, the main tickborne epidemiological system involves *F. tularensis tularensis* and rabbits (Lagomorpha), particularly *Sylvilagus,* as reservoirs. Some of the ticks that transmit the disease to humans are shown in tables 2 and 3. The bird-rabbit tick *Haemaphysalis leporispalustris,* however, is probably essential in the maintenance of the disease among its hosts [9, 70]. In Europe, the animal reservoir for tularemia has yet to be clearly demonstrated [146].

Clinical characteristics, diagnosis, and treatment. The incubation period of tularemia averages 4–5 days, ranging from 1 day to 21 days [9, 145]. The disease is of variable severity, depending on the organism involved and the route of inoculation. The onset of the disease is abrupt, and signs include chills, fever, headache, vomiting, anorexia, and fatigue. Pulse-temperature dissociation is a classic finding. The most frequently seen and most characteristic form of the disease is the ulceroglandular form, in which a papule develops at the tick bite site, becomes pustular and then ulcerated (inoculation eschar), and is associated with regional lymphadenopathy. Glandular, oculoglandular, pharyngeal, typhoidal (illness with fever, prostration), pleuritic, and pneumonic (indicating a poor prognosis) presentations may also occur.

Without treatment, the disease usually extends over a period of 2–3 months. Mortality has been estimated to be ~7%, but it was probably as high as 33% before the introduction of antibiotics [9, 70, 145]. The disease is most frequently diagnosed by use of serological tests [9, 70, 145], although organisms may be isolated from blood cultures, sputum samples, or samples of CSF and from biopsies of inoculation eschars or regional lymph nodes. An isolation method with use of shell vials has also been reported [75]. Organisms may be identified in tissues or in cultures by use of direct immunofluorescence or molecular methods, including PCR and gene sequencing [91, 92]. Although doxycycline and chloramphenicol may be used to treat the disease, streptomycin and aminoglycosides (gentamicin) are the drugs of first choice and should be used for 7–14 days [70].

Q Fever

Query (Q) fever, first recognized in Australia by Derrick in 1935 [147, 148], is a ubiquitous zoonotic disease caused by *C. burnetii.* Although the organism has been found to infect >40 species of ticks of 12 genera throughout the world, the role of ticks in human infections is probably minimal, and Q fever is usually acquired by the ingestion or inhalation of virulent organisms from infected mammals and their products, most frequently goats, sheep, and cats [148–151]. The feces of ticks infected with *C. burnetii* have very high concentrations of viable organisms, which may persist for long periods in the environment, and thus ticks may play an important role in the dissemination of the organism [9]. The clinical signs, diagnosis,

and treatment of Q fever have been extensively reviewed recently [149, 150] and are therefore not discussed further here.

LIMITATIONS OF SEROLOGICAL TESTING

Serological tests are the most available and simplest diagnostic tests available for the study of infectious diseases. Numerous serological tests have been described, including complement fixation, indirect hemagglutination, latex agglutination, ELISA, immunoperoxidase, immunoblot assays, and immunofluorescence, which is the reference method in most laboratories for bacterial tickborne diseases. The major limitation of serological tests is the cross-reactivity that might be present between antigens of pathogens within the same genus and also in different genera. For example, the Weil-Felix test, the first serological assay for rickettsioses, is based on the detection of antibodies to various *Proteus* species, which contain lipopolysaccharide antigens that cross-react with rickettsial antigens [79]. Rickettsiae have been shown to cross-react among them and with *Proteus* and *Legionella* species [79, 80]. Similarly, serological cross-reactivity is present between the members of the *B. burgdorferi* complex and also between these organisms and other infectious agents, including relapsing fever borreliae, *Treponema pallidum, Ehrlichia* species, *Helicobacter pylori, Leptospira* species, Epstein-Barr virus, and HIV [131, 132, 152]. A number of cross-reactions have also been reported among ehrlichiae—for example, between *E. chaffeensis* and the agent of HGE and between *E. chaffeensis* and veterinary pathogens, such as *E. canis* and *Cowdria ruminantium,* the agent of heart water in cattle [123]. Further, *F. tularensis* has been shown to cross-react with *Brucella, Proteus,* and *Yersinia* species [70].

Because of the cross-reactivity between pathogens, serological results need to be interpreted very carefully to prevent mistakes being made in the discovery and description of the epidemiology of tickborne bacterial diseases. In 1987, Maeda et al. [109] reported the first human case of infection with *E. canis,* the agent of canine monocytic ehrlichiosis, on the basis of the results of serological tests and direct examination of leukocytes. It was subsequently shown that the causative agent was in fact a new ehrlichial pathogen, *E. chaffeensis,* which was isolated in 1991 in the United States and found to be morphologically identical to *E. canis* and to cross-react extensively in serological tests [110].

In 1994, *E. chaffeensis* was suspected to have occurred in New England on the basis of results of serological testing and the presence of morulae in the polymorphonuclear leukocytes of a febrile patient. The known vector of the disease, *A. americanum,* is, however, not found in that region, and *E. chaffeensis* is known to infect mainly monocytes. During this time, we reported the risk of cross-reactions with a newly described ehrlichia, and thereafter the HGE agent was described [153].

In Europe, monocytic ehrlichiosis due to *E. chaffeensis* has been suspected in several patients on the basis of results of serological tests [114]. Because of the presence of HGE in Europe and the presence of serological cross-reactions, it has yet to be proven that human monocytic ehrlichiosis occurs in Europe. On a similar note, we also probably overestimate the presence of human monocytic ehrlichiosis in Africa [154]. To date, the agent has yet to be isolated there, and its known vectors do not occur on the continent. Other ehrlichiae of veterinary importance, including *E. canis* and *C. ruminantium,* are widely distributed on the continent, and these organisms are known to cross-react with *E. chaffeensis* in serological tests [155].

A number of syndromes have been misdiagnosed as rickettsioses, Lyme disease, or ehrlichioses on the basis of results of serological tests. As examples, *Rickettsia* species have been suspected to cause Lyme disease [156] and to be involved in multiple sclerosis, myocardial infarction, and schizophrenia [11], and *E. canis* has been suspected to be a cause of Kawasaki disease [157]. Currently, there are numerous reports of new diseases, new distributions of diseases, and new clinical forms of known diseases based on serological data [11]. It should be noted, however, that although serological tests can be very useful diagnostic tests and are often the only evidence of a disease that can be obtained, they should be only one step in diagnosing or recognizing a tickborne disease. There should be supporting clinical evidence, such as erythema migrans or a spotted fever with eschar, and also supporting epidemiological evidence, for example, trips to rural areas of endemicity for tickborne diseases [123, 131, 132]. Positive results of serological tests for spotted fever rickettsiae may then be interpreted as an infection due to *R. rickettsii* in United States, *R. sibirica* in eastern Russia, *R. africae* or *R. conorii* in Africa, and *R. conorii* or *R. slovaca* in Europe.

As demonstrated above, the use of a serological test as an epidemiological tool may lead to a disease being thought to occur in an area where it has not been clearly described. The results of serological studies have to be interpreted after the following questions are considered: Have the bacteria been isolated from or their specific gene sequences identified in ticks and/or humans in the study area? Is the known tick vector prevalent in the area? Is the known reservoir of the disease present in the study area? If these questions cannot be answered clearly, the conclusion of seroepidemiological studies can be only that the agent or a serologically related organism occurs in the study area. The presence of the disease may then be suspected, but its presence cannot be proven on the basis of serological evidence alone.

HOW TO TREAT TICK BITES

Removing ticks from the skin. Patients may present with attached ticks, and removing these ticks may not be easy. It is

best to use blunt, rounded forceps, and a magnifying glass may be helpful if immature ticks are found. The forceps are used to grasp the mouthparts of the ticks as close as possible to the skin, and the tick is then pulled upward, perpendicular to the skin, with a continuous and steady action (figure 18) [23]. Specific instruments are commercially available and may be particularly useful for removing nymphal stages (Take Care [Tropenzorg]; Pro-Tick Remedy [SCS Limited]). Usually any mouthparts of the ticks retained in the skin are eliminated uneventfully by the body. Shave incisions close to the skin may also be used. After removal of the ticks, a disinfectant should be applied to the bite site and the tick stored at −20°C in case the patient subsequently develops a disease that requires the tick for detection or isolation of the causative agent. Other methods of removing ticks, such as using the fingers instead of forceps or using lighted cigarettes, petroleum jelly, or suntan oil to kill the ticks in situ, should be avoided, because they may increase the risk of regurgitation by the tick and, consequently, the transmission of infectious agents [23].

Antibiotic prophylaxis after tick bites? Most tick bites are uncomplicated and result only in benign cutaneous inflammatory reactions that may be pruritic for a few days. Sometimes a granuloma may develop, supposedly as a result of mouthparts being retained at the feeding site.

As described above, the bacterial agents transmitted by ticks are susceptible to antibiotics, particularly doxycycline, which may be used prophylactically after a tick bite to reduce the risk of disease transmission. However, there are no data to indicate that antimicrobial prophylaxis is beneficial for tick-bitten patient to prevent Lyme disease, tularemia, and rickettsioses, and its efficacy in an animal model of Rocky Mountain spotted fever has not been demonstrated [42, 70, 71]. Testing for the presence of antibodies against tickborne bacteria (borreliae, rickettsiae, *F. tularensis, C. burnetii,* ehrlichiae) at presentation and 3–6 weeks later, and treating it only if there is clinical or serological evidence of infection is not recommended because of the low sensitivity, low positive predictive value, and the cost of the tests. Finally, clinicians have to observe the patient and treat only if a disease occurs. It must be kept in mind that the risk of transmission of a bacterial disease by a tick increases with the duration of attachment and generally requires >24–48 h. The degree of tick engorgement or the time since tick exposure and discovery of the tick may be used to establish the likely duration of attachment and the risk of disease transmission.

Prevention of tick bites. Tick bites are best prevented by people avoiding tick-infested areas. When this is not possible, tick bites may be prevented by the wearing of long trousers that are tucked into boots. At the present time, the best method to avoid tick bites has 2 components: application of a topical deet (*N,N*-diethyl-*m*-toluamide) repellent to exposed skin and treatment of clothing with permethrin. This system is currently used by the US Army and by numerous armies throughout the world to protect their soldiers [158, 159, 160]. It yields protection results at nearly 100% and is also effective against biting flies, gnats, chiggers, fleas, and mosquitoes.

Tick repellents that contain deet are the most effective and can be applied to the skin [23]. The optimal concentration ranges from 15% to 33%, which allows for high performance and a high margin of safety. The performance drops off when concentrations of >35% are used. Although subsequent uses may cause irritation in some people, there are no data to suggest long-term dangers of deet products when they are used in accordance with label directions [161]. These products are available in a wide variety of formulas (such as lotions and sprays) that can address specific needs of users, including individual travelers, persons working or recreating in the outdoors, and even young children, who should use less concentrated products (⩽7%). Some of them provide up to 12 h of protection from 1 application, and long-acting formulations are being developed [162].

However, the application of repellents to exposed skin provides little protection against ticks, because they can crawl underneath clothing and bite untreated portions of the body. Thus, treating clothing with permethrin (a pyrethroid that kills ticks on contact) is recommended to complement the treated skin. It is not licensed for direct application to the skin. This chemical is virtually nontoxic to humans and can be applied to clothing in a pressurized spray formulation. Major brands include Duranon, Sawyers, and Permanone. It can be used with any age group. Furthermore, it remains effective for up several weeks and through weekly washings. Nets can be treated with permethrin for additional protection while sleeping.

Finally, checking clothing regularly while in tick-infested areas is highly recommended to back up the few hours of protection provided by the insect repellents. It is also recommended that the entire body be carefully screened for parasites by campers while they are staying in and after the leave infested areas. Any tick found should be removed immediately.

CONCLUSIONS AND PERSPECTIVES

Since the beginning of the 1980s, more than 15 new tickborne bacterial diseases have been described in the world. Numerous factors may explain the increased number of emerging tickborne diseases or their increased incidence. People are undertaking more outdoor activities, which result in increased contact with ticks and tickborne pathogens. The heightened awareness of tickborne diseases among primary care physicians, further studies of potential pathogenic bacteria found in anthropophilic ticks, and the use of new molecular biology techniques have all greatly facilitated studies of the epidemiology of emerging human tickborne diseases all over the world. Moreover, a num-

ber of *Rickettsia*, *Borrelia*, *Ehrlichia,* and even *Bartonella* species have been found in ticks only, and their pathogenicity in humans is yet to be determined [86].

Clinicians should be aware of the clinical signs of tick-transmitted diseases, because morbidity and mortality as a result of such diseases increases substantially if there are delays in diagnosis and treatment. Tickborne diseases occur in rather distinct geographic areas, and reporting these diseases to the health department would enable the public to be informed about the risks of disease in tick-infested areas and the means of preventing infections. We anticipate that the number of known tickborne diseases will probably increase in the future.

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