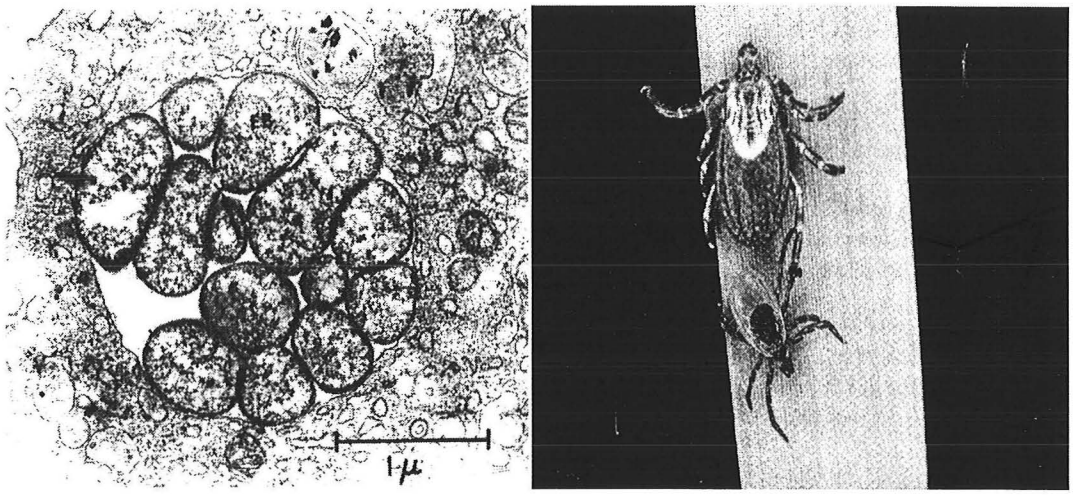
THE EMERGENCE OF EHRLICHIOSIS:

DOUBLE-TROUBLE FROM TICKS



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Sexually Transmitted Diseases

INTRODUCTION

Less than a decade ago, conventional wisdom held that, through the use of vaccines and ever more powerful antimicrobial agents, medical science was on the verge of eradicating infectious diseases as significant threats to public health (1). This sanguine notion, however, has been resoundingly dispelled by the recognition of an ever-expanding spectrum of "emerging infectious diseases" (EID), a term which an expert committee of the Institute of Medicine defined in 1992 as "new, reemerging, or drug-resistant infections whose incidence in humans has increased during the past two decades or whose incidence threatens to increase in the near future" (1). EID now encompasses the many bacterial, viral, and eukaryotic pathogens which were either unknown a short while ago or which have re-emerged to fill the vacuum created as "traditional" infectious diseases come under control or are eradicated. So important has the field of EID become that the Centers for Disease Control and Prevention/National Center for Infectious Diseases recently launched an entire journal, aptly named Emerging Infectious Diseases, devoted to the subject.

Ehrlichiosis, the topic of today's Internal Medicine Grand Rounds, typifies the phenomenon of the emerging infectious disease (1,2). It is vector-borne and zoonotic, like many EID (1,3). Until 1986, it was considered to be of interest solely to veterinarians and was unheard of among practicing physicians (4,5). In the brief span of 11 years, two forms of the disease, human monocytic ehrlichiosis (HME) and human granulocytic ehrlichiosis (HGE), have been identified; their respective pathogens, *E. chaffeensis* and the still unnamed HGE agent, have been identified and partially characterized; and approximately 800 cases, many of them fatal, have been reported throughout the United States. Thus, in a short time, ehrlichiosis has emerged from virtual obscurity to gain recognition as a major tick-borne illness (2,6,7). The reason for the sudden emergence of these two infections is not entirely understood but it is believed to be a combination of true increases in incidence in combination with an enhanced ability to detect these fastidious micro-organisms using molecularly based techniques, particularly polymerase chain reaction (PCR).

TICK-BORNE DISEASES AND PATHOGEN TRANSMISSION

Before discussing HME and HGE in depth, some background information on ticks and tick-borne diseases is necessary. In the United States, more vector-borne diseases are transmitted by ticks than by any other arthropod (7). Furthermore, in true EID fashion, the significance of ticks as disease vectors is increasing as a result of many natural and human-made demographic changes, including the migration of affluent humans from urban to rural areas (8,9). The net effect of these changes is the repopulation of habitats previously devoid of ticks by appropriate host animal species such as deer, small rodents, and humans (10,11). It is worth emphasizing that all tick-borne diseases are zoonotic and that humans are incidental

hosts unessential for perpetuation of the respective pathogen; rather. Humans unluckily acquire these diseases when they intrude upon the vector's habitat and substitute for the natural mammalian host as the source of a blood meal (12,12).

Ticks are obligate hematophagous members of the class Arachnida, a group of arthropods that also includes mites, spiders, and scorpions. Of the three families of ticks, the Ixodidae and the Argasidae, are known to transmit pathogens to humans (7). Ixodid ticks are called hard ticks because they possess a dorsal, sclerotized shield (the scutum) which limits abdominal expansion during feeding. The life-cycle of all ticks consists of four stages: embryo, larva, nymph, and adult. The life-cycle of most hard ticks extends over a two year period with a blood meal needed for each stage of morphogenesis (7). The timing of the blood meal differs for each stage and determines the seasonal distribution of a stage-specific pathogen being transmitted. Among the 13 genera of hard ticks, *Amblyomma, Dermacentor,* and *Ixodes,* transmit disease within the United States (7) (Table 1) . Of the five genera of soft ticks, only ticks of the genus *Ornithodoros* transmit pathogens to humans within the United States (Table 2). As can be seen in Table 1, hard ticks are of much greater medical significance than soft ticks. Obviously, the geographic distribution of a tick vector determines the distribution of the pathogen it distributes and even within endemic areas tick-borne diseases are highly focal (13). A corollary of this notion is that a pathogen cannot become established in a new geographic area if the conditions for establishing an enzootic cycle do not exist.

In the United States, various species of ticks transmit bacterial, viral, and piroplasmal pathogens (Table 1) (7). Three of the diseases in Table 1 harbor special relationships with ehrlichiosis which will be discussed during this presentation:

1. Rocky Mountain Spotted Fever (RMSF). Ehrlichiae and rickettsiae are taxonomically related obligate intracellular pathogens. The diseases they cause share many attributes but also have significant differences. *Amblyomma americanum*, the Lone Star tick, is the vector for both *Rickettsia rickettsi* and *E. chaffeensis*.

2. Lyme Disease and Babesiosis. *Borrelia burgdorferi*, *Babesia microti*, and the agent of HGE share tick vector, *Ixodes scapularis,* and enzootic cycle (14,15). Lyme disease has been intensively studied during the past decade and provides an invaluable framework for analogous studies of HGE. The potential for coinfection and consequent co-morbidity from these three diseases also is being increasingly recognized (15).

TABLE 1. MAJOR TICK-BORNE DISEASES IN THE UNITED STATES

DISEASE	CAUSATIVE AGENT	MAJOR VECTOR	REGION
Lyme disease	*Borrelia burgdorferi*	Ixodes	Northeast, Upper Midwest
Relapsing Fever	Borrelia species	Ornithodoros	West
Tularemia	*Francisella tularensis*	Dermacentor, Amblyomma	Arkansas, Missouri, Oklahaoma
Rocky Mountain Spotted Fever	*Rickettsia rickettsi*	Dermacentor, Amblyomma	Southeast, West, South central
Human monocytic ehrlichiosis	*Ehrlichia chaffeensis*	Amblyomma, Dermacentor	Southeast, South central
Human granulocytic ehrlichiosis	HGE agent (*E. equi*?)	Ixodes	Northeast, Upper Midwest
Colorado tick fever	Coltivirus species	Dermacentor	West
Babesiosis	*Babesia microti*	Ixodes	Northeast
Tick paralysis	Neurotoxin	Dermacentor, Amblyomma	Northwest, South

In the flat (i.e., unfed) tick, ehrlichia are quiescent within the midgut epithelium and are probably less infectious (16). Ingestion of the blood meal stimulates a burst of bacterial replication. By analogy with *B. burgdorferi* (17,18), the blood meal also presumably stimulates a number of antigenic changes ("activation") which enable the bacterium to migrate from the midgut, through the hemolymph, to the salivary glands from whence it can be transmitted in infectious form to the mammalian host (16). This entire process takes many hours so that removal of the tick early after attachment can abort transmission of the pathogen. In contrast to hard ticks, Argasidae or soft ticks feed frequently and their blood meals typically last 30 minutes or less. Pathogens transmitted by soft ticks are already in the salivary gland in an infectious state at the time of feeding and, consequently, transmission is much harder to interrupt (7).

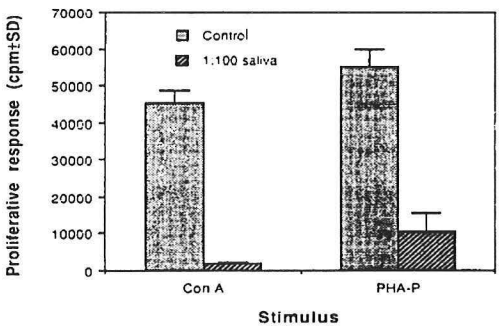

Figure 1. *Ixodes scapularis* **saliva inhibits T cell proliferation to mitogens.**

Finally, some aspects of the tick-host interface also need to be mentioned given their potential relevance to transmission of ehrlichiae. Ticks are not merely "crawling syringes" that transmit pathogens simply by injecting them into the microvasculature. Rather, they use their mouth parts to dissect within the tissues, creating hemorrhagic pools within the dermis from which

they feed. These pools must be constantly replenished from nearby blood vessels in order to feed to repletion (19). Maintenance of blood flow and prevention of clotting during the extended period required for feeding are critical problems which ticks overcome via the presence in saliva of components which exert a variety of pharmacological (e.g., vasodilatory, anti-platelet, and anti-coagulant) activities (19,20). The long period of attachment enables the host to induce a complex cellular and humoral local inflammatory response which theoretically could act to limit tick feeding (21). Ticks counter this by secreting salivary components with potent immunosuppressive (Fig. 1) and anti-inflammatory (Fig. 2) activities (21-23). All vector-borne pathogens, including ehrlichiae, exploit these pharmacological and immunosuppressive activities in order to gain access to the mammalian host en route to fulfilling their parasitic destinies. It is even possible (though unproven) that ehrlichiae first gain access to leukocytes during this local cellular response. In view of all of these phenomena, it cannot be presumed that needle inoculation is equivalent to tick inoculation, a point which must be borne in mind when evaluating experimental studies. Lastly, it should be pointed out that these findings have important ramifications for the development of strategies to control tick-borne diseases. Investigators have shown that immunization against tick salivary proteins can abrogate both feeding and pathogen transmission (21).

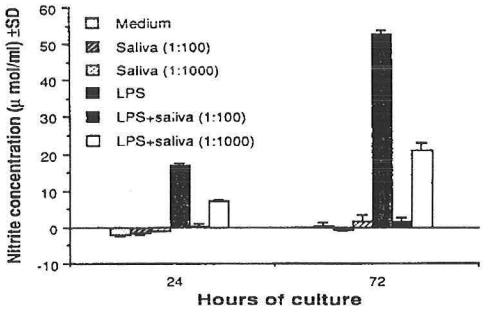

Figure 2. ***Ixodes scapularis*** **saliva inhibits nitric oxide production by macrophages.**

MICROBIOLOGY

Ehrlichiae are small cocci or coccobacilli that average 0.5 to 1.5 μm in length. All ehrlichiae species are obligate intracellular pathogens of granulocytes or monocytes that grow within membrane bound inclusions called morulae (Latin for "mulberry") (Fig. 3) (2,24). Individual ehrlichiae are called elementary bodies. Growth within membrane-bound inclusions distinguishes ehrlichiae from rickettsiae, which grow freely within the cytoplasmic compartment (25). Another distinctive feature of ehrlichiae is their preferential use of glutamine as a primary energy source; this is in contrast to rickettsiae which preferentially utilize glutamate (24,26). Ehrlichiae are similar to conventional gram-negative bacteria in that they possess outer and cytoplasmic membranes; they differ from gram-negatives, however, in that their outer membranes are said to lack lipopolysaccharide or lipooligosaccharide (24). The lack of this highly potent proinflammatory glycolipid may have biological significance. *In vitro* studies have shown that ehrlichiae fail to induce human

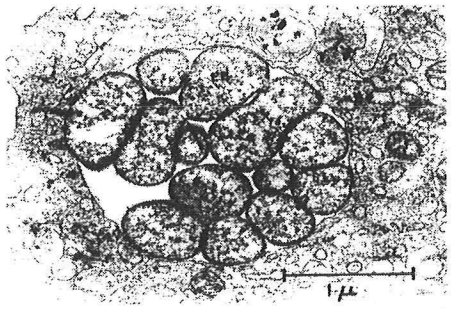

Figure 3. Transmission electron micrograph of ***E. canis*** **inside an inclusion body contained within a cultured monocyte. Note the double-membrane structure of the bacteria and the surrounding membrane of the inclusion body. By light microscopy, this would be seen as a morula.**

monocytes to produce seminal inflammatory mediators such as TNFα, IL-6, or GM-CSF, even though they do stimulate the production of IL-1β, and IL-10 (27).

MOLECULAR AND CELLULAR BIOLOGY

Very little is known about ehrlichiae at the molecular level. Immunodominant antigens of several ehrlichial species have been identified by immunoblot analysis (24,28). Cloning and sequence analysis revealed that a highly immunogenic 58-kDa antigen of *E. chaffeensis* is a homolog for the *E. coli* GroEL heat shock protein (29). More recently, the GroEL homolog from the HGE agent also was cloned and sequenced (30). Sequence analysis of the gene encoding an immunodominant 120-kDa *E. chaffeensis* antigen, a putative adhesin, revealed that it possesses five tandem 80 amino acid repeats (31).

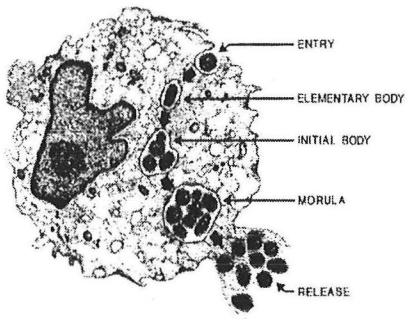

Figure 4. Intracellular growth cycle of ehrlichia.

Presently, only a modest amount of information exists regarding the specific mechanisms or sorting pathways which ehrlichiae exploit in order to convert the hostile environment of the phagosome into a milieu conducive for growth and replication. It seems likely, however, that they share pathogenic strategies with other bacteria which have adapted successfully to "life within the vacuole" (32). The developmental cycle of ehrlichiae is depicted in figure 4 (5). Elementary bodies are taken up into the cell by phagocytosis involving specific receptor-ligand interactions between proteinase-accessible (i.e., surface-exposed) structures on both bacteria and host cells (33). Monodansylcadaverine inhibits bacterial uptake, indicating the involvement of receptor-mediated endocytosis; internalization, in contrast, was not affected by cytochalasin D, a compound which disrupts actin filaments (33). Tyrosine phosphorylation also is required for infection inasmuch as phospho-tyrosine kinase inhibitors (e.g., genistein and herbimycin A) prevent uptake and replication; phosphorylated proteins of 54- and 52-kDa are thought to be involved in the relevant signaling pathways (34). The absence of phagolysosomal fusion is indicated by the failure of phagosomes to acquire lysosomal markers (35,36). Inhibition of phagolysomal fusion requires viable bacterium inasmuch as it does not occur with latex beads or when infected cells are incubated with oxytetracycline, an inhibitor of bacterial protein synthesis (35). The individual bacterial cells within the vacuole then divide by binary fission to produce a microcolony recognized in Wright stained smears as a morula. Morulae break up into elementary bodies, ready to begin the cycle anew, when the infected cell lyses. Ehrlichia readily proliferate within resident murine peritoneal macrophages but cannot replicate within macrophages from ehrlichiae-recovered mice or within macrophages pre-treated with interferon-γ (37).

TAXONOMY

The genus *Ehrlichia* was established in 1945, in honor of the great German microbiologist Paul Ehrlich, to distinguish ehrlichiae from the other medically significant obligate intracellular pathogens recognized at the time (*Rickettsia*, *Chlamydia*, and Casually) (5). Prior to the molecular era, phylogenetic relationships among members of the *Ehrlichia* genus were based upon morphology, host-cell tropism, and serological studies. Phylogenetic analysis based upon 16S ribosomal RNA (rRNA) sequences (38), however, has made it possible to determine with great precision the taxonomic relationships among ehrlichiae species and related protobacteria (particularly rickettsiae) (Fig. 5). All recognized ehrlichial species fit into three genogroups or clusters, each of which contains one of the species pathogenic for humans: (a) the *E. canis* group (which contains *E. chaffeensis*), (b) the *E. phagocytophila* group (which contains the agent of HGE), and (c) the *E. sennetsu* group (Fig. 5) (2,6). It is interesting to note that the taxonomic scheme derived by rRNA sequence analysis is consistent with relationships derived by serological grouping (6,28). For example, prior to isolation of *E. chafeensis*,it was noted that sera from patients with HME reacted with *E. canis*. Subsequent PCR-based analysis of rRNA genes revealed that *E. canis* and *E. chafeensis* 16S rRNAs are greater than 98% identical (39,40).

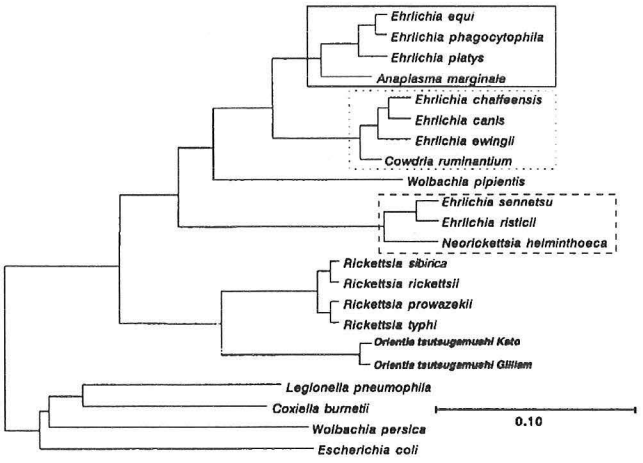

Figure 5. Dendrogram representing the phylogenetic relationships of ehrlichiae and other *Protobacteria* as determined by 16S rRNA gene sequence analysis. The three clusters enclosed in rectangles indicate organisms designated as *Ehrlichia*.

In contrast to *E. chaffeensis*, investigators have not been able to agree on the taxonomic status of the agent of HGE. The original 16S rRNA sequence reported for the HGE agent was highly similar, but not identical, to the GenBank sequences for *E. equi* and *E. phagocytophilia* (41); this finding suggested that the three organisms were very closely related but not necessarily identical. Strong serological cross-reactivity of HGE patient sera with *E. equi* and *E. phagocytophilia* also supported this interpretation of the genetic data (42,43). There is now substantial evidence, however, that HGE may, in fact, be caused by an ehrlichial species (i.e., *E. equi*) which is chiefly a pathogen of horses and other animals (i.e., dogs). 16S rRNA sequences of ehrlichia in the peripheral blood of naturally infected horses and dogs from several geographical areas have been found which are identical to the HGE agent sequences (44-46). Both *E. equi* and the HGE agent appear to be transmitted by the same hard tick, *Ixodes scapularis* in the same geographical areas (47) (48). Horses injected with blood from HGE patients developed a granulocytic ehrlichiosis which could be serially transmitted to

other horses (49). The HGE agent could be transmitted to dogs and rabbits by challenge with field-collected ticks (50). The close genetic relationship of these three ehrlichia also has been confirmed by sequencing an entirely different genetic target (the *groESL* heat shock operon) (51). Although the 16S rRNA genes of *E. equi* and *E. phagocytophila* are 99.9% identical, the latter is thought to cause disease only in European ruminants. It is for this reason that many authorities consider the HGE agent to be identical to or a strain of *E. equi* rather than *E. phagocytophilia* (49). An interesting implication of this line of reasoning is that there appear to be biological distinctions between these two pathogens which cannot be detected by existing molecular taxonomic techniques.

A PRIMER OF EHRLICHIAL DISEASES

DISCOVERY. Ehrlichiae first came to medical attention in 1910 when Theiler described *Anaplasma marginale,* the etiologic agent of malignant anaplasmosis, an economically important disease of cattle. In 1925, Cowdry described *Cowdria ruminantium*, the agent of heartwater, a South African disease of cows, goats, and sheep transmitted by hard ticks of the genus *Amblyomma*. As shown in figure 5, phylogenetic analysis based upon 16S rRNA gene sequence analysis indicates that both of these agents are actually *Ehrlichia* species (6,52). Table 2 presents key basic information on the leukocytotropic ehrlichial species of major veterinary and medical importance.

TABLE 2. LEUKOCYTOTROPIC EHRLICHIAL SPECIES AND DISEASES

SPECIES	MAMMALIAN HOST	CELL INFECTED	DISEASE
E. canis	Dog	monocyte	Tropical canine pancytopenia
E. equi	Horse	granulocyte	equine granulocytic ehrlichiosis
E. risticii	Horse	monocyte	Potomac Horse Fever
E. phagocytophila	Ruminants	granulocyte	Tick-borne fever
E. sennetsu	humans	monocyte	Sennetsu fever
E. chaffeensis	humans	monocyte	Human monocytic ehrlichiosis
HGE Agent (*E. equi*?)	humans (Dog/horse?)	granulocyte	Human granulocytic ehrlichiosis

VETERINARY PATHOGENS

E. canis. Canine monocytic ehrlichiosis was first described in 1935 by Donatien and Lestoguard at the Pasteur Institute in Algeria (53). They noted that experimental dogs housed at the Institute, particularly dogs infested with the tick *Rhipicephalus sanguineus* occasionally

developed a severe febrile illness characterized by anemia (53). Giemsa-stained blood smears showed small rickettsia-like organisms (originally named *Rickettsia canis*) inside monocytes (53). Soon after its discovery, *E. canis* infection was documented in the Mediterranean area, India, and various parts of Africa (54). In 1957, Bool and Sutmoller (55) identified *E. canis* in the monocytes of severely ill dogs in Aruba, thereby marking its recognition in the Western hemisphere. In 1969, Ewing (54) associated *E. canis* with monocytic ehrlichiosis of dogs in Oklahoma; by this time *E. canis* was recognized as a globally distributed pathogen. Ehrlichial disease gained considerable notoriety as a result of a devastating epizootic of tropical canine pancytopenia which killed as many as 300 sentry dogs (mostly German shepherds) used by U.S. troops in Vietnam from 1968 to 1970 (56,57). In 1971, a new ehrlichial species, *E. canis,* was successfully isolated in primary canine monocytes (58). Subsequently, it was found that *E. canis* could be serially propagated without loss of infectivity in canine monocytes and, monocyte cell lines (59). By the late 1980's, *E. canis* was known to be widely distributed in the United States (60).

<u>*E. equi*</u>. In 1969, Stannard et al. (61) summarized their observations on four spontaneous and eight experimental cases of equine granulocytic disease in California. Clinical manifestations were fever, ataxia, anorexia, petechiae, and edema in association with pancytopenia. Microscopic examination of the granulocytes of infected horses revealed typical ehrlichial inclusions, and the etiologic agent was named *E. equi* (62). *E. equi* has a broad host range and, in addition to horses, is capable of infecting burros, sheep, goats, dogs, cats, monkeys, baboons, and probably humans (63). In fact, the pathogen's extremely broad host range is one piece of evidence used to support the contention that it is identical to the agent of HGE. In the Northeast and Midwest, *E. equi* is transmitted by *I. scapularis*; in California the vector is *I. pacificus.*

<u>*E. phagocytophila.*</u> *E. phagocytophila,* was discovered by Gordon in 1940 (64). This European pathogen of ruminant neutrophils causes tick-borne fever of sheep, cattle, bison, and deer and is transmitted by *Ixodes ricinus* (5).

<u>*E. risticii*</u>. In 1979, eight horses and ponies stabled on farms near the Potomac River in Montgomery County, Maryland developed a disease characterized by fever, anorexia, distal edema of the limbs, watery diarrhea, and dehydration. Between 1979 and 1984, 400 cases of Pontiac Horse Fever (PHF) were documented in Maryland, and additional cases were reported from 29 other states (65,66). Eventually, a new species of ehrlichia, *E. risticii*, was isolated from the blood of horses with PHF and named *E. risticii* to honor the many contribution of Miodrag Ristic to the study of rickettsial diseases (67,68). Laboratory studies with *E. risticii* have been an important source of information regarding ehrlichial disease pathogenesis and ehrlichial cell biology. In contrast to *E. equi*, which is granuclocytotropic, *E. risticii* infects equine monocytes.

HUMAN PATHOGENS

<u>*E. sennetsu*</u>. In 1953, *E. sennetsu* was isolated from the blood, bone marrow, and lymph

node of a Japanese patient with a mononucleosis-like syndrome (69). A similar microorganism was isolated independently from another patient in Japan with a similar syndrome (70). Preliminary characterization of the agent of Sennetsu fever suggested that it was a new species of rickettsiae, and it was named *Rickettsia sennetsu* in 1956 (71). Subsequent work by Hoilien et al. (72) and Holland et al. (73) established that it resembled *E. canis* with respect to morphology, growth cycle, and monocyte tropism. Subsequent sequence analysis of its 16S rRNA gene revealed that it was most closely related to *E. risticii* (2). Serologic data suggest that a focus of Sennetsu fever also exists in Malaysia (5). Sennetsu fever and *E. sennetsu* have never been identified outside of the Far East.

<u>*E. chaffeensis*</u>. For more than thirty year years, *E. sennetsu* was thought to be the sole ehrlichial pathogen of humans. The demise of this misconception began April 14, 1986 when a 51 year man was admitted to a Detroit hospital with an acute febrile illness manifested by encephalopathy, hepatitis, renal failure, anemia, and thromobocytopenia two weeks after planting trees in rural Arkansas (4). A presumptive diagnosis of Rocky Mountain Spotted Fever (RMSF) was made, but acute and convalescent serologies for *R. rickettsi* were nonreactive. The presence of inclusion bodies in lymphocytes, atypical lymphocytes, neutrophils and monocytes one week after the onset of symptoms suggested canine ehrlichiosis; this deduction appeared to be supported by a markedly elevated acute titer to *E. canis* (4). Subsequent seroepidemiological surveys revealed that this new and occasionally fatal human ehrlichiosis was widely distributed throughout the United States, and was particularly prevalent in the Southeast and Oklahoma (74-78).

Several laboratories tried to isolate the responsible agent following the presentation of the index case. In 1990 alone, a group from the CDC recorded 37 unsuccessful isolation attempts (40)! Because *E. canis* had been isolated in primary canine monocytes, investigators had been trying to isolate the human pathogen by cultivation with human monocytes. Ironically, the breakthrough occurred in 1991, when leukocytes from a febrile Army reservist at Ft. Chaffee, Arkansas were layered onto a monolayer of DH82 canine histiocytoma cells (40), the same cell line which was being used at the CDC to continuously passage *E. canis* (2). Twenty-four additional attempts at isolation using the same approach were unsuccessful. The isolated organism also reacted strongly with sera from ehrlichiosis patients, further implicating it as the etiologic agent of HME. DNA sequencing of the PCR-amplified 16S ribosomal RNA (rRNA) gene from one of the isolates revealed that it was greater than 98.2 identical to the 16S rRNA gene of *E. canis* (39,40). The new ehrlichial species was designated *E. chaffeensis* (39).

<u>HGE Agent</u>. In 1994, Bakken et al. (42) reported 12 cases of ehrlichiosis in patients from the Upper Midwest. Although the clinical presentation of this illness was indistinguishable from HME, several lines of evidence indicated that these individuals were afflicted by an ehrlichial pathogen distinct from *E. chaffeensis:* (i) morulae were observed exclusively in the cytoplasm of neutrophils, (ii) convalescent sera did not recognize *E. chaffeensis* whereas 9 of 10 sera reacted with *E. phagocytophila* and/or *E. equi,* (iii) morulae in postmortem tissue from one patient reacted with antibodies against *E. equi* but not *E.*

chaffeensis, and (iv) PCR products were obtained from most patients using *E. phagocytophila/E. equi*-specific primers whereas no *PCR* products were obtained with primers specific for *E. chaffeensis*. Sequence analysis of a PCR product obtained from a Wisconsin patient with a similar syndrome confirmed that it was caused by an organism closely related to *E. phagocytophila* and *E. equi* (41). These findings stimulated a race among several laboratories to cultivate the HGE agent. In a report published in the New England Journal of Medicine, Goodman and co-workers (47) described the cultivation of the HGE agent in undifferentiated HL60 promyelocitic cells inoculated with blood from three patients with HGE, only one of whom had circulating morulae. The sequences of the 16S rRNA genes amplified from all three isolates were identical and differed by only a single basepair from the *E. equi* sequence. Since then, investigators in New York also reported isolating the HGE agent in undifferentiated and differentiated (i.e., granulocytic) HL60 cells (46).

PATHOLOGY AND PATHOGENESIS

General features. The pathogenesis of ehrlichiosis is poorly understood. According to current notions, tick inoculation of ehrlichiae into the dermis is followed by dissemination via the microvasculature or lymphatics. Exactly when ehrlichiae take up residence within leukocytes is unclear, though, as mentioned earlier, this theoretically could happen very early by taking advantage of the cellular inflammatory response induced at the site of the tick bite. In any event, the ehrlichiae then spread throughout the reticuloendothelial cell system as well as to other organs. It is clear that pathogenic mechanisms operative during ehrlichiosis differ considerably from those of rickettsial infection despite their many similar clinical features and the relatively close taxonomic relationship of their etiologic agents. Thrombosis, endothelial cell hypertrophy and hyperplasia, and vasculitis, characteristic features of rickettsial infection (25), are rare in ehrlichiosis because the vascular endothelial cell is not the primary target of ehrlichial infection (24). Ehrlichia do cause cytopatholytic effects when grown in cell culture (79); however, it is uncertain whether this is the major mechanism for tissue damage *in vivo*. In fact, one of the enigmas of ehrlichiosis is that clinical manifestations often seem disproportionate to the sparse total body burden of ehrlichiae (estimated by microscopic analysis of blood smears and tissues). This disparity has fueled speculation that host immunity or inflammatory responses influence the severity and outcome of infection (80). This contention has recently received strong support by the demonstration that *E. chaffeensis*-antibody complexes induce enhanced cytokine production by monocytic cells and that this effect requires binding of immune complexes to the Fcγ receptor (81).

Pathologic findings in humans. Immunohistologic studies have demonstrated that both *E. chaffeensis* and the agent of HGE are capable of establishing infection in many organs and tissues (80). Typically, the heaviest burden of infection is seen in organs of the reticuloendothelial system (i.e., liver, spleen, lymph nodes). Focally heavy infection involving inclusion-bearing macrophages or granulocytes in pulmonary vasculature, adrenal glands, or

kidneys also has been described. Many other organs have perivascular lymphohistiocytic infiltrates, reflecting the system distribution of the infection and/or immune and inflammatory responses of the host (6,82). Bone marrows from isolated HME cases have revealed a variety of abnormalities, including marrow hypoplasia (83), normocellularity (77), and hypercellularity (77), making it difficult to discern a single underlying pathophysiologic mechanism. Hemophagocytic syndrome caused by inclusion-bearing monocytic cells also has been described (84). The largest hematopathological series of HME was reported by Dumler and co-workers (80). Of the 12 specimens examined, granulomatous changes were present in nine specimens. In most patients (eight), the marrow was hypercellular, although normocellularity and hypocellularity also were observed (two each). Erythrophagocytosis, plamacytosis, and lymphoid aggregates also were observed in a substantial proportion of patients. Megakaryocytosis was observed in seven of the cases. These findings, taken as a whole led Dumler and co-workers (80) to speculate that leukopenia and thrombocytopenia result from peripheral destruction and sequestration of platelets and white blood cells (80).

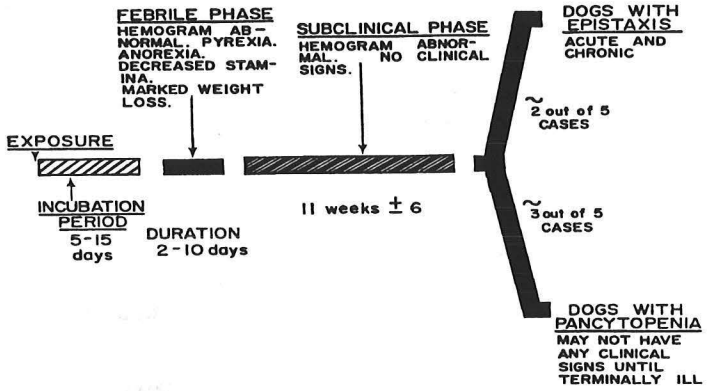

Figure 6. Clinical course of tropical canine pancytopenia.

Experimental *E. canis* infection. Experimental inoculation of dogs with *E. canis* closely mimics naturally acquired disease (85,86). One interesting observation is that the course and outcome of infection are highly breed-dependent. Both beagles and German shepherds develop an acute febrile disease with transient pancytopenia approximately 10 days following inoculation. However, beagles usually recover from the acute phase and go on to develop mild persistent infection with cyclic variations in blood counts. In contrast, after a period of apparent recovery, German shepherds usually enter a third, terminal stage of severe pancytopenia (particularly leukopenia and thrombocytopenia), hemorrhage, peripheral edema, emaciation, and secondary bacterial infections (Fig. 6). Although epistaxis may be the first clinical sign of disease, it is actually an indicator of advanced infection and often a harbinger of a fatal outcome due to either hemorrhage or secondary bacterial infection (Fig. 6).

Pathologic examination of chronically infected dogs reveals widespread perivascular plasma cell infiltrates (85,87,88), while immunohistochemical staining reveals widely disseminated inclusion-bearing monocytoid cells, particularly in the lungs, spleen and mesenteric lymph nodes (89). Examination of bone marrow from dogs with severe pancytopenia showed pronounced hypoplasia, depletion of megakaryocytes and granulocytic precursors, and occasionally loss of sinusoidal architecture; all of these features suggest that the pancytopenia of the final stage is largely a production defect. However, peripheral destruction may still be

contributory. Smith et al. (90) found that the lifespan of platelets in chronically infected dogs is considerably shorter than in uninfected animals (4 days vs. 9 days). Also suggesting that the pancytopenias of the initial and terminal stages have different pathogenic mechanisms is the finding that the hematologic abnormalities of the acute stage resolve promptly with tetracycline, whereas the hematologic abnormalities of the third stage are very slow to resolve. In both beagles and German shepherds, persistent infection occurs despite a robust antibody response to *E. canis* (91) as would be expected with an intracellular pathogen. Formerly, it was thought that human ehrlichiosis was always self-limited and/or acute and that chronic infection, comparable to that in dogs, was not relevant to human disease. However, this notion is contradicted by a number of well-documented cases of chronic HME (92,93). The existence of a chronic stage (which may be difficult to discern) may explain the disparate pathological findings described earlier.

HGE. Animal models for HGE are presently under development. Preliminary studies with a murine model indicate that both inbred and outbred mice are competent for infection with the HGE agent and that infection tends to be low-grade (94). This is consistent with adaptation by the bacterium for survival in an enzootic horizontal transmission cycle between ticks and a natural mammalian reservoir. Immunodeficient SCID mice develop more severe infection as determined by persistence and tissue levels of organisms (94). Recently reported *in vitro* studies showing that the HGE agent can infect CD34+ HLA-DR+ bone marrow progenitors (95), suggest that the hematologic abnormalities of HGE also originate from infection in the marrow. These investigators also reported that the HGE agent could infect granulocytic as well as monocytic precursors (95). The ability of the agent of HGE, as well as *E. chaffeensis*, to infect more than one type of leukocytic precursor could explain why the leukopenia of both HME and HGE involves multiple white blood cell lines (96-98).

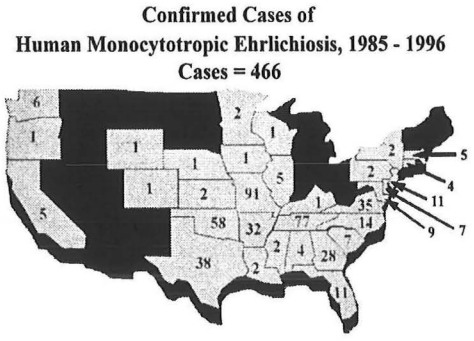

Figure 7

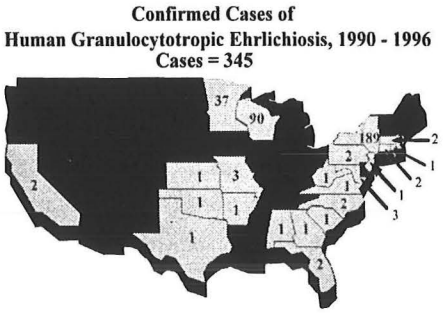

Figure 8

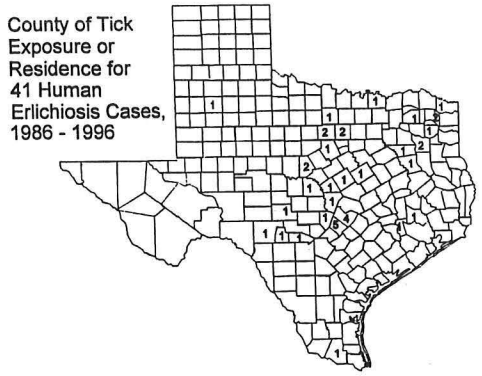

Figure 9

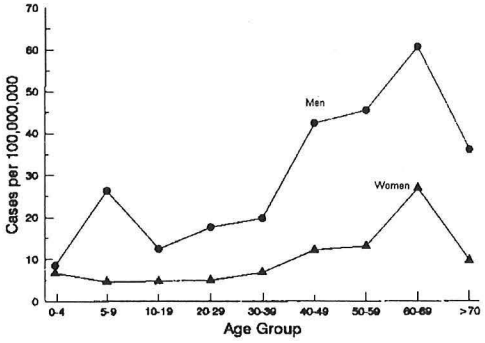

Figure 10. Age and sex distribution of HME.

EPIDEMIOLOGY AND ECOLOGY

Currently, there is no nationwide system or requirement for reporting either HME or HGE. Ehrlichiosis, however, is now reportable in some states, including Texas (as of mid-1996) (99). Serological surveys suggest that the (39)approximate 466 HME and 345 HGE confirmed cases reported to the CDC from 1986 to 1996 probably represent the proverbial tip of the iceberg (2). Further contributing to the likelihood of a substantial degree of under-reporting is the fact that most physicians are unaware of these diseases and undoubtedly are not making the diagnosis. (2). Figures 7 and 8 show the distribution of HME and HGE cases throughout the United States. Consistent with the distribution of their arthropod vectors, HME is found predominantly in southern and south central states, while HGE is observed mainly in the upper Midwest, New York, and New England. Areas of overlap also are noted. However, two findings suggest that many of the presumed HME cases in these overlap areas (100) may actually have been HGE: (i) HGE patient sera can cross-react with *E. chaffeensis* (99,101) and (ii) in one study from Connecticut, HGE agent , but not *E. chaffeensis*, was detected by PCR in field-collected ticks (100,102).

<u>HME</u>. Cases of HME have been reported from thirty states (2,99,103). In Oklahoma, the state with the highest number of confirmed cases (96), seroprevalence surveys demonstrated that the disease is at least as prevalent as RMSF (76). A prospective surveillance study of febrile hospitalized patients in southern Georgia showed that the prevalence of HME was seven to eight times higher than that of RMSF (75). Seroconversion rates of 1.31% were found in military personnel assigned to Ft. Chaffee during an observation period consisting of only weeks to months (104). All ehrlichiosis cases reported in Texas are thought to be HME (99). Of the thirty-nine cases which have been documented here since 1985, 34 were acquired in-state (99). A map showing the distribution of Texas cases (Fig. 9) reveals that many occurred in proximity to the Dallas-Ft. Worth area.

A large majority of patients with symptomatic HME report exposure to ticks or tick-bite within the three weeks preceding the onset of illness (77,96,104). As expected for a tick-borne illness, cases are predominantly rural and seasonal, with most (>90%) occurring between April and September, the months when ticks are most active (6). The median age of patients in two large series was 42 and 44 years, respectively, with a striking male preponderance (77,96). Interestingly, despite their similar modes of acquisition, patients with ehrlichiosis are significantly older than patient with RMSF (76). In two large studies, age greater than 60 years was a risk factor for severe disease (Fig. 10) (77,96). Regarding the relatively high incidence among elderly individuals, one of the most interesting (and humorous) epidemiological analyses involved an outbreak in a golf-oriented retirement community in Tennessee. Among men who golfed, the risk of infection was significantly greater for players who reported higher golf scores. The golfing practice that was most strongly associated with infection was retrieving a golf hit off the course rather than using a new ball (105).

***E. chaffeensis* has been detected in two tick species, *Amblyomma americanum* (the Lone Star Tick) and *Dermacentor variabilis* (the American dog tick) (106,107) both of which are found in Texas. *Amblyomma americanum* appears to be the more important of the two vectors (108). There is now reasonably strong evidence that white-tailed deer are the natural reservoir for *E. chaffeensis:* (a) seropositivity rates to *E. chaffeensis* are very high (greater than 40%) among deer in endemic states (13); (b) deer are susceptible to experimental infection with *E. chaffeensis* (109); (c) ticks allowed to feed on needle-inoculated deer could transmit *E. chaffeensis* to naive deer but not to dogs (110); (d) *E. chaffeensis* has been isolated in culture or detected by PCR in white-tailed deer populations which are heavily infested with *Amblyomma americanum* (111); and (e) mice inoculated intraperitoneally with material from *E. chaffeensis*-infected deer were unable to transmit the infection to naive ticks (111).**

HGE. Sizable numbers of cases of HGE have been reported from Massachusetts (101), Wisconsin and Minnesota (97), New York (98,112), Connecticut (113) and Rhode Island (50). Similar to HME, HGE shows a striking male and age preponderance (97). HGE occurs mostly during May, June, and July (the period of peak seasonal activity of *I. scapularis* nymphs) with a secondary peak in October, November, and December (peak activity for *I. scapularis* adults (97,98). In one large study, most patients admitted to frequent contact with animals, particularly dogs and horses, as well as heavy tick exposure (97). The remarkably similar geographic and seasonal distributions of HGE and Lyme disease suggested that these two diseases may share a common tick vector (10). Indeed, recent studies have identified HGE agent DNA in *Ixodes scapularis* ticks in Wisconsin, Connecticut, Massachusetts, and New York (14,48,102,114) and in small rodents in Minnesota (115). In one study, HGE agent DNA was not detected in *Dermacentor variabilis* ticks collected from the same region in which PCR-positive *I. scapularis* ticks were obtained (48). A geographic overlap in the distribution of Lyme disease and HGE also exists in Europe where *E. phagocytophila* and *Borrelia burgdorferi* are known to be transmitted by the same tick vector, *Ixodes ricinus* (116,117). Given that *Ixodes* ticks are globally distributed in temporal climates (dubbed "Cold Zones" by Persing (15)), it is likely that co-distribution of these two pathogens is world-wide.

TABLE 3. CLINICAL AND LABORATORY ABNORMALITIES IN EHRLICHIOSIS

SIGN/SYMPTOM/ LAB FINDING	% ABNORMAL
Fever	97
Headache	81
Chills or rigors	67
Myalgia	62
Malaise	60
Nausea	62
Anorexia	60
Vomiting	57
Diarrhea	38
Abdominal pain	22
Rash	40
Cough	39
Dyspnea	23
Lymphadenopathy	24
Confusion	24
Leukopenia	67
Thrombocytopenia	58
Abnormal LFTs	80
Elevated creatinine	29

In an elegant study, Telford and co-workers (14) presented compelling evidence that the agent of HGE is perpetuated in a deer tick-rodent cycle. They showed that (i) mice inoculated with blood from an HGE patient living on Nantucket Island developed granulocytic ehrlichiosis (interestingly, outbred *Peromyscus leukopus* (white-footed) mice developed lower levels of infection than inbred murine strains; (ii) ticks which fed on inoculated mice were able to efficiently transmit the infection to uninfected mice. (iii) salivary glands from ticks collected in the patient's backyard contained ehrlichial inclusions and were infectious for mice and (iv) ehrlichiae-infected mice from another Nantucket field site were able to xenodiagnostically infect ticks.

CLINICAL MANIFESTATIONS

The clinical manifestations of HME and HGE are indistinguishable and, for this reason, will be discussed jointly. Most CDC-confirmed cases of ehrlichiosis have involved patients with moderate to severe illness, and it is from these that a profile of the ehrlichiosis syndrome has emerged (Table 3). It must be emphasized, however, that the spectrum of illness is now known to be quite broad and includes asymptomatic and mild infections as well as the life-threatening severe sepsis-like syndromes (2,78,100).

Symptoms of ehrlichiosis typically begin 6 days to 10 days following a tick-bite (42,98), although incubation periods as long as 30 days have been noted (42). The signs and symptoms are nonspecific and protean but usually include fever, chills, headache, malaise, and myalgia at the outset (Table 3). Other symptoms, such as cough, abdominal pain, diarrhea, and vomiting also are relatively common but may be delayed in appearance (96). A paucity of physical signs is the rule. It is often said that ehrlichiosis differs from RMSF in that rash with the former is uncommon. This is not exactly true. Rash is noted in up to 40% of patients (as opposed to the 90% or greater incidence in RMSF) and, as with RMSF, its appearance is often delayed until several days into the illness (96,118). The rash of ehrlichiosis is typically maculopapular, less commonly petechial or scarlatiniform , and, unlike the early rash of RMSF, often sparing of the palms and soles (78,96,97,118,119). The rash of ehrlichiosis does not progress to the ecchymotic lesions characteristic of severe RMSF (118). There is also a suggestion in the literature that rash is relatively common in children with ehrlichiosis(77); vesicular lesions in children also have been described (118). The less frequent and less dramatic dermatologic manifestations of ehrlichiosis are important distinguishing clinical features from RMSF (Table 4).

During the first few days of illness, it is not unusual for laboratory values to be normal or

near-normal (Fig. 11). As the disease progresses, leukopenia, thrombocytopenia, anemia and mild to moderate elevations in transaminases and lactate dehydrogenase become apparent (Fig. 11) (96,98). It is interesting to note that the time course for the development of anemia is quite different from those for leukopenia and thrombocytopenia (Fig. 11) (96). Leukocyte counts typically nadir at values above 1500, platelet counts at values of 50,000. Although thrombocytopenia is common in both ehrlichiosis and RMSF, leukopenia is uncommon with RMSF (Table 4). Laboratory evidence of disseminated intravascular coagulation also is observed in a significant proportion of cases, occasionally in association with clinically significant hemorrhage (96). CSF abnormalities usually consist of a lymphocytic pleocytosis with mild to moderate increases in total protein (i.e., an aseptic meningitis picture); borderline hypoglycorrhachia also can be seen (119,120). CT scans with contrast are unremarkable even in patients with frank CNS involvement (120). To my knowledge, no data on MRI findings are available.

TABLE 4. COMPARISON OF EHRLICHIOSIS AND RMSF

	EHRLICHIOSIS	RMSF
Tick Exposure	Common	Common
Seasonal Distribution	Yes	Yes
Geographic Distribution	South/South Central U.S. (HME), Northeast/Upper Midwest (HGE)	South/ South Central U.S.
Acute Onset	Yes	Yes
Rash	(40%) Maculopapular, occas. petechial	(90%) Maculopapular to ecchymotic
Leukopenia	Common	Rare
Thrombocytopenia	Common	Common
Morulae	Yes	No
LFT Abnormalities	Common	Common
Vasculitis	No	Yes
CNS Involvement	Common	Common

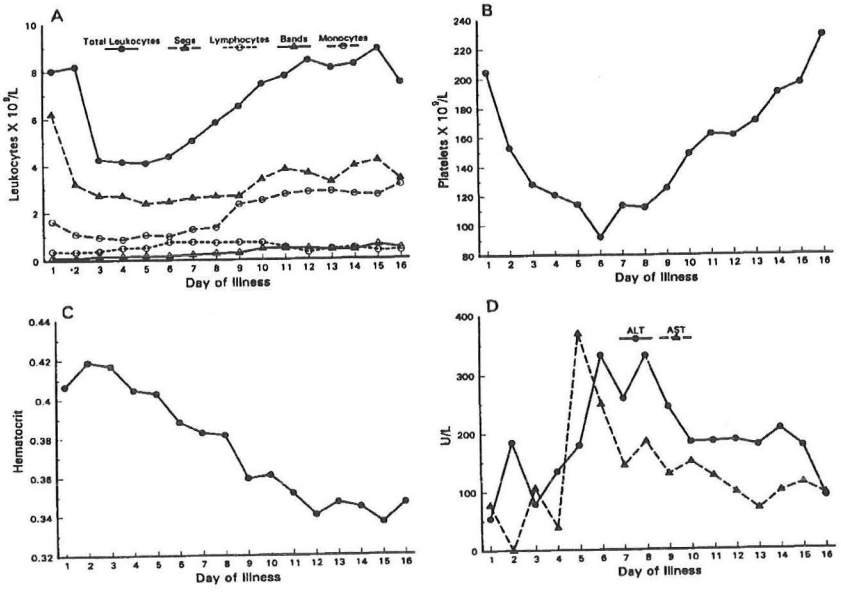

Figure 11. Time course of major laboratory abnormalities in ehrlichiosis

Patients with HME or HGE may be subject to a number of complications. These include pneumonia with or without respiratory insufficiency (ARDS) (96,97,119,121), myocarditis (122,123), renal insufficiency (96,97,119), and central nervous system abnormalities (encephalopathy/encephalitis) (119,120). Ratnasamy and co-workers (120) found that 15 of 57 patients with confirmed HME or HGE presented with symptoms or signs suggestive of neurological involvement, including severe

headache, confusion, lethargy, ataxia, hyperreflexia, photophobia, central nerve palsy, seizures, and nuchal rigidity. Eight of the 15 had abnormal cerebrospinal fluid values; altered mental status was the best predictor of CSF abnormalities. Although lacking rigorous controls, they noted a striking predominance of HME among ehrlichiosis patients with CNS dysfunction. Lastly, although HME and HGE are considered acute infections, reports of persistent disease, even after aggressive antimicrobial therapy, also have appeared, primarily in elderly patients (92,93,124). *E. chaffeensis* was detected in a postmortem examination of a patient who was infected 68 days previously and was treated with full courses of both doxycycline and chloramphenicol (93).

In addition to RMSF, the differential diagnosis of ehrlichiosis also includes bacterial sepsis, leptospirosis, and viral meningoencephalitis. Because of the overlap in vectors and geographic distribution, Lyme disease is often considered when patients present with HGE. However, in the absence of coinfection, the clinical features of the two infections usually can be differentiated (Table 5). While Lyme disease often is associated with headache, myalgias, and arthralgias, it is usually an indolent process and far less likely to present as a severe, acute febrile illness. Frank arthritis can occur early in Lyme disease but is not a feature of HGE. The localized, rash of Lyme disease, erythema migrans, also should be relatively easy to differentiate from the more diffuse rash of ehrlichiosis (125). Disseminated erythema migrans, on the other hand, might be more easily confused with the rash of ehrlichiosis. Abnormalities of the peripheral nervous system, such as facial nerve palsy and meningoradiculitis, are common with Lyme disease but atypical for ehrlichiosis; central nervous system disease in early Lyme disease are usually an aseptic meningitis. Lastly, leukopenia and thrombocytopenia are unusual laboratory abnormalities in Lyme disease, and their presence, even in a patient with typical Lyme disease, should prompt a work-up for concomitant HGE.

TABLE 5. COMPARISON OF EHRLICHIOSIS AND LYME DISEASE

	EHRLICHIOSIS	LYME DISEASE
Acute Febrile Illness	Yes	No
Tick Exposure	Common	Common
Seasonal Distribution	Yes	Yes
Geographic Distribution	South, South Central U.S. (HME), Northeast/Upper Midwest (HGE)	Northeast/Upper Midwest
Rash	Maculopapular, occas. petechial	Erythema Migrans
Neurological Abnormalities	Diffuse CNS	Cranial/peripheral nerve, aseptic meningitis
Arthritis	No	Common
Leukopenia	Common	No
Thrombocytopenia	Common	No
Morulae	Yes	No
LFT Abnormalities	Common	Uncommon

Tick-borne fever was recognized as a co-factor for the development of louping ill tick-borne encephalitis in goats; animals became ill and died of encephalitis only when coinfected with *E. phagocytophila* (64). Since then, numerous examples of severe and fatal coinfections that occur concomitantly with or immediately following tick-borne fever have been described, the most severe being a disseminated staphylococcal infection called pyemia. These findings have raised the question of whether ehrlichiosis is immunosuppressive in humans. This is indeed a plausible notion considering that ehrlichiosis involves widespread dissemination of bacteria throughout the reticuloendothelial cell system. While controlled studies have not been done,

it is interesting to note that opportunistic infections have been reported in severely ill ehrlichiosis patients (97,113). The possibility for immunosuppression by ehrlichiosis is especially relevant to the issue of coinfection with babesia and/or *B. burgdorferi.* Concern has been raised that concurrent ehrlichiosis could enhance the severity of babesiosis and/or Lyme disease, contribute to refractoriness to antibiotics, or interfere with the development of a diagnostic immune response (10).

DIAGNOSIS

Except for the instances in which morulae are observed in circulating peripheral blood leukocytes (see below), rapid diagnosis of both HME and HGE is not possible. Consequently, ehrlichiosis, like RMSF, should be suspected and empirically treated in any previously healthy individuals who develops an acute influenza-like illness three weeks or less following outdoor activity in an endemic area during the appropriate time of the year. Stated otherwise, accurate diagnosis of ehrlichiosis begins with careful history-taking, paying particular attention to recent outdoors activity, tick exposure, and travel history. Suspicion should be heightened further if characteristic laboratory abnormalities are present. The need to obtain an accurate travel history cannot be over-emphasized. It must be reiterated that the index case of ehrlichiosis (4) was acquired in Arkansas but presented in Detroit (4) and that more than 10% (4 of 39) of confirmed ehrlichiosis cases in Texas were acquired out of state (also Arkansas)(99). Experience with RMSF has long taught that temporizing or not thinking of the diagnosis is fraught with the potential for a poor, even fatal, outcome.

Failure to recognize the disease's propensity to mimic other entities also can be costly. Marty et al. (126) described a fatal HME case in which the presence of thrombocytopenia, encephalopathy, and renal failure led to a diagnosis of thrombotic thrombocytopenic purpura and treatment with high dose corticosteroids! Toxic shock syndrome also can be mimicked when severe ehrlichiosis presents with a diffuse maculopapular or scarlatiniform rash (119). It also must be emphasized that different diagnostic tests are required to diagnose HME and HGE and that the diagnostic approach should be tailored to the region in which infection was presumably acquired. For example, given the rarity of HGE in Texas (99), it would be wasteful to work-up all Parkland Memorial Hospital patients suspected of having ehrlichiosis for both HGE and HME. The following five modalities are available to establish a specific diagnosis of HME or HGE:

1. <u>Recovery by *in vitro* cultivation</u>. Recovery of pathogens from clinical specimens is the traditional gold standard for microbiological diagnosis. However, even in the best of hands, isolation of *E. chaffeensis* is both insensitive and time consuming; successful isolations have been accomplished with only two patients and, in each case, more than 30 days of cultivation were required (40) (6). Recovery of HGE using HL60 cells, on the other hand, appears to be reasonably sensitive (46,98) but is still logistically complex for most medical centers.

2. Detection of morulae. Detection of morulae in peripheral blood smears or buffy coat preparations is the most rapid means of ma king a specific diagnosis. Morulae are visualized as purple-stained dots or clusters of dots in the cytoplasm of leukocytes stained by Romanowsky-type techniques (e.g, Giemsa, Wright, or Diff-Quik stains). The method's utility, however, is limited to the acute febrile stage of infection (prior to treatment) when organisms are most prevalent. Identification of morulae is highly insensitive in HME even with exhaustive search of buffy coats from patients presumed to have high burdens of *E. chaffeensis* (6,103). On the other hand, morulae are found in up to 75% of HGE patients(42,97) but their detection is rather (42,97,98). Morulae in CSF mononuclear cells also have been observed in cytospin preparations from 4 of 21 HME patients with CNS involvement (120,127).

3. Serodiagnosis. Detection of specific antibodies against ehrlichial pathogens by indirect immunofluorescent assay (IFA) has long been the mainstay of diagnosis. Nevertheless, this modality is limited by the fact that acutely ill patients often have negative serological results while elevated titers are often obtained too late to be of practical clinical benefit. Diagnostic increases in antibody titers usually appear by the third week after onset of illness; a precipitous decline in antibody also usually occurs in the year following illness (2). Although *E. canis* functioned reasonably well as a surrogate antigen for *E. chaffeensis,* comparative tests have shown that *E. chaffeensis* is more sensitive for detecting homologous antibody during the early stages of the disease (103). Currently, *E. equi* is used as diagnostic antigen for HGE; assays based upon cultivated HGE agent are currently under development (128). The current CDC recommendations for serological diagnosis of HME and HGE call for a four-fold increase in titer or a single titer $\geq$ 1:128 for a patient with a clinically compatible history. A minor controversy in serodiagnosis is whether antibodies in the sera of HGE and HME patients cross-react with the heterologous antigen (97,101), thereby creating the possibility for mistakes in diagnosis. The present consensus appears to be that unidirectional cross-reactivity for HGE does exist (99). In other words, some HGE patient sera will cross-react with *E. chaffeensis*, though at lower titer than against *E. equi*, but that HME patient sera will not cross-react with *E. equi*. Recombinant antigens (e.g., the 120-kDa protein with tandem repeats) also show potential as serodiagnostic reagents (129).

4. PCR. Highly specific PCR assays using primers derived from the variable regions of the 16S rRNA gene have been developed to detect both *E. chaffeensis* (106,130) and the HGE agent (41,97). (130)The sensitivity of PCR on peripheral blood during the acute phase of illness appears to be at least 75% (98)and probably closer to 90% (6,43), far surpassing the 40% positivity rate of IFA in early disease (130). Interestingly, Everett et al. (130) also found that eight of 30 patients with HME were PCR-positive but failed to seroconvert during convalescence, suggesting that serodiagnosis may be less sensitive than is generally assumed. PCR-positivity of CSF in HME patients with CNS involvement also has been reported (120,130).

5. Immunocytochemical detection. Immunocytochemical procedures are useful for confirming that morulae-like structures seen in tissues or body fluids are, in fact, ehrlichiae.

The sensitivity of immunocytochemistry drops considerably, however, when applied to specimens in which morulae are not readily detectable by routine microscopy (80,131).

TREATMENT

Conventional antimicrobial agents, such as beta-lactams and aminoglycosides, are ineffective against ehrlichiae *in vitro* (132,133) and lack clinical efficacy as well (96,98). Consequently, the importance of recognizing possible cases of ehrlichiosis derives chiefly from the fact that appropriate antimicrobial therapy requires agents which are not normally used in the treatment of possible sepsis. Doxycycline therapy for both HME and HGE is associated with rapid defervescence and a high cure rate (96-98). The drug also has excellent *in vitro* activity against both *E. chaffeensis* and the HGE agent (132,133). The major controversy in ehrlichial therapeutics centers about the use of chloramphenicol, a drug which is considered highly efficacious for RMSF. In *in vitro* tests, chloramphenicol has fared poorly against both *E. chaffeensis* and the agent of HGE (132,133). Nevertheless, despite clearcut therapeutic successes, (132) most authorities are wary of the drug because of its poor *in vitro* activity and anecdotal treatment failures, and they recommend against its use as a first-line agent, even for children (6). Doxycycline is a desirable first line agent for ehrlichiosis because it also will successfully treat coinfection with Lyme disease, whether symptomatic or asymptomatic at the time of presentation(134) (125).

Presently, only a small amount of information is available to guide the choice of alternative antibiotics. Rifampin has excellent activity against both ehrlichial pathogens (132,135) though one would hesitate to use it alone for fear of developing resistance. Ciprofloxacin has some activity against both pathogens though there is no clinical experience with the drug (132,133). Other antimicrobials commonly used for intracellular pathogens (e.g. erythromycin/azithromycin, trimethoprim-sulfamethoxazole, clindamycin) appear to lack anti-ehrlichial activity (132,133).

COINFECTION

As noted earlier, the possibility for coinfection with Lyme disease, ehrlichiosis, and babesisos was suggested as soon as it became apparent that these three infections share a common vector and geographical distribution. In fact, in the past several years, substantial evidence has arise that coinfection with these three pathogens does occur. Serologic surveys in Lyme disease endemic areas have identified individuals (approximately 10%) with antibodies against more than one tick-borne pathogen (136,137). Coinfected ticks have been identified by several groups of investigators, and the rates of tick coinfection appear to rougly parallel the rates for coinfection of humans (14,15,114,138). Lastly, well documented cases of coinfection also have been reported (139-141), confirming that *Ixodes scapularis* is capable of simultaneously transmitting more than one pathogen. Consistent with the fact that coinfection with *B. microti*

and the HGE agent is least common in ticks, thus far, a case of HGE and babesiosis coinfection has not yet been reported. Although HME and RMSF also share vectors and occur in many of the same regions of the country (Table I), to my knowledge, coinfection with these two diseases has not been reported.

Physicians need to be aware that patients may present with signs and symptoms of more than one disorder. Broad use of doxycycline is certainly the optimal therapeutic approach in instances where coinfection is possible (although it must be pointed out that this agent will not cure babesiosis). Whether infection with one agent can modify the clinical course and/or response to therapy of one of the others is an area of controversy and intensive investigation, fueled by the limited evidence that both HGE and babesisos are immunosuppressive (15). In this regard, Kraue et al. (139) reported that babesiosis can enhance the severity and duration of Lyme disease. Combined babesisos and ehrlichiosis in dogs also is more severe than either infection alone (142). Immunosuppression by the HGE agent or *B. microti* also raises the concern that diagnostic antibody responses will be blunted or abrogated. Studies are ongoing in endemic areas to evaluate all of these possibilities.

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