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Human Ehrlichiosis and Anaplasmosis

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OVERVIEW

Human ehrlichiosis and anaplasmosis are acute febrile tick-borne diseases caused by various members from the genera *Ehrlichia* and *Anaplasma* (Anaplasmataceae). Human monocytotropic ehrlichiosis was first reported in the United States in 1987, but during the ensuing 20 years it has become the most prevalent life-threatening tick borne disease in the US. The emergence of ehrlichiosis and anaplasmosis is become more frequently diagnosed as the cause of human infections, as animal reservoirs and tick vectors have increased in numbers and humans have inhabited areas where reservoir and tick populations are high.

Ehrlichia chaffeensis, the etiologic agent of human monocytotropic ehrlichiosis (HME) is an emerging zoonosis that causes clinical manifestations ranging from a mild febrile illness to a fulminant disease characterized by multi-organ system failure. The primary tick vector of HME is the Lone Star tick (*Amblyomma americanum*). *A. phagocytophilum* causes human granulocytotropic anaplasmosis (HGA), previously known as human granulocytotropic ehrlichiosis (HGE). *Anaplasma phagocytophilum* is transmitted by *Ixodes scapularis*, which also transmits agents that cause Lyme disease and babesiosis.

HME and HGA have similar clinical presentations including fever, headache, leukopenia, thrombocytopenia and elevated liver enzymes. Symptoms typically begin a median of 9 days following tick bite, with the majority of patients seeking medical attention within the first 4 days of illness. Neurologic manifestations are most frequently reported with HME. In this article, we review recent advances in the understanding of ehrlichial diseases related to microbiology, epidemiology, diagnosis, pathogenesis, immunity, and treatment of the two prevalent tick-borne diseases found in the United States, HME and HGA.

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MICROBIOLOGY

The agents of human tick-borne ehrlichiosis include *Anaplasma* (formerly *Ehrlichia*) *phagocytophilum*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and recently reported *E. canis* (Table 1). These pathogens are members of the family Anaplasmataceae, in the order Rickettsiales, and they are classified as α-proteobacteria (1–4). The evolutionary relationships determined by 16S ribosomal RNA gene (*rrs*) and *groESL* comparisons indicate that *Ehrlichia* and *Anaplasma* spp., share a common ancestor with other obligate intracellular pathogens such as *Wolbachia*, *Neorickettsia*, *Orientia*, and *Rickettsia* (3–7). In addition to causing human disease, *Ehrlichia* species are important veterinary pathogens. Canine ehrlichiosis, first described in 1935 in Africa, is caused by *Ehrlichia canis* and is transmitted by the Brown Dog tick, *Rhipicephalus sanguineus* (6,7). *Ehrlichia ewingii*, an agent that is transmitted by *Amblyomma americanum*, infects granulocytes and causes human ehrlichiosis ewingii (HEE) (8–10). Recent phylogenetic studies have concluded that the economically important veterinary pathogen *Ehrlichia* (formerly *Cowdria*) *ruminantium* (described in 1925) belongs to the genus *Ehrlichia* (10–16) (Figure 1).

Agents of human tick-borne ehrlichioses are small (approximately 0.4–1.5 μm), obligately intracellular Gram negative bacteria that replicate in membrane-bound compartments inside host granulocytes *(A. phagocytophilum* and *E. ewingii)* or mononuclear phagocytes *(E. chaffeensis* and *E. canis)* (17,18) (Figure 2). Ehrlichiae replicate within the host vacuoles forming microcolonies called morulae, derived from the Latin word “morus” for mulberry (18–20). All *Ehrlichia* species pathogenic for humans can be cultivated in cell culture except *E. ewingii* (Figure 3A–C).

Ehrlichia and *Anaplasma* exist intracellularly in two morphologically distinct ultrastructural forms dense-cored cells (DC) (0.4–0.6 μm) and reticulate cells (RC) (0.4–0.6 μm by 0.7–1.9) (Figure 4) (20). DCs are smaller and have an electron dense chromatin while the larger RCs have uniformly dispersed nucleoid filaments and ribosomes. *In vitro* kinetic analyses have shown that DC ehrlichiae predominate during the first 24 hour post-infection, suggesting that dense core forms are critical for bacterial adhesion and internalization. By 48 h post-infection, RC forms of ehrlichiae that divide by binary fission predominate. At 72h after infection, the RC ehrlichiae mature into dense-cored cell forms, correlating with the time when DC ehrlichiae are released to begin a new cycle (21,22) (Figure 4). Consistent with their life cycle, DC and RC forms of ehrlichiae differentially express two tandem repeat containing proteins (TRP); TRP120 and TRP47. The TRP47 is a secreted effector protein that interacts with numerous host cell proteins involved in cell signaling, transcriptional regulation and vesicle trafficking. (23–26).

Ehrlichia and *Anaplasma* species have relatively small genomes (0.8–1.5Mb) that have undergone several types of reductive evolutionary processes as they have lost redundant genes and developed dependence on the host cell for necessary functions (27). *Ehrlichia* have a small subsets of genes associated with host-pathogen interactions including tandem repeat containing proteins and ankyrin repeat proteins. Other common features of the *Ehrlichia* genomes include low GC content and high proportion of non-coding sequences. *E. chaffeensis* and *A. phagocytophilum* also have genes for synthesis of all nucleotides, vitamins and cofactors (27).

Ehrlichia and *Anaplasma* have the characteristic Gram negative cell wall structure, but lack important cell membrane components including lipopolysaccharide and peptidoglycan (28). However, the ehrlichial cell wall is rich in cholesterol, which is derived from the host cell and appears to be important for ehrlichial survival and entry into mammalian cells. Recent study has demonstrated that *A. phagocytophilum* exploits host cell cholesterol derived from the low

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density lipoprotein receptor (LDLR)- mediated uptake pathway and LDLR regulatory system, to accumulate cholesterol in their inclusions to facilitate replication (29). In addition to the possible role of cholesterol in supporting bacterial cell wall and promoting internalization, it is possible that cholesterol rich cell walls may also function as ligands for stimulation of innate and acquired immune responses. In support of this conclusion, a recent report showed that heat-killed *Ehrlichia muris* are recognized by mouse CD1d-restricted natural killer T-cells, which recognize lipids and glycolipid (30).

E. chaffeensis, *E. ewingii*, *E. canis*, and *A. phagocytophilum* have immunodominant outer membrane proteins, which are members of Pfam PF01617 and constitute the OMP-1/MSP2/P44 families (31–39). Evaluation of different *E. chaffeensis* isolates demonstrated a differentially expression of p28/30-Omp proteins in infected macrophages and tick cell cultures. In infected macrophages, the dominant *E. chaffeensis* expressed proteins are the products of the p28-Omp 19 and 20 genes (40,41). In cultured tick cells derived from *E. chaffeensis* vector, *Amblyomma americanum* and non-vector (*Ixodes scapularis*) ticks, *E. chaffeenesis* expression consists only of the p28-Omp 14 protein (40,41). It is postulated that this differential expression of proteins within the p28/p30-Omp locus may be critical for the adaptation of *Ehrlichia* species to their different hosts (mammals and ticks). These observations support the long evolutionary relationship between the bacterium, its vector and its niche within host monocytes. How differential expression of these proteins provides an advantage to the organism within these extremely different environments is not known. Thus, *in vivo* investigations of ehrlichial infection in animal models and natural tick vectors will be necessary to determine *in vivo* the biological significance of these *in vitro* observations. To this end, a recent study by Ganta *et al.* (42) determined that C57BL/6 mice have different responses to infection depending on the source of the inoculum. ISE6 tick cell-derived *Ehrlichia* inoculated into mice results in a more persistent infection, which includes relapses of increasing bacterial load On the other hand, the *A. phagocytophilum* genome has three *omp-1*, one *msp2,* two *msp2* homologs, one *msp4*, and 113 *p44* loci belonging to the OMP-1/MSP2/P44 superfamily (37–40). P44 plays a role in the binding of *A. phagocytophilum* to surfaces of neutrophils and in antigenic variation and evasion of host immune responses. The *P44s* are diverse, include several paralogs *(p44-1 to p44-65)* expressed in mammals and ticks and confer antigenic environmental adaptation, especially during tick transmission (37–40).

Ehrlichiae also express several secreted ankyrin, tandem repeat (23–25,43–46) and putative lipoptoteins (28,29,35) that are major targets of the humoral immune response. Several genes code for major immunoreactive proteins of *E. chaffeensis* including ankyrin (200 kDa) and tandem repeat containing proteins (120-, 47- and 32-kDa proteins) (23–25). These proteins contain major antibody eptiopes that are molecularly distinct and elicit strong species-specific host immune responses. The TRP47 has recently linked with host-pathogen interactions that suggest a complex network of interactions between TRP47 and the host (25) (Figure 5). Another major immunoreactive protein includes TRP32 (variable-length PCR target protein) *E. chaffeensis*, which has one conformational and one continuous epitope within the 90-bp TRs (24). TRP32 repeats vary in number among *E. chaffeensis* isolates; hence, it has been utilized as a molecular target for differentiation of *E. chaffeensis* isolates (24).

Ehrlichia and *Anaplsama* also contain genes for type IV secretion systems, which are structures known to use a complex of transmembrane proteins and a pilus to mediate the translocation of macromolecules across the cell envelopes of both gram negative and gram positive bacteria (47–49,27). Genes for Type IV secretion are cotranscribed with genes for enzymes, such as superoxide dismutase (*sodB*), that catabolize reactive oxygen species. However, whether these are secreted by type IV secretion complexes needs to be investigated. Interestingly, among the genes that encode the classic type IV secretion system, VirB is common to all *Ehrlichia* species and has been associated with secretion of toxins (47–49). One substrate, Ank A of *A.*

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phagocytophilum, of the type IV secretion system has been identified. AnkA is secreted into host cell cytoplasm where it interacts with host cell tyrosine kinase Abl and phosphotase SHP-1 and eventually transported into the host cell nucleus. There it interacts with nuclear chromatin and appears to target gene regulatory regions (44–46). Other virulence genes, such as those that encode two-component regulatory systems have also been described and studied, and appear to be involved in intracellular survival by inhibiting lysosomal fusion. Further comparison of ehrlichial genomes will provide insight and facilitate investigations of bacterial virulence factors, disease pathogenesis, and mechanisms of immune modulation, and will provide targets for vaccines or new antimicrobial therapies.

EPIDEMIOLOGY

HME Epidemiology

HME was first described in 1986, and now more than 2,300 cases have been reported to the CDC in the past 19 years (50–52). While the average incidence of HME in the United States is estimated at 0.7 cases/million population, this incidence is based on passive surveillance and is likely a significant underestimation of the actual disease incidence (Figure 6A). Active surveillance in endemic areas has suggested rates of HME of 100–200 cases per a population of 100,000 (51,52). The true incidence of human infection with *E. chaffeensis* is likely to be much higher, as two-thirds of the infections are either asymptomatic or minimally symptomatic (53–59). A sero-prevalence study found that 20% of the children residing in endemic areas had detectable antibody to *E. chaffeensis*, without prior history of clinical disease (58,59).

Similar to other tick borne diseases, the distribution of the arthropod vectors and vertebrate reservoirs correlates with the human disease incidence (60,61). States with the highest reported rates of HME include Mississippi, Oklahoma, Tennessee, Arkansas, and Maryland (62) (Figure 6B). The dominant zoonotic cycle of *E. chaffeensis* involves a reservoir of many persistently infected white-tailed deer (*Odocoileus virginianus*) and the tick vector, *Amblyomma americanum*, prevalent throughout the southeast and southcentral United States (63–66) (Figure 7A). Other reservoirs such as dogs and coyotes and other tick vectors including *Ixodes pacificus* (67), *Ixodes ricinus* (68), *Haemophysalis yeni* (69), *Amblyomma testudinarium*, *Amblyomma maculatum* and *Dermacentor variabilis* (69) may also have a limited role in human transmission. Similar to other ticks, *Amblyomma* ticks have three feeding stages (larval, nymph, and adult); each developmental stage feeds only once. Trans-stadial (i.e. larva-nymph-adults) transmission of *Ehrlichia* occurs during nymph and adult feeding stages because larvae are uninfected. In contrast to *Rickettsia* spp., *Ehrlichia* are not maintained by trans-ovarial transmission (Figure 7B).

There have been case reports of patients co-infected with both *E. chaffeensis* and *R. rickettsii*, which, although spread by different tick vectors, share a common geographic distribution (70). Among cases of HME reported to the CDC between 2001 and 2002, 61% were male, and 95% of cases self-identified as Caucasian (62). The median age for infection was 53 years; however the age-specific incidence was highest in the group aged 70 years and above. While cases were reported year-round, the greatest number of cases occurred during the period of May through August, corresponding to periods of abundant tick populations and human outdoor recreation.

Epidemiology of HGA

In the early 1990s, patients from Michigan and Wisconsin with a tick bite history experiencing a febrile illness similar to HME were described (71,72). These cases were distinguishable by the presence of inclusion bodies in granulocytes rather than monocytes, causing this syndrome initially to be termed human granulocytic ehrlichiosis (HGE). The disease has recently been

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renamed human granulocytic anaplasmosis, or HGA, after phylogenetic analysis reclassified *Ehrlichia phagocytophilum* as a member of the genus *Anaplasma* (72,73).

More than 2,900 cases of HGA have been reported to the CDC between 1994 and 2005, with the annual number of cases of HGA exceeding that of HME at an estimated annual incidence of 1.6 cases per million in the US. The highest annual incidence rates of HGA in the United States have been reported in Connecticut (14–16 cases per 100,000), Wisconsin (24 to 58 cases per 100,000 population), and New York state (2.7/100,000) (64) (Figures 8A and B). Active surveillance in endemic areas has identified incidence rates of >50 cases per 100,000 population (73–75). As with *E. chaffeensis*, serosurveillence studies suggest that asymptomatic disease is common (74–75).

A. phagocytophilum is transmitted by *Ixodes scapularis* (Figure 9A) in New England and North Central United States, *Ixodes pacificus* (Figure 9B) in the western United States, *I. ricinus* in Europe, and *I. persulcatus* in Asia. *Ixodes scapulais* is also the tick vector for *Borrelia burgdorferi*, *Babesia microti*, and tickborne encephalitis viruses, and therefore ~10% of patients with HGA have serologic evidence of coinfection with Lyme disease, or babesiosis (76). The reservoir for *A. phagocytophilum* is primarily small mammals such as the White-footed mouse (*Peromyscus Leucopus*); Dusky-footed Wood rats (*Neotoma fuscipes*) or others such as such as *Apodemus*, *Microtus* or *Clethrionymus* species, with humans serving as dead-end hosts (72). Transmission of *A. phagocytophilum* from tick-mammalian reservoir to humans is similar to that of *E. chaffeensis*.

Demographic characteristics of HGA patients are similar to HME patients. The median age is 51, with more than 95% of cases are reported in Caucasians, with a slight male predominance (62). Cases occur year-round, with a peak incidence during June and July, perhaps reflecting the shorter arthropod season in these northern states or the relative importance of the nymphal stage of *Ixodes* ticks in disease transmission. Given the ubiquity of the tick vector, it is not surprising that cases of HGA have been confirmed world-wide, including Europe and Asia (China, Siberian Russia, and Korea) (Figure 10A and B).

Epidemiology of HEE

Ehrlichia ewingii was exclusively a canine pathogen until a series of four human cases of *E. ewingii* infection were described in 1999 (5,8,9). The epidemiology of HEE remains poorly defined due to the lack of a specific serologic assay for this organism and the absence of a dedicated reporting system for this infection. Most infections reported to date have occurred in patients with HIV (5,8,9), or who were immunosuppressed following organ transplantation (77). *Amblyomma americanum*, the primary vector for *E. chaffeensis*, is also the primary vector for *E. ewingii.* Most cases of HEE have been reported in Tennessee, Missouri, and Oklahoma. However, *E. ewingii* infection in deer, dogs and ticks have been described throughout the range of the Lone Star tick, suggesting that human infection with this pathogen might be more widespread than is currently documented (72).

CLINICAL PRESENTATION

HME General Clinical Features

Human monocytotropic ehrlichiosis is a more severe disease than HGA or HEE, with 42% of cases requiring hospitalization, and a case-fatality rate of 3% (1,3,51–54,65). The median age of patients with either infection is ~50 years, and slightly more males (57% – 61%) are infected than females. Up to 17% of patients develop life-threatening complications, although severe disease and death are more common in immunocompromised patients (5,19,62). HME can be fatal in immunocompetent patients and manifested as a multisystem disease resembling toxic or septic shock syndrome, or Rocky Mountain spotted fever, except for the infrequent

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occurrence of rash (78–80). Other life-threatening manifestations include cardiovascular failure, aseptic meningitis, hemorrhages, hepatic insufficiency or failure, interstitial pneumonia, and adult respiratory distress syndrome (79–82). The severity of the disease is greater in elderly and immunocompromised patients; however, HME can be fatal even in immunocompetent patients. Several studies have reported an association between the use of sulfonamide antibiotics and severe manifestations of *Ehrlichia* (53,83). Whether this represents a causal relationship is unknown. A study of HME among transplant patients found no difference in severity of illness among patients taking prophylactic sulfa-antibiotics (53,77).

Fever is an almost universal symptom (97%), followed by headaches (80%), myalgias (57%), and arthralgias (41%) (49–51,74). A skin eruption is relatively common among children with HME, occurring in 66% of pediatric cases compared to 21% of adults (51,52). A rash is present in 10% of cases of HME and can be maculopapular, petechial, or be characterized by diffuse erythroderma (80), but typically spares the face, palms, and soles of the feet. Nausea, vomiting, abdominal pain and cough are variably present. Gastrointestinal symptoms such as nausea, vomiting, diarrhea and anorexia are sometimes reported in the course of HME, mainly in children. Other frequently observed signs and symptoms in children and pregnant women with HME are altered mental status and abdominal pain that can be severe mimicing acute appendicitis..

Although the clinical manifestations of *E. chaffeensis* infection are nonspecific, laboratory abnormalities provide important diagnostic clues. A prospective cohort study of patients in an endemic area presenting with a febrile illness following a tick-bite, found a significantly lower WBC (mean 4.6×10^9 cells/L), neutrophil (mean 2.6×10^9 cells/L), and platelet count (mean 172×10^9 cells/L) among patients with HME than non-infected patients, and elevated transaminase levels were present in 83% of cases (72). In pediatric patients, mild hyponatremia has been reported in 50% of the cases (51,52,72), but this finding has been less frequently noted among infected adults.

HME Neurologic Features

The most frequent neurologic manifestation of HME is meningitis or meningoencephalitis. Central nervous system involvement is identified in approximately 20% of patients with HME (82), and in some cases may be associated with seizures and coma. Uncommon complications include cranial nerve palsy, with onset after initiation of effective antimicrobial therapy being reported (79–82). Long-term neurologic sequelae in children are uncommon, but include cognitive delays, fine motor impairment, and persistent foot drop. Subjective neurocognitive deficits following meningoencephalitis have also been reported in adults (72,82–87).

Among patients with HME who undergo lumbar puncture, CSF pleocytosis is identified in approximately 60% (82). While most samples have a lymphocytic predominance, a neutrophilic or mixed picture is found in a third of cases (82). The CSF WBC count is typically < 100 cells/mm^3, and protein may be mildly elevated. Morulae are rarely identified in CSF monocytes by Giemsa stain (1,80–82). Radiographic imaging may be normal, or may show leptomeningeal enhancement. Bilateral medial temporal lobe enhancement has been reported for a case with PCR evidence of *E. chaffeensis* and *A. phagocytophilum* coinfection (88). Electroencephalogram may show nonspecific slowing (82). Although pathologic review of brain tissue from patients with HME neurologic dysfunction is limited, one study reported atypical lymphoid infiltration of the leptomeninges and Virchow-Robin space, with sparing of the brain parenchyma, while others have not shown CNS pathology (82).

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HGA General Clinical Features

HGA resembles HME with respect to the frequency of fever, headache, and myalgias, but rash is uncommon, noted in less than 10% of patients (44,50,75,81). As with HME, leukopenia, thrombocytopenia, and elevations in transaminases are important clues to the diagnosis. HGA tends to be a less severe illness than HME, although life-threatening complications including acute respiratory distress syndrome, acute renal failure, and hemodynamic collapse have been reported.

HGA Neurologic Features

Central nervous system involvement is uncommon in HGA, with meningoencephalitis reported in only approximately 1% of cases (88,90). In contrast, a number of different peripheral nervous system manifestations have been described, including brachial plexopathy, cranial nerve palsies, and demyelinating polyneuropathy (1,88,90), and bilateral facial nerve palsy (88,90), where recovery of neurologic function may be delayed over several months. As the geographic distribution of *B. burgdorforii* is similar to *A. phagocytophila*, patients should be tested for co-infection since Lyme disease has similar neurologic manifestations. Although the cause of neurologic dysfunction in HGA is not yet known, it is thought to be due to complicating opportunistic infections, or concomitant co-infection with *B. burgdorforii*. Lumber puncture is performed less frequently for HGA than HME. Reported CSF abnormalities include lymphoctyic pleocytosis and moderate elevation in proteins (87,88,90).

HEE General Clinical Features

Little is known of the clinical spectrum of HEE due to the paucity of reported cases. Symptoms appear to be similar to those described for HME and HGA. Despite the fact that the majority of HEE infections have been in immunocompromised hosts, the clinical manifestations appear to be milder (77,84). Findings of leukopenia, thrombocytopenia, and abnormal liver function tests are variably present (77,84).

HEE Neurologic Features

Headache is a frequent symptom in HEE, and may be associated with meningitis, but the frequency of this finding and the spectrum of neurologic manifestations are unknown. One instance of neutrophilic pleocytosis in a patient with HEE has been reported (8).

Pathogenesis

Following tick bite, *Ehrlichia* and *Anaplasma* enter circulation where they multiply within their target cells monocytes/macrophages and polymorphonucelar leukocytes, respectively. *Ehrlichia* and *Anaplasma* enter through receptor-mediated endocytosis via a glycophosphoinositol anchored receptor within caveolae- or lipid rafts. *Ehrlichiae* were found to localize exclusively within endosomes that avoid phagolysosomal pathways (91). *Ehrlichia* and *Anaplasma* multiply within endosomes ultimately reprograming host cell defense mechanisms and processes in order to facilitate their survival.

Pathogenesis of HME

Following entry into mononuclear phagocytes, *E. chaffeensis* inhibits phagolysosome fusion involving genes controlled by a two-component regulatory system. *E. chaffeensis* also suppresses and induces host genes to facilitate their intracellular survival (91–93). Microarray analysis of THP-1 cells infected with *E. chaffeensis* revealed downregulation of Th1 cytokines such as IL-12 and IL-18, which are important inducers of adaptive Th1 mediated immune responses, and genes such as SNAP 23 (synaptosomal-associated protein, 23 kDa), Rab5A (member of RAS oncogene family), and STX16 (syntaxin 16), which are involved in membrane

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trafficking, while upregulating apoptosis inhibitors and cell cyclins (93). Recently an immunoreactive, secreted 200 kDa ankyrin protein (Ank200) of *E. chaffeensis* has been shown to be translocated to the host cell nucleus where it binds gene regulatory regions within *Alu* elements and is thought to play a role in host cell gene regulation (25). *E. chaffeensis* also circumvents host defenses by inhibiting the signal transduction pathway (Jak/Stat) of interferon-γ–mediated anti-ehrlichial activity, and increasing transferrin receptor delivery of iron to the ehrlichial vacuole. *In vitro* studies have shown that *E. chaffeensis* down-regulates surface expression of toll-like receptors (TLR) 2 and 4 and CD14 on infected target cells, and inhibits activation of several transcription factors that are involved in the induction of proinflammatory innate immune responses (92,93). The mechanism by which down-regulation of TLR 2 and 4 benefits survival within the macrophage is not understood, as pathogen-associated molecular patterns (PAMPs) have not been identified in ehrlichiae, and as mentioned above, the traditional ligands for these receptors, peptidoglycan and LPS, that activate TLR-2 and -4, respectively, are not present in the bacterium (28). However, it is postulated that loss of traditional PAMPs in Ehrlichia genome may enable them to persist within their tick vectors, without inducing strong innate defenses that are normally elicited by these patterns (94).

Studies with *Ehrlichia muris* have demonstrated that activation of innate lymphocytes such as natural killer T cells (NKT) occurs in a manner that is independent of TLRs, but dependent upon CD1d expression on antigen presenting cells such as the dendritic cells. Although NKT stimulation by *Ehrlichia* results in the production of IFN-γ and elimination of intracellular ehrlichiae (30), they also contribute to the development of *Ehrlichia* induced toxic shock-like syndrome in murine models of fatal monocytic ehrlichiosis (95).

Frequent pathologic findings of HME include granuloma formation, myeloid hyperplasia, and megakaryocytosis in the bone marrow (79–81,88). Some patients develop erythrophagocytosis and plasmacytosis, suggesting a compensatory response. Other pathologic findings in patients with severe HME include focal hepatocellular necrosis; hepatic granulomas; cholestasis; splenic and lymph node necrosis; diffuse mononuclear phagocyte hyperplasia of the spleen, liver, lymph node, and bone marrow; perivascular lymphohistiocytic infiltrates of various organs including kidney, heart, liver, meninges and brain; and interstitial mononuclear cell pneumonitis (96–98). The severe pathology and multi-organ involvement in severe and fatal HME in immunocompetent patients is thought to be related to dysregulation of the host immune response that leads to tissue damage and eventually multi-system organ failure (99). This conclusion is based on the finding that in fatal disease in the form of toxic shock-like syndrome, uninfected hepatocytes undergo apoptosis without evidence of ehrlichial infection (96–98). Similarly, analysis of hepatic tissues from autopsy cases in immunocompetent patients with HME showed lymphohistiocytic foci, centrilobular and/or coagulation necrosis, Kupffer cell hyperplasia, and marked monocytic infiltration, while *Ehrlichia*-infected cells are rarely identified (96–98,100). In contrast, an overwhelming ehrlichial burden in the organs was observed in HME patients who are immunocompromised due to other infections such as HIV or chemotherapy (96,98). The hypothesis that severe and fatal *Ehrlichia*-induced toxic shock like syndrome is due to an immunopathologic mechanism and is supported by studies in murine models of fatal ehrlichiosis, where lethal ehrlichial infection with virulent *Ehrlichia* species named *Ixodes Ovatus Ehrlichia* (IOE) (101,102) results in a progressive, fatal ehrlichiosis that mimics toxic shock-like syndrome (103). Characteristic of this disease in murine model of fatal monocytotropic ehrlichiosis include focal hepatic necrosis and apoptosis compared to formation of granuloma in animal model of mild monocytotropic ehrlichiosis (Figure 11), liver dysfunction marked by elevated liver enzymes (AST &ALT), significant leucopenia and lymphopenia, and CD4+T cell apoptosis (103–106). Further analysis indicated that severe and fatal primary HME in animals is due to the early overproduction of pro-inflammatory cytokines (e.g. TNF-α), and anti-inflammatory IL-10 cytokines. Interestingly, CD8+T cells appear to

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play a pathogenic role in HME where fatal disease is correlated with significant expansion of cytotoxic CD8+T cell producing TNF-α and IFN-γ. Mice that lack CD8+T cells and infected with virulent *Ehrlichia* species are protected against fatal disease, showing decreased tissue injury, normal CD4+T cell populations and increased protective CD4+Th1 responses, which suggest that CD8+T cell mediate lymphopenia and tissue damage in fatal HME in immuocompetent host (105). Consistent with pathogenic role of CD8+T cells in fatal murine ehrlichiosis, Dierberg and Dumler (107) reported a significantly greater amount of hemophagocytosis (macrophage activation) and an increased number of $CD8^+$ cells associated with low bacterial burden in the lymph nodes of patients who died of HME (Figure 12A-C). Thus, both human and animal model data suggest that organ pathology and fatal disease is indeed due to immune mediated pathology.

Pathogenesis of HGA

A. phagocytophilum is observed predominantly in neutrophils in the peripheral blood and tissues from infected individuals. *A. phagocytophilum* has the unique ability to selectively survive and multiply within cytoplasmic vacuoles of PMN cells by delaying their apoptosis through upregulation of anti-apoptotic bcl-2 family member bfl-1 (A1), and blocking anti-FAS (CD95/Apo-1)-induced programmed cell death of human neutrophils (108,109). *A. phagocytophilum* uses multiple evasion strategies to inhibit neutrophil anti-microbial functions (110,111). Some studies have suggested that one of the mechanisms by which *A. phagocytophilum* avoids the toxic effects of neutrophils is its ability to inhibit the fusion of the lysomes with the cytoplasmic vacuoles and by arresting or inhibiting other signaling pathways related to respiratory burst (108–113). The mechanism by which A. *phagocytophilum* inhibits phagosome–lysosome fusion remains to be elucidated. *A. phagocytophilum* appears to modulate host cell gene transcription through AnkA, a secreted protein which is transported to the infected host cell nucleus through binding to protein: DNA complexes in neutrophil nuclei (114). Further studies are required to establish the effects of AnkA binding on neutrophil functions. The molecular basis by which *A. phagocytophilum* affects respiratory burst of granulocytes shown to be due to the down-regulation of $gp91^{phox}$ and rac2, two important components of NADPH oxidase (111,113). Paradoxically, another study showed that infection with *A. phagocytophilum* induced protracted degranulation in human neutrophils (115), which was attributed to inflammatory tissue injury. In addition, *A. phagocytophilum* upregulates the production of chemokine IL-8 as well as pro-inflammatory cytokines. This increased proinflammatory activity and chemokines may facilitate the recruitment of additional host neutrophil cells and localized tissue injury when neutrophils are unable to generate effective antimicrobial responses (116).

Pathologic findings in patients with HGA and animal models include normocellular or hypercellular bone marrow, erythrophagocytosis, hepatic apoptosis and periportal lymphohistiocytic infiltrates, focal splenic necrosis, and mild interstitial pneumonitis and pulmonary hemorrhage (96–98). Similar to HME, HGA hematologic abnormalities including marked leukopenia and thrombocytopenia. Although the immune mechanisms that account for severe and fatal HGA are not completely understood, there is some evidence of immunosuppression in patients with HGA (79,87,90,92). This conclusion is supported by the high number of fatal cases due to secondary opportunistic infections and organ failures (81). The mechanism by which this immune suppression occurs in HGA is not yet completely defined.

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RE-INFECTION AND IMMUNITY

HME

Immunity to primary *E. chaffeensis* infection in humans has not been investigated, but comparative murine models have provided some insight. A role for cell-mediated immunity has been suggested based on the severity of *E. chaffeensis* infection in HIV-infected patients and the lympho-proliferative responses observed in patients following recovery from HME (54,56,86,117). However, the relative importance of cell-mediated and humoral immunity has not been firmly established (118). Studies using murine models have suggested that protective immunity during primary and secondary ehrlichial infection is mediated by cellular immunity, mainly $CD4^+$ T cells producing IFN-γ (i.e. Th1 cells) (103–106,119–122). IFN-γ production by CD4+Th1 cells likely leads to macrophage activation and induction of bactericidal mechanisms such as production of reactive oxygen species, which in turn lead to bacterial elimination (120–122). In addition to IFN-γ, murine studies have shown that proinflammatory cytokines such as IL-12p40 and TNF-α are also important factors in the clearance of *Ehrlichia* and protection. Humoral immunity appears to also play a role in protection against *Ehrlichia* infection as evidenced by significant seroconversion in patients who recover from disease. In murine studies, *Ehrlichia*-specific antibodies, mainly IgG2a (Th1-dependent Ig subclass), protect SCID mice from severe *E. chaffeenesis* infection (103,122,123)

It is unknown whether patients that recover from HME are immune or susceptible to reinfection. Such evidence is limited to a single report of re-infection with a genetically distinct *E. chaffeensis* strain in a liver transplant recipient receiving immunosuppressive therapy (124). In murine studies, the development of heterologous protection model of ehrlichiosis has provided a mechanism to investigate immunity, including memory immune responses (103, 125). These studies indicate that prior infection of immunocompetent mice with *E. muris* that cause persistent asymptomatic infection in mice and strong cell mediated immune responses (Figure 11B), protect against secondary infection with highly virulent *Ehrlichia* species (IOE) which cause fatal disease in uninfected host (103,125). Heterologous protection against *Ehrlichia* is associated with minimal tissue injury, development of well-defined granulomas, expansion of IFN-γ producing effector memory CD4+ and CD8+type-1 cells and substantial production of *Ehrlichia* specific IgG2a antibodies. Vaccines are currently unavailable for HME.

HGA

Currently, little is known about immunity following an *A. phagocytophilum* infection. Although infection may result in long-term immunity, there have been rare reports of laboratory-confirmed reinjection. Thus, individuals who live in endemic areas and are at risk of exposure to infected ticks should be vigilant about avoiding tick bites and other tick-borne pathogens. Similar to immunity against *E. chaffeensis*, protective immunity against *A. phagocytophilum* is mediated by cellular and humoral immune mechanisms (43,75). It is generally believed that individuals who develop high titer antibodies are protected against re-infection; patients previously infected with *A. phagocytophilum* develop high titer antibodies that may last for as long as 3 years. Whether this persistence of antibodies is due to persistent infection or re-infection is not clearly determined. Similarly, it is not known if previous infection of human leads to antigen specific memory T and B cell responses that protect the individual against reinfection. Vaccines are currently unavailable for HGA.

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DIAGNOSIS

HME and HGA

Diagnosis of HME and HGA rests primarily on clinical suspicion due to the limited availability of rapid diagnostic tests such as PCR, and the absence of detectable serum antibodies at the time of clinical presentation (4 days after the onset of clinical illness) (19,117). The prognosis worsens if treatment is not administered or delayed (5,51,52,72,117) and therefore, it is important that empiric therapy with doxycycline be started for any patient with compatible clinical and laboratory findings. Initial diagnosis of ehrlichiosis can be based on non specific biochemical and hemaotological findings. However, confirmatory tests should be performed at different intervals after the onset of illness.

Hematologic and Biochemical Abnormalities

Presumptive diagnosis of HME is based on clinical manifestation, clues from medical history such as history of tick bite and outdoor activities, as well as specific laboratory abnormalities. Pancytopenias are a hallmark laboratory feature of HME early in the course of the illness (126). Anemia occurs within 2 weeks of illness and influence 50% of patients. Mild to moderate leukopenia with largest decline in lymphocyte population is observed approximately in 60 to 70% of patients during the first week of illness (51,52,72). Interestingly, during convalescence, a significant increase in lymphocyte count, i.e. relative and absolute lymphocytosis, is seen in most patients and is characterized predominantly by the expansion of activated γδ T cells (118). Marked thrombocytopenia is one of the pathognomonic findings in HME, which is usually detected in 70 to 90% of patients during their illness. Mildly or moderately elevated hepatic transaminase levels are detected in ~ 90% of patients associated with increased levels of alkaline phosphatase and bilirubin in some patients. Mild to moderate hyponatremia has been reported in as many as 50% of adult patients and 70% of pediatric patients (52,72,117).

Other laboratory abnormalities that occur in severe disease are specific to the organ involved. Examples are increased serum creatinine, lactate dehydrogenase, creatine phosphokinase, amylase, and electrolyte abnormalities including hypocalcemia, hypomagnesemia, hypophosphatemia, prolonged prothrombin times, increased levels of fibrin degradation products, metabolic acidosis, profound hypotension, disseminated intravascular coagulopathy, hepatic and renal failure, adrenal insufficiency, and myocardial dysfunction (49,50,75).

Specific Laboratory Diagnostic Tests

A diagnosis of HME can be confirmed by several laboratory methods. These tests include serological detection of specific antibodies, detection of morulae in peripheral blood or in CSF leukocytes, detection of ehrlichial DNA by PCR of blood or CSF, direct detection of ehrlichiae in tissue samples by immunohistochemistry, and isolation of bacteria.

Serologic Testing

Serologic testing of IgM and IgG antibodies specific to *E.chaffeensis* using indirect immunofluorescence assay (IFA) is the “gold standard” and is most frequently utilized confirmatory tests for HME (72,90,127). Paired sera collected during a 3- to 6-week interval represent the preferred specimens for serologic evaluation of HME. A single IgG antibody titer of at least 256; seroconversion from negative to positive antibody status (with a minimum titer of 64), a 4-fold rise in titer during convalescence is indicative of HME when acute- and convalescent-phase samples are compared (127,128). Although serology is one of major diagnostic criterion for ehrlichiosis, it has several limitations that should be considered: 1) IgG IFA test is negative in as many as 80% of patients during the first week of illness and the IgM titers may also be uninformative at this time. Thus a negative serologic result for the acute-

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phase sample does not exclude the diagnosis; 2) A high rate of false positive serology usually occurs due to cross reactive antigens shared by *Ehrlichia* and *Anaplasma* that induce cross reactive antibodies. Because of this cross-reactivity among ehrlichial species, sera should be tested against both *E. chaffeensis* and *A. phagocytophilium* antigens when ascribing a specific etiology; 3) Failure to seroconvert in some cases can be attributed to immune impairment; and 4) Early treatment with a tetracycline-class antibiotics occasionally reduces or abrogates the antibody response to *E. chaffeensis* (32,79,98,129).

Blood Smear Staining

Diagnosis of HME can be accomplished by staining of blood smears from peripheral blood, bone marrow or CSF to detect morulae. Smears can be stained with Wright's, Diff-Quik, or Giemsa stains (19,28). Although this method is rapid, it is relatively insensitive compared to other confirmatory tests, especially in immunocompetent patients where severe disease is usually associated with very low bacterial burden in blood and peripheral organs. Morulae are detected within monocytes in only about 3% of patients with HME. In contrast, blood smear is more useful for HGA diagnosis where 25% – 75% of patients have morulae in peripheral blood examinations, and sensitivity is highest during the first week of infection (130,131).

PCR Amplification

Due to its high specificity (60–85%) and sensitivity (60–85% for *E. chaffeensis* and 67–90% for *A. phagocytophilum*) as well as rapid turnaround time, diagnosis of ehrlichial infection by PCR has become the test of choice for confirming serology indicating HME and HGA (130–132). PCR is the only definitive diagnostic test for *E. ewingii* infection since the bacteria is unculturable, although the sensitivity and specificity of this approach is unknown. Multiplex or multicolour testing capable of detecting several related etiologic agents in a single test has been described (135). PCR of whole blood is commercially available, and allows rapid diagnosis of infection in up to 85% of cases (130). Blood samples should be collected in EDTA or sodium citrate anti-coagulants and obtained before or at the initiation of therapy to increase sensitivity. However, since doxycycline treatment is effective only at early stages of infection, treatment should start as soon as possible while waiting for laboratory result. PCR detection is particularly important for detection of ehrlichial infection at early stages when antibody levels are very low or undetectable. While PCR of CSF may be positive, the sensitivity is lower than for whole blood, likely due to the significantly lower volume of infected cells (2,4,130, 131). Several PCR targets have been employed to conserved genes among different *Ehrlichia* isolates including the *rrs* (16S rRNA) and *groESL* heat shock operon (133). Other genes have been utilized such as genus-specific disulfide bond formation protein gene (*dsb*), the *E. chaffeensis*-specific 120-kDa and TRP32 protein (VLPT) genes, and the 28-kDa outer membrane proteins (p28) (134).

Isolation

Similar to other infectious diseases, cultivation of *Ehrlichia* is the gold standard in diagnosis of HME and HGA however, primary isolation may take up to several weeks. The sensitivity of *E. chaffeensis* isolation compared with PCR amplification is very low (3,11,49). In contrast, the sensitivity of culture for detection of *A. phagocytophilum* can be equivalent to that of PCR and blood smear examination (69). Similar to PCR amplification and blood smear examination, prior doxycycline treatment diminishes the sensitivity of culture to a greater degree. Due to lower sensitivity of this method, therapeutic decisions must often be based on a high index of clinical suspicion and laboratory evidence of the infection, such as PCR assays and peripheral blood smears (69,74).

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Immunohistochemistry

Immunohistichemical staining of the formalin fixed biopsy or autopsy tissues is another confirmatory method for diagnosis of *Ehrlichia* and *Anaplasma* infection. The IHC method is most useful in documenting the presence of organisms in patients before the initiation of antibiotic therapy or within the first 48 hours after antibiotic therapy has been initiated. IHC techniques also are available for diagnosing cases of ehrlichiosis and anaplasmosis from bone marrow biopsies and tissue obtained at autopsy of fatal cases, including the spleen, lymph nodes, liver, and lung (51,52,79).

HEE Diagnosis

A diagnosis of HEE is suggested by visualization of intracytoplasmic morulae in neutrophils in a patient with residence or travel to an area of HME (rather than HGA) endemnicity. Morulae may be visualized in both blood and, rarely, CSF (8,9,11). While there is no specific serologic assay for *E. ewingii*, there is significant serologic cross-reactivity to *E. chaffeenesis* (8,9). It is conceivable that in the absence of visualization of morulae in granulocytes or confirmatory PCR for *E. ewingii*, a proportion of cases meeting serologic criteria for HME actually represent HEE infection. A specific PCR for *E. ewingii* exists, but is limited to research laboratories. Similar to the PCR for *E. chaffeensis* and *A. phagocytophilium*, sensitivity is maximal early in the course of the illness, prior to antibiotic therapy.

Differential Diagnosis

The differential diagnosis of HME, HGA and HEE at early stages of the disease where the symptoms and signs of disease are non specific and patient presents with fever, headache, myalgia, and malaise, may include various viral syndromes, Rocky Mountain spotted fever, upper respiratory illness, urinary tract infection, and sepsis. If history of tick bite and outdoor activities exist with these symptoms, the physician should consider other tick-borne febrile illnesses such as Rocky Mountain spotted fever, relapsing fever, tularaemia, Lyme borreliosis, Colorado tick fever, and babesiosis (52,127). CNS signs and symptoms with CSF pleocytosis suggest viral or bacterial meningoencephalitis. Other diseases that share clinical and laboratory findings of ehrlichial disease, particularly if patients presented with rash are meningococcemia, toxic shock syndrome, murine typhus, Q fever, typhoid fever, leptospirosis, hepatitis, enteroviral infection, influenza, bacterial sepsis, endocarditis, Kawasaki disease, collagen-vascular diseases, and immune thrombocytopenic purpura (72).

Treatment

Most patients with HME or HGA respond well to tetracyclines if administrated early in illness. *In vitro* antimicrobial susceptibilty testing has shown an excellent sensitivity of all *Ehrlichia* and *Anaplasma* species to doxycycline. *In vivo*, doxycycline is preferred over tetracycline because it has fewer side effects and better patient tolerance (90,136). Doxycycline remains the treatment of choice in pediatric patients, despite the risk of dental discoloration in this age group (137). This drug is bacteriostatic in its activity against rickettsial organisms.

Pregnant patients with ehrlichial infection represent a particular challenge, as doxycycline is contraindicated. In this population, as well as in patients with a specific contraindication to doxycycline, rifampin (adults: 300 mg twice daily; children < 100lb 10 mg/kg twice daily) may be substituted (138–140). *In vitro* susceptibility testing has shown that *E. chaffeensis* is resistant to representatives of most classes of antibiotics including aminoglycosides (gentamicin), fluoroquinolones (ciprofloxacin), penicillins (penicillin), macrolides and ketolides (erythromycin and telithromycin), and sulfa-containing drugs (co-trimoxazole) (87). Chloramphenicol is an alternative drug that has been considered for treatment of HGA or HME. However, this drug is associated with various side effects and might require

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monitoring of blood indices, and therefore, it is no longer available in the oral form in the United States.

HME Treatment

Doxycycline is the recommended treatment for HME. Response to treatment is typically rapid, and fever persisting >72 hours after initiation of treatment strongly suggests an alternative diagnosis. The recommended dose is 100 mg per dose administered twice daily (orally or intravenously) for adults or 2.2 mg/kg body weight per dose administered twice daily (orally or intravenously) for children weighing <100 lbs. (45.4 kg). While no studies have specifically addressed duration of treatment, most authorities advocate continuing antibiotics for 3 to 5 days after defervescence (90,137), and perhaps longer (e.g., total of 10–14 days) if there is CNS involvement (141).

HGA Treatment

Therapeutic considerations for HGA are similar to HME, with doxycycline remaining the drug of choice for both pediatric and adult cases. If coinfection with *B. burgdorferii* is suspected based on characteristic skin findings or elevated antibodies, doxycycline should be continued for at least 10 days for adults (89,142). In *B. burgdorferii* coinfected children < 8 years of age, doxycycline should be continued until the patient is afebrile for three days, with the remainder of the 14 day course completed with an alternative agent active against *B. burgdorferii* (e.g., amoxicillin or cefuroxime axetil) to minimize the risk of dental discoloration (10,11,49,72). Patients who fail to respond clinically to doxycycline monotherapy after 72 hours should be evaluated for an alternative diagnosis or the possibility of *Babesia* co-infection.

HEE Treatment

There are no prospective studies evaluating treatment of *E. ewingii*, however doxycycline is considered the treatment of choice in both adults and children. When therapy with this agent is started promptly, outcomes are uniformly excellent (8,9,11). Considerations in dosing and administration of doxycycline are discussed in the section on HME management.

Prevention

Preventive antibiotic therapy for ehrlichial infection is not indicated for patients who have had recent tick bites and are not ill. Avoidance of tick bites and immediate removal of ticks remains the ultimate prevention approach. Individuals who live in endemic areas should wear light colored clothes during outdoor activities, which allow the person to see crawling ticks (143). Adults who are at high risk of getting bitten by ticks should apply Chemo-prophylactic repellents such as DEET (n, n-diethyl-m-toluamide) to exposed skin that prevents tick attachment. Individuals should carefully inspect their bodies, hair, and clothes for ticks upon return from potentially tick-infested areas and should immediately remove any attached ones. Studies have shown that a period of 4–24 hours of infected ticks attached to the host may be required before effective transmission of *Ehrlichia* and *Anaplasma* occur (143–45). Therefore, immediate and complete removal of attached ticks is critical for prevention of transmission and infection.

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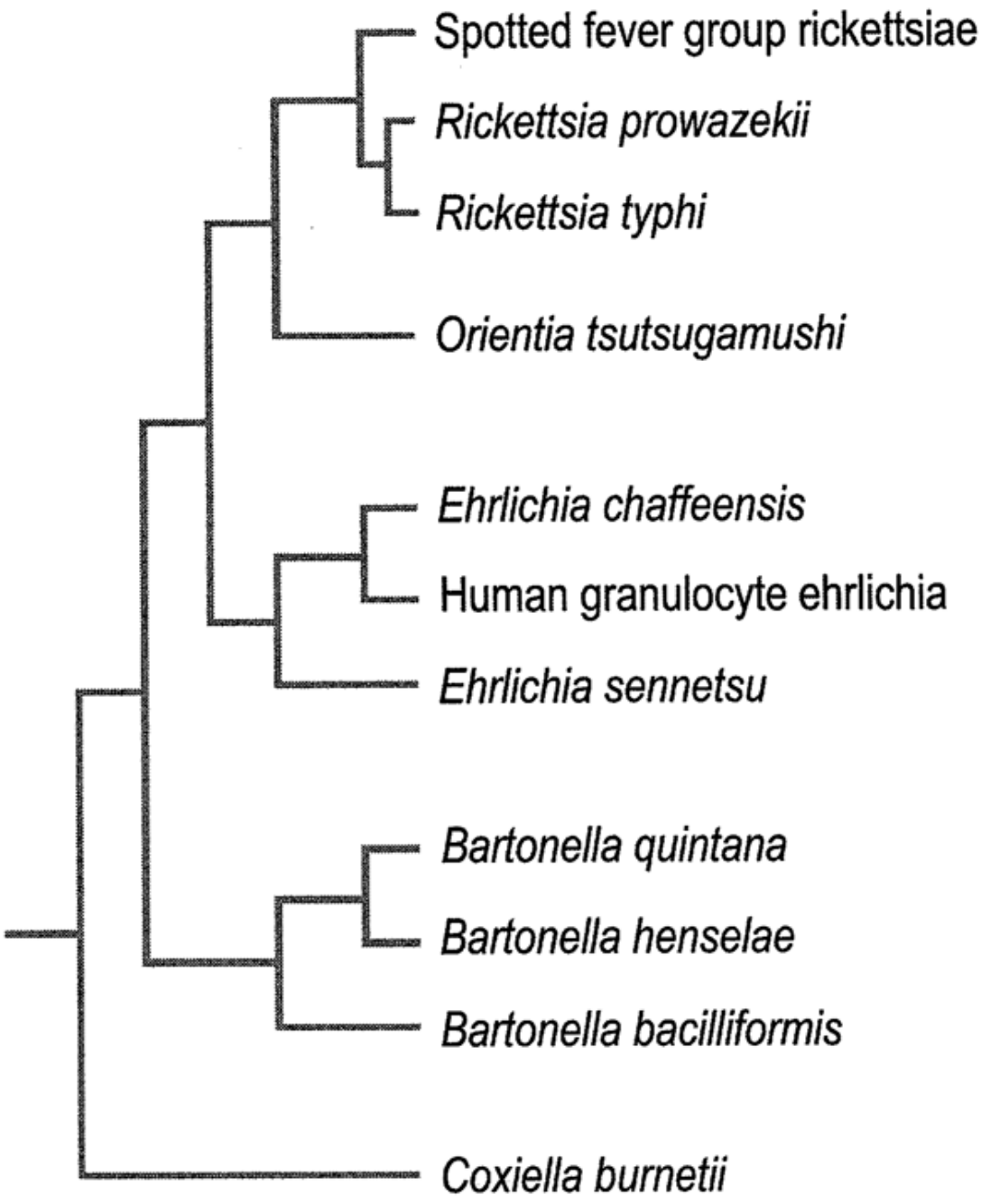

Figure 1.
Phylogenetic relationships between rickettsias based on 16S rRNA gene sequences. (*From* Mason PR, Kelly PJ. Ch. 235: Rickettsia and Rickettsia-Like Organisms. In: Cohen & Powderly, editors. Infectious Diseases, 2nd ed. Mosby; 2004. Permission requested from *Elsevier*.)

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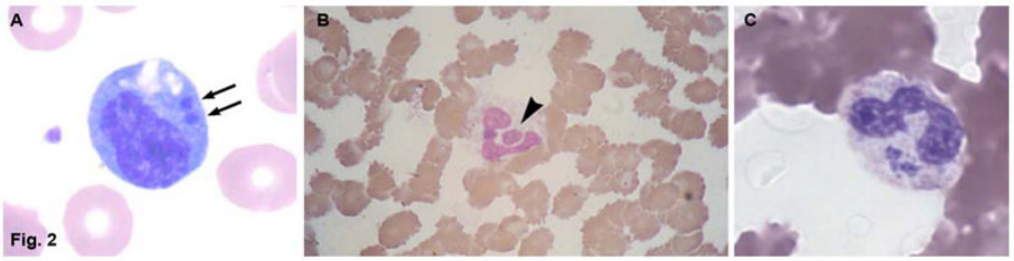

Figure 2.

Peripheral blood leukocytes containing ehrlichial morula in patients with human monocytic ehrlichiosis (**A**) and human granulocytic anaplasmosis (**B and C**). **A and B**, a morula (arrow) containing *Ehrlichia chaffeensis* in a monocyte in patient with HME. **B and C**; a morula (arrowhead) containing Anaplasma phagocytophilum in a neutrophil in patient with HGA. Wright stains, original magnifications ×1,200. (**A** *From* Walker DH, Dumler JS. Ch. 190: *Ehrlichia chaffeensis* (Human Monocytotropic Ehrlichiosis), *Anaplasma phagocytophilum* (Human Granulocytotropic Anaplasmosis), and Other Ehrlichiae. In: Mandell, Bennett, & Dolin, editors. Principles and Practice of Infectious Diseases, 6th ed. Church Livingstone; 2005. Permission requested from *Elsevier*. **B** *From* Siberry GK, Dumler JS. Ch. 228: Ehrlichiosis and Anaplasmosis. In: Kliegman, editor. Nelson Textbook of Pediatrics, 18th ed. Saunders; 2007. Permission requested from *Elsevier*. **C** *From* Walker DH, Paddock CD, Dumler JS. Emerging and Re-emerging Tick-Transmitted Rickettsial and Ehrlichial Infections. Medical Clinics of North America, 2008; 92(6). Permission requested from *Elsevier*.)

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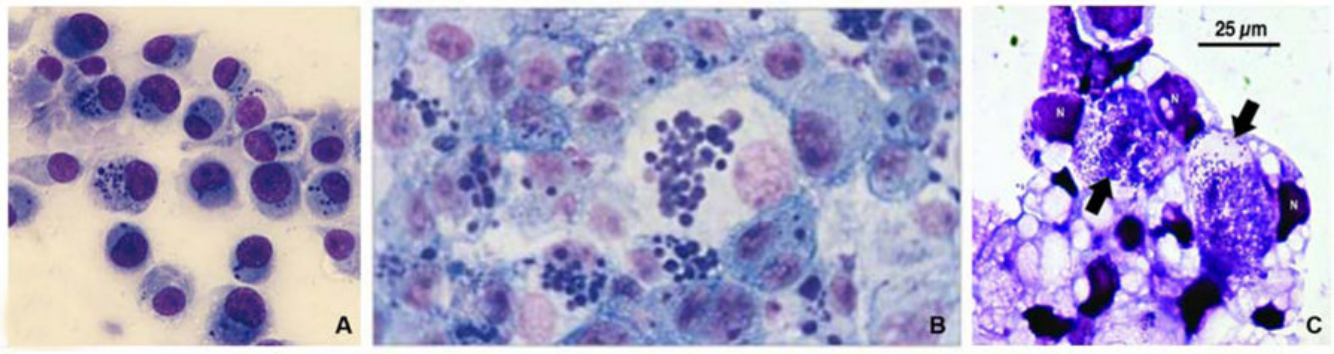

Figure 3.

Light microscopic picture of canine monocytes (DH82) are heavily infected *in vitro* with *E. chaffeenesis* (**A**) and *E. canis* (**B**). Typical ehrlichial inclusions (morulae) are present inside the cytoplasm of infected cells (Giemsa staining. Orginal magnification × 200. (**C**) Giemsa-stained ISE6 cells infected with *A. phagocytophilum* strain isolated from a female *I. scapularis* tick. *Anaplasma* organisms exist in large intracellular vacuoles (arrow). N, host cell nucleus. Giemsa stain. (**A** Courtesy of Ismail N. **B** *From* Mason PR, Kelly PJ. Ch. 235: *Rickettsia* and *Rickettsia*-Like Organisms. Cohen & Powderly: Infectious Diseases, 2nd ed. 2004; permission requested from Elsevier. **C** *From* Massung RF, Levin ML, Munderloh UG et al. Isolation and Propagation of the Ap-Variant 1 Strain of *Anaplasma phagocytophilum* in a Tick Cell Line. J of Clin Micro, 2007; 2138–2143. Permission requested from JCM.)

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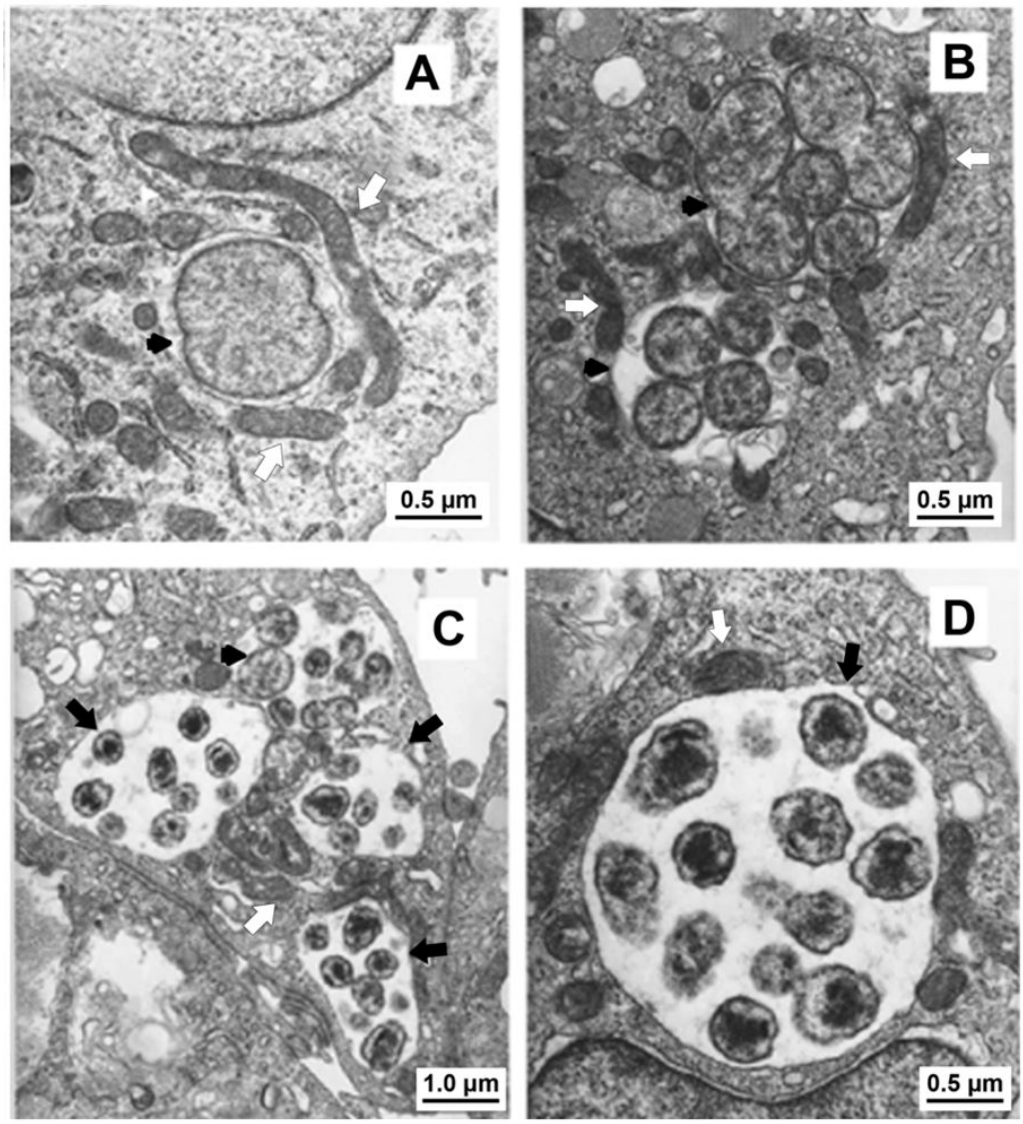

Figure 4. Electron micrographs of *E. chaffeensis* interaction with DH82 cells

(**A**) At 24h post-infection, a single reticulate cell (RC) divide by binary fission (black arrowhead). (**B**) At 48h post-infection, two morulae (black arrowheads) contain RC. (**C**) At 72 h post infection, *Ehrlichia* have matured into dense core (DC). Three morulae contain DC (black arrows), and one morula contains RC (black arrowhead). (**D**) High power of a morula containing DC at 72 h post infection. Mitochondria surrounding morulae were indicated by white arrows. (*From* Zhang J-Z, Popov VL, Gao S, et al. The developmental cycle of *Ehrlichia chaffeensis* in vertebrate cells. Cellular Microbiology, 2006; 9(3):610–618. Permission requested from Blackwell Publishing Ltd.)

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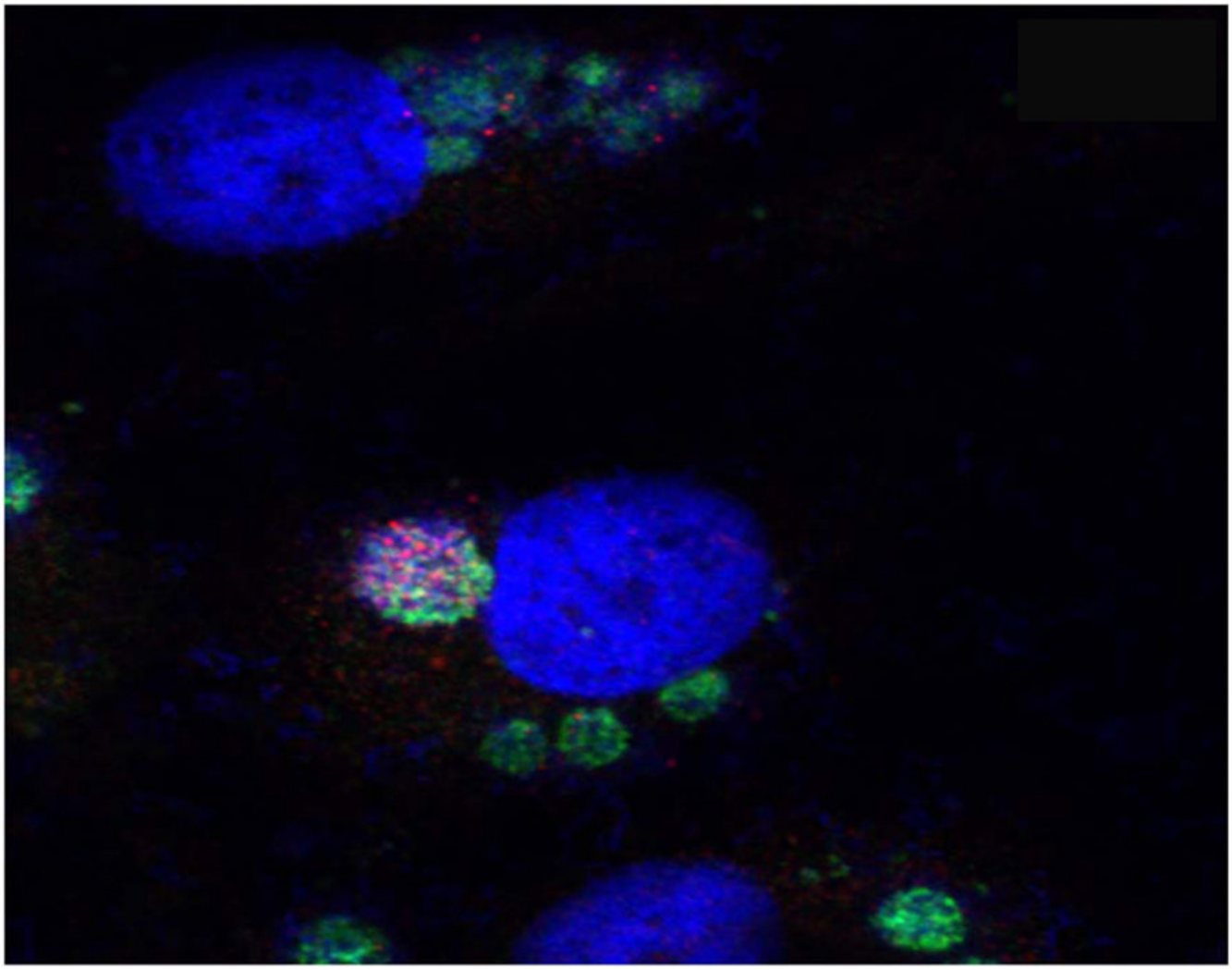

Figure 5.
Ehrlichia TR47: ultrastructure and confocal microscopy. Differential expression of TRP47 (red) on dense-cored *E. chaffeensis* cultured *in vitro* (DH82 cells) as visualized using three color scanning laser confocal fluorescent microscopy. *E. chaffeensis* infected cells were dually stained with rabbit anti-*Ehrlichia* disulfide bond formation protein (Dsb) (green-Alexa Fluor 488), and mouse anti-*E. chaffeensis* TRP47 (red-Alexa Fluor 568). Host cell nuclei were counterstained with 4′, 6′-diamidino-2-phenylindole, dihydrochloride (DAPI) and images merged. (Courtesy of McBride JW.)

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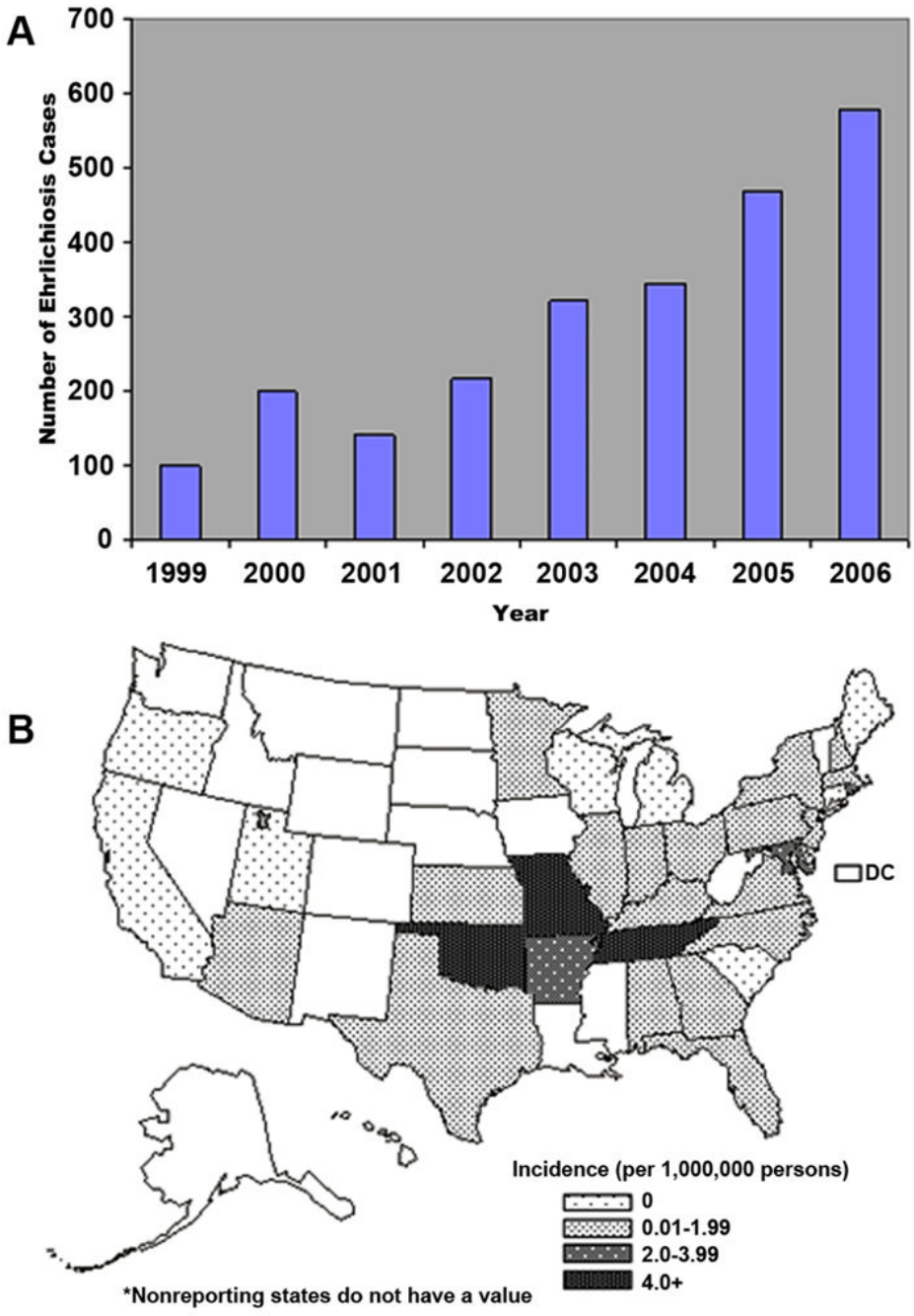

Figure 6.

A) Number of ehrlichiosis cases caused by *E. chaffeensis* reported to CDC by State Health Department from 1999–2006. **B**) Average reported annual incidence of human monocytic ehrlichiosis by state –United States, 2001–2002 and nationwide. (Open access: published by the Centers for Disease Control and Prevention, a U.S. Government agency and are in the public domain and can be used without permission.)

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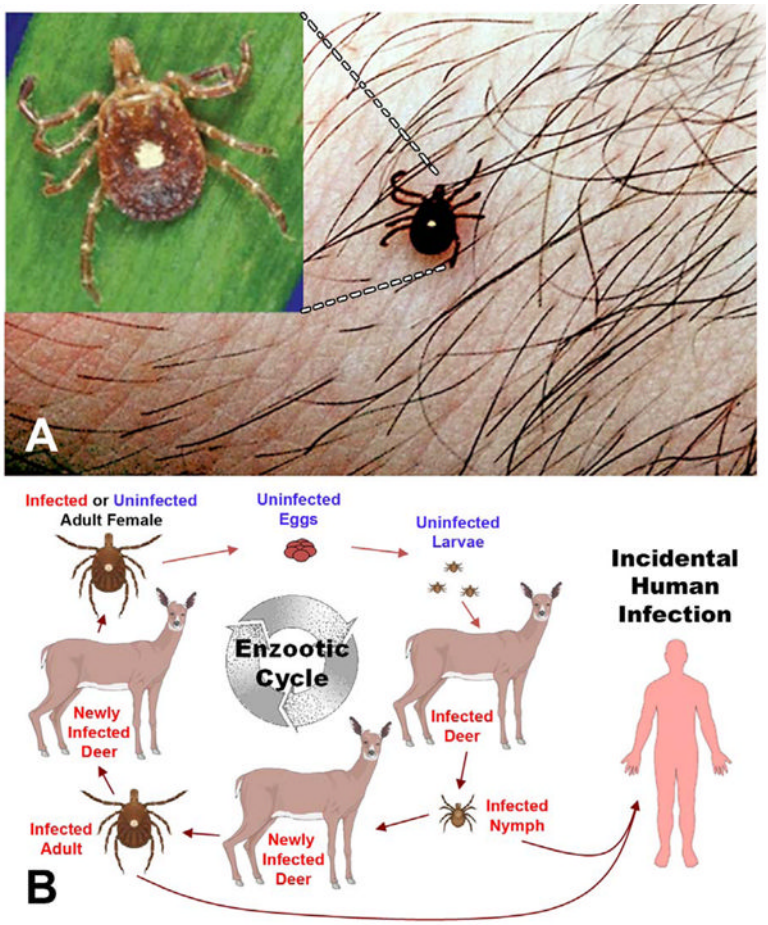

Figure 7.

A) Lone Star tick; *Amblyomma americanum* that transmit the agent of human monocytic ehrlichiosis **B**) Life Cycle of monocytotropic *E. chaffeensis.* (Courtesy of Bloch KC, and open access: published by the Centers for Disease Control and Prevention, a U.S. Government agency and are in the public domain and can be used without permission)

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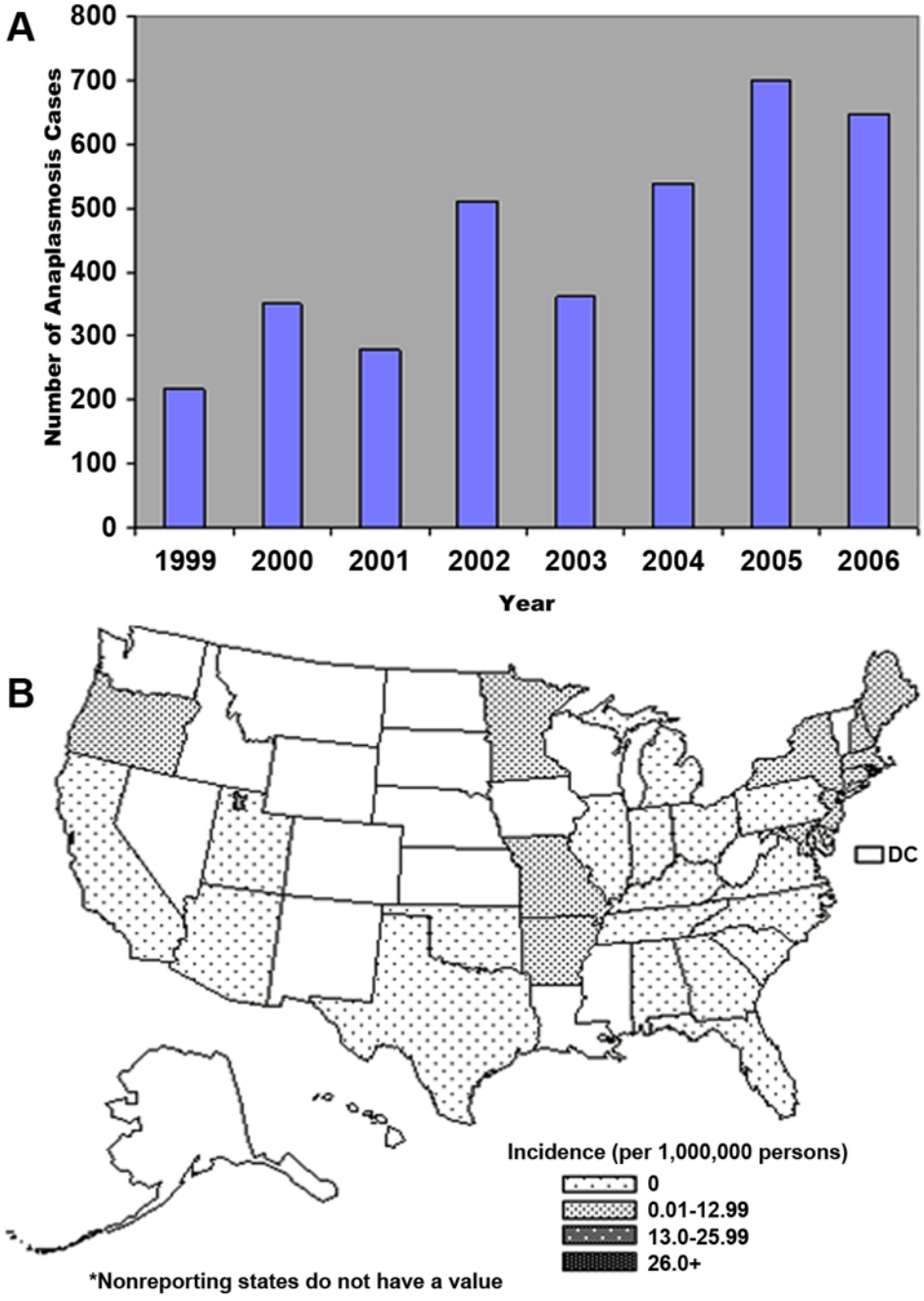

Figure 8.

A) Number of ehrlichiosis cases caused by *A. phagocytophilum* reported to CDC by State Health Department from 1999–2006. **B**) Average reported annual incidence of human monocytic ehrlichiosis by state –United States, 2001–2002 and nationwide. (Open access: published by the Centers for Disease Control and Prevention, a U.S. Government agency and are in the public domain and can be used without permission.)

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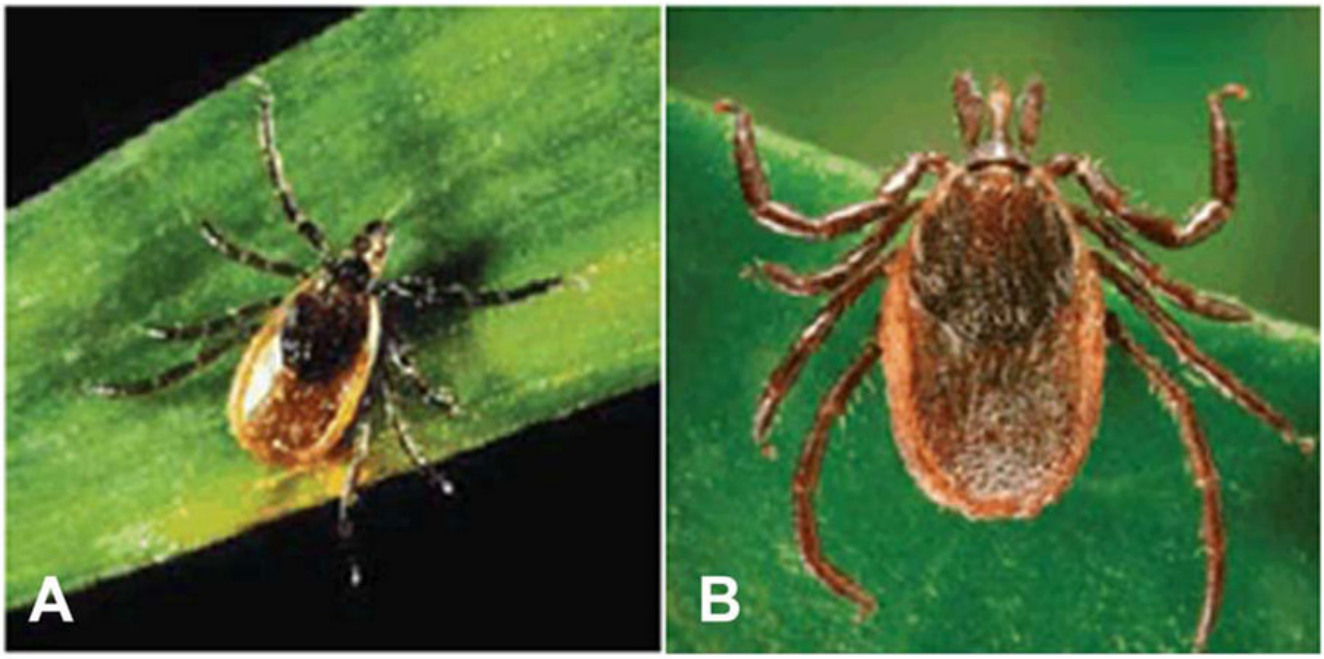

Figure 9.

A) Blacklegged tick; *Ixodes Scapularis* that transmit the agent of human granulocytic ehrlichiosis and Lyme disease. **B**) Western black-legged tick; *Ixodes Scapularis* that transmit the agent of human granulocytic ehrlichiosis (**A & B** Open access: published by the Centers for Disease Control and Prevention, a U.S. Government agency and are in the public domain and can be used without permission)

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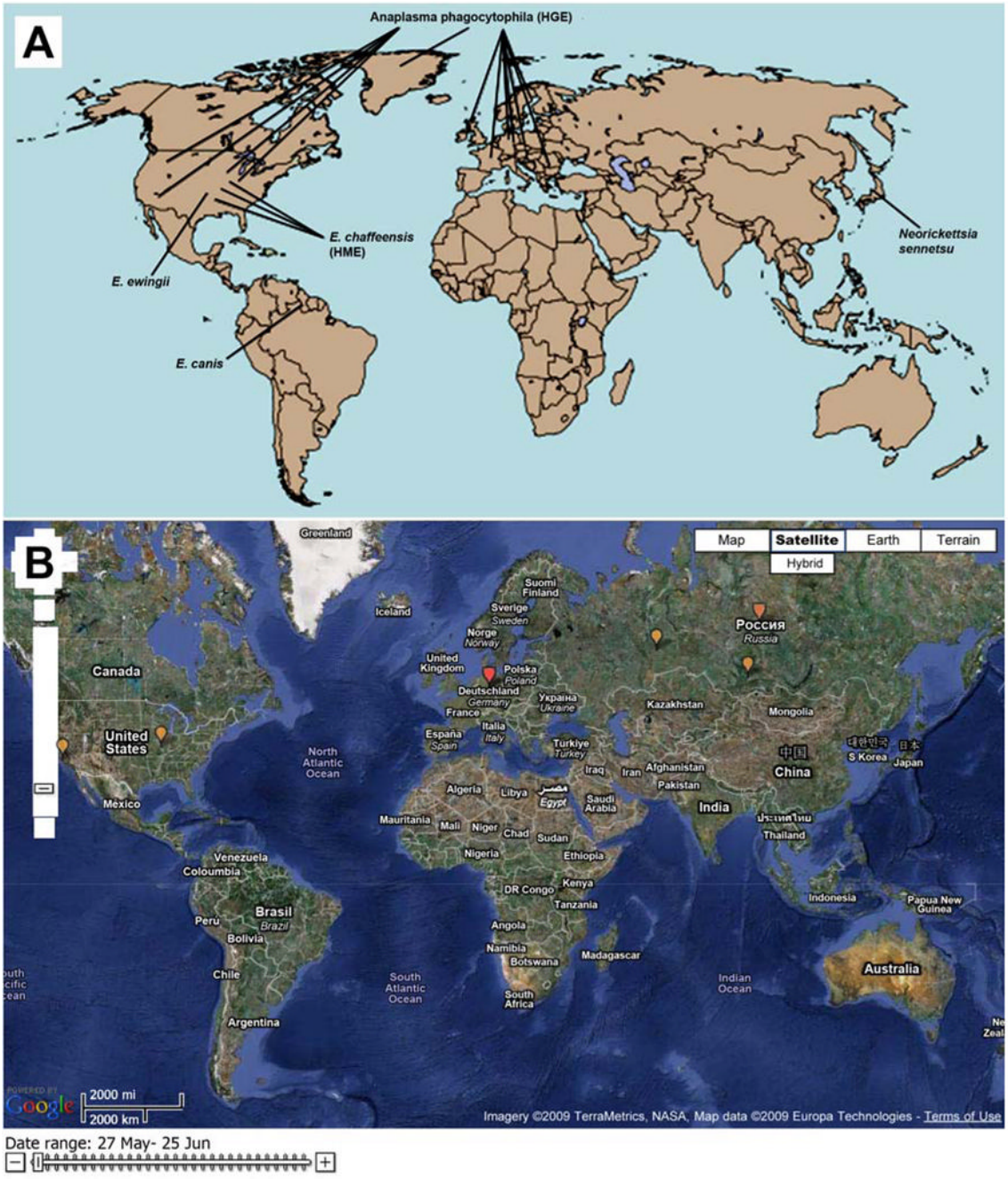

Figure 10.

A) Worldwide distribution of *Ehrlichia* and *Anaplasma*. HGA: human granulocytic anaplasmosis; HME: human monocytic ehrlichiosis. **B**) Global health map shows worldwide distribution of HGA and HME marked by yellow dots. (**A** *From* Raoult D. Ch. 348: Rickettsioses. In Goldman, editor. Cecil Medicine, 23rd ed. Saunders, 2007. Permission requested from Elsevier. **B** *From* "Worldwide Outbreak of *Ehrilichia*" tracking map from http://www.healthmap.com [accessed June 15, 2009]. Permission granted.)

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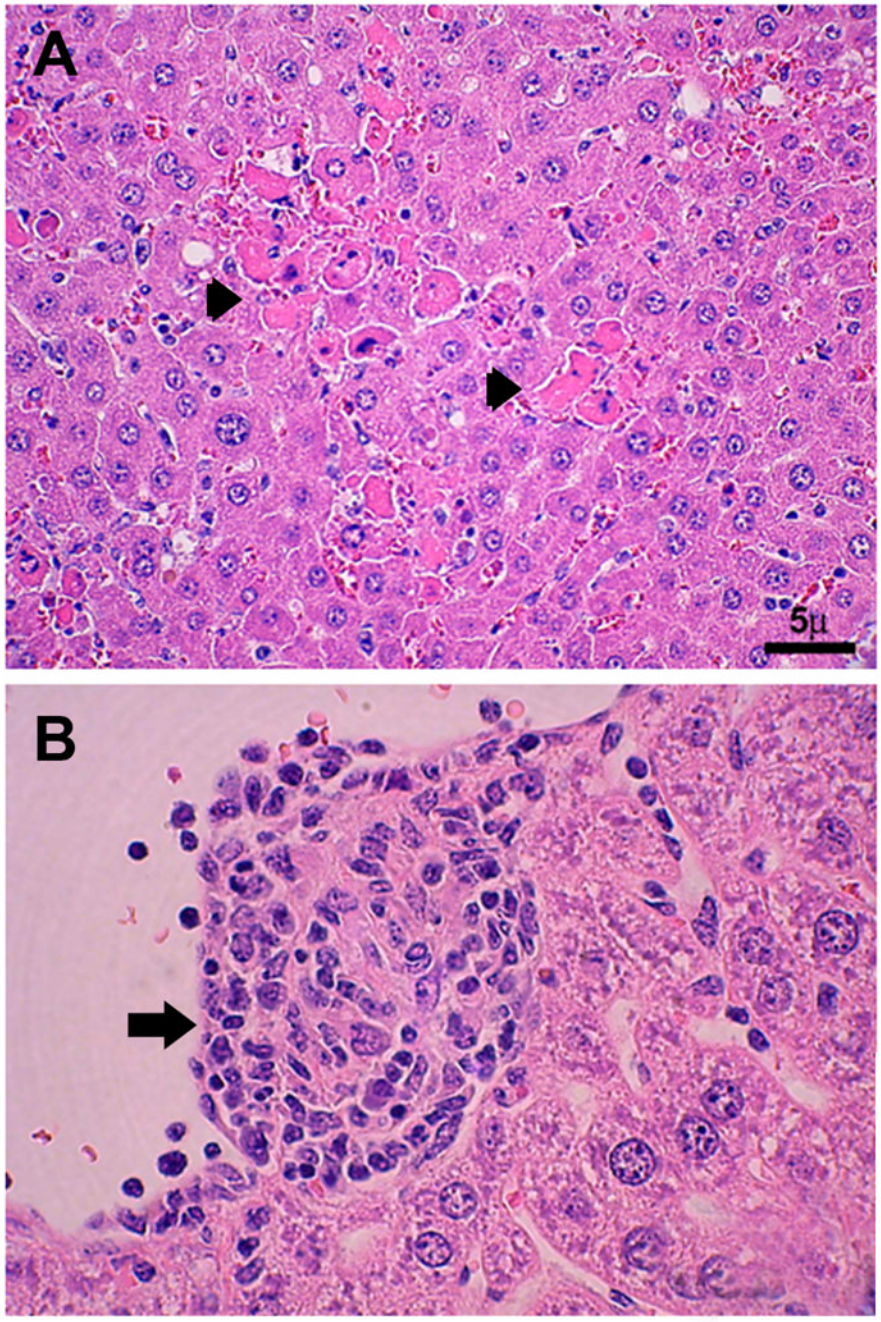

Figure 11.

A) Hepatic histopathology in the murine model of fatal monocytic ehrlichiosis caused by systemic infection with virulent moncytic *Ehrlichia* (IOE). H&E staining shows extensive focal necrosis and apoptosis of hepatocytes (arrows). **B)** Hepatic histopathology in the murine model of mild monocytic ehrlichiosis caused by infection with mildly virulent *Ehrlichia muris*. H&E staining *shows* formation of well formed granuloma (arrowhead). (*From* Ismail N, Soong L, McBride JW, et al. Overproduction of TNF-alpha by CD8+ type 1 cells and down-regulation of IFN-gamma production by CD4+ Th1 cells contribute to toxic shock-like syndrome in an animal model of fatal monocytotropic ehrlichiosis. J Immunol, 2004; 172(3): 1786–800. Permission granted.)

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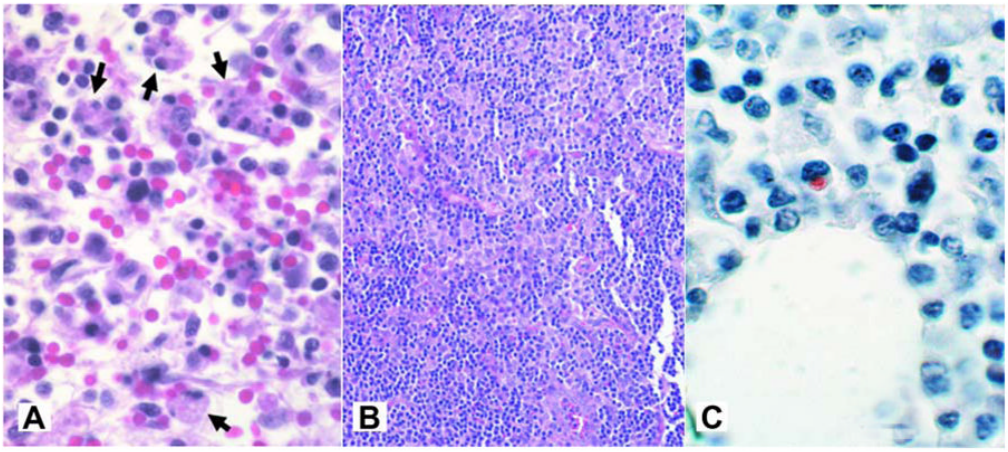

Figure 12.

A) Hemophagocytosis (arrows) in lymph nodes from a patient with HME (H&E; original magnification 240×). **B**) High cellularity in lymph node from patient with HME (magnificationX 64). **C**) Immunohistochemical staining of lymph nodes from patient with HME shows typical low bacterial burden (immunoperoxidase with hematoxylin counterstain; original magnification 240×). (*From* Dierberg KL, Dumler JS. Lymph node hemophagocytosis in rickettsial diseases: a pathogenetic role for CD8 T lymphocytes in human monocytic ehrlichiosis (HME)? In: BMC Infect Dis. 2006; 6:121. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.)

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Table 1

Ehrlichiae and Anaplasmae Species Causing Medical and Veterinary Diseases

Genus	Human or animal disease	Target Cells	Geographic Distribution	Vector	Reservoir
1-Ehrlichia					
Ehrlichia chaffeensis	Human monocytic ehrlichiosis (HME)	Monocytes/Macrophages	southeast, south central, and Midwest states	Lone star tick *Amblyomma*	Deer
Ehrlichia canis	Canine ehrlichiosis, HME	Monocytes/Macrophages	southeast, south central, and Midwest states	*Rhipicephalus*	Dogs
Ehrlichia ewingii	Human ewingii ehrlichiosis (HEE)	Neutrophils	Southeast, south central, and Midwest states	Lone star tick *Amblyomma Dermacenter variabilis*	Dogs, Deer
Ehrlichia muris	Murine monocytic ehrlichiosis, possibly HME	Monocytes/Macrophages	Unknown	Ticks *(Ixodes persulcatus, Haemaphysalis flava)*	Apodemus mice, vole
Ehrlichia ruminantium	Heartwater in Cattle	Monocytes/Macrophages Endothelium	Africa	Lone star tick *Amblyomma*	Cattle, sheep, goats
2-Anaplasma					
Anaplasma marginale	Bovine Anaplasmosis	Erythrocytes		Unknown	Cattle, wild ruminants
Anaplasma phagocytophilum	Human granulocytic anaplasmosis (HGA)	Neutrophils	Northeastern and north central states and northern California	Ixodes	white-footed mice, wood rats, mice, horses, dogs, cats, sheep, cattle, white-tailed deer
3-Neorickettsia					
Neorickettsia helminthoeca	No human infection	Monocytes/Macrophages	Unknown	Fish	Dogs
Neorickettsia sennetsu	Human infection Infectious mononucleosis like syndrome	Monocytes/Macrophages	Unknown	Ticks *(Boophilus, Rhipicephalus,* and others)	No animal reservoir
Neorickettsia risticii	No human infection Cause rickettsial disease in horses	Macrophages, enterocytes, Ticks *(Ixodes persulcatus, Haemophysalis flava)*mast cells	Unknown	Trematode larvae	Horse

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