EHRLICHIOSES, RICKETTSIOSES AND ANAPLASMOSIS
IN THE UNITED STATES:
CURRENT STATUS AND OPPORTUNITIES FOR NEW VACCINES

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EHRlichioses, Rickettssioses and Anaplasmosis
in the United States: Current Status and Opportunities for New Vaccines

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EHRlichioses

Introduction

Ehrlichia are obligately intracellular gram-negative bacteria that are associated with emerging, tick-transmitted, life-threatening zoonoses in humans. Human monocytotropic ehrlichiosis (HME) and human ehrlichiosis ewingii (HEE) are now well established zoonoses in the United States caused by E. chaffeensis and E. ewingii, respectively. The first recognized case of human ehrlichiosis occurred in 1986 in a patient that acquired the infection in Arkansas (Maeda et al., 1987), and a previously unknown pathogen, E. chaffeensis, was later identified as the etiologic agent (Anderson et al., 1991). As epidemiologic and ecologic understanding of E. chaffeensis biology has developed, it is now considered a prototypical emerging pathogen (Paddock and Childs, 2003). Shortly after the emergence of E. chaffeensis, the canine pathogen, E. ewingii, associated with granulocytic ehrlichiosis in dogs, was molecularly identified in four patients from Missouri presenting with fever, headache and thrombocytopenia (Buller et al., 1999). The emergence of human ehrlichioses has been attributed to changes in biological, demographic and environmental factors, and these factors in addition to increased surveillance and diagnostic capability are likely to result in increasing recognized incidence of human ehrlichiosis in the future (Paddock and Childs, 2003). Thus, there is an immediate need for effective vaccines now for human ehrlichiosis and into the foreseeable future. In this review, we summarize the current status of vaccines and examine the status of new prospects for vaccine development for human ehrlichiosis.
Etiologic agents of the human ehrlichioses

_Ehrlichia_ species are in the α subdivision of _Proteobacteria_ and members of the family _Anaplasmataceae_, which also includes the genera _Anaplasma_, _Wolbachia_, and _Neorickettsia_. Organisms in the genus _Ehrlichia_ include _E. chaffeensis_, _E. ewingii_, _E. canis_, _E. ruminantium_, and _E. muris_. _Ehrlichia_ replicate in a membrane-bound cytoplasmic vacuole forming a microcolony called morula. Multiple morulae (1.0 to 6.0 µm) are often present in an infected cell, and by light microscopy they appear as dark blue-to-purple intracytoplasmic inclusions demonstrated by Romanovsky-type stains (Rikihisa, 1991). Morphologically individual ehrlichiae are coccoid and coccobacillary and exhibit two ultrastructural cell types, a larger reticulate cell (RC) (0.4 to 0.6 µm by 0.7 to 1.9 µm) and a smaller dense-cored cell (DC) (0.4 to 0.6 µm in diameter). Both forms have a gram-negative cell wall, characterized by a cytoplasmic membrane and rippled outer membrane separated by a periplasmic space. Reticulate cells are pleomorphic and have uniformly dispersed nucleoid filament and ribosomes, and DC ehrlichiae are typically coccoid and have centrally condensed nucleoid filament and ribosomes (Popov et al., 1995; Popov et al., 1998). Small and large morulae containing both RC and DC or exclusively containing DC or RC ehrlichiae usually in loosely packed clusters can be observed within a single infected cell (Popov et al., 1995; Popov et al., 1998). The intramorular space in some morulae contains a fibrillar matrix of ehrlichial origin (Popov et al., 1995). The DC ehrlichiae are infectious and attach to the host cell surface where they are rapidly internalized and transition into a replicating RC forms. RC replicate, doubling every 8 hrs, and then mature to DC within 72 hr after initial cell contact (Zhang et al., 2007).

The intracellular niche occupied by _Ehrlichia_ has resulted in reductive evolutionary processes and corresponding severe loss of genes associated with metabolic processes provided by the host cell. Hence the genome sizes (~1-1.5 Mb) of _Ehrlichia_ are relatively small compared to extracellular bacteria. The genomes of three _Ehrlichia_ species have been sequenced (Collins et al., 2005; Dunning Hotopp et al., 2006; Mavromatis et al., 2006) and exhibit a high degree of genomic synteny, low G+C content (~30%) and one of the smallest genome coding ratios that is attributed to long non-coding regions and numerous long tandemly repeated sequences (TRs) (Frutos et al., 2006). These long non-coding regions and low G+C content in other related _Rickettsiales_ members are speculated to represent degraded genes in the final stages of elimination and excess GC-to-AT mutations (Andersson and Andersson, 1999). Another feature of _Ehrlichia_ genomes is the presence of a large number of long period TRs that appear to have evolved after divergence of the species (Frutos et al., 2007).

The identified agents of human ehrlichiosis in the United States include _E. chaffeensis_ and _E. ewingii_. _E. chaffeensis_ exhibits tropism for mononuclear phagocytes and causes mild to life-threatening disease in humans and mild to severe disease in dogs (Breitschwerdt et al., 1998b; Dawson and Ewing, 1992). _E. chaffeensis_ is maintained in nature in a zoonotic cycle potentially involving many vertebrate species, and is transmitted primarily by the lone star tick, _Amblyomma americanum_ (Paddock and Childs, 2003).

_E. ewingii_ is an established canine pathogen first described in 1971 (Ewing et al., 1971). _E. ewingii_ exhibits host cell tropism for granulocytes (neutrophils), and is also transmitted by the lone star tick, _A. americanum_ (Anziani et al., 1990). Dogs are a reservoir for _E. ewingii_, and many individuals with documented infections reported contact with dogs before onset of symptoms (Buller et al., 1999). Most cases of HEE are manifested in immunocompromised
patients, and thus, *E. ewingii* appears to be an opportunistic pathogen (Buller et al., 1999; Paddock et al., 2001).

*E. canis* is the type strain for the genus *Ehrlichia* and is the primary etiologic agent of canine monocytic ehrlichiosis (CME), a serious and sometimes fatal, globally distributed disease of dogs (Keefe et al., 1982). *E. canis* is transmitted by the brown dog tick, *Rhipicephalus sanguineus* (Groves et al., 1975), and infects monocytes/macrophages in dogs. *E. canis* was initially described in dogs in the United States in 1963 (Ewing, 1963), but received more attention after its identification as the agent responsible for outbreaks of a cryptogenic hemorrhagic disease called tropical canine pancytopenia in American and British military dog units on duty in southeast Asia (Huxsoll et al., 1969; Seamer and Snape, 1970; Wilkins et al., 1967). Human infections with *E. canis* have been reported in Venezuela (Perez et al., 1996; Perez et al., 2006). The clinical manifestations of acute infection with *E. canis* are similar to those observed in humans infected with *E. chaffeensis*.

**Epidemiology and public health importance**

Approximately 2,500 cases (passive surveillance) of HME have been formally reported to the Centers for Disease Control from 1999 to 2006, and HME and HEE are Nationally Notifiable Diseases on the public health information network. However, the incidence is likely underestimated since active surveillance studies performed in HME-endemic areas in Missouri, Tennessee and Georgia have revealed an incidence that is 10-100 times higher than reported by passive surveillance (Olano et al., 2003b). HME is a seasonal disease with most reported cases occurring in the spring and summer coinciding with higher tick activity, although cases can occur in the fall in more southern latitudes. The geographic distribution of HME follows the distribution of its vector *A. americanum* (lone star tick), that begins in west central Texas and extends east along the Gulf Coast, north through Oklahoma and Missouri, eastward to the Atlantic Coast and proceeds northeast through New Jersey, encompassing all the south central, southeastern and mid-Atlantic states. The main zoonotic reservoir is the white-tailed deer (*Odocoileus virginianus*), but other potentially important reservoirs are naturally infected with *E. chaffeensis* including goats, domestic dogs, and coyotes (Breitschwerdt et al., 1998b; Dugan et al., 2000; Kocan et al., 2000). The states with highest incidence include Arkansas, North Carolina, Missouri, Oklahoma, and New Jersey (McQuiston et al., 1999). Outside the USA, HME has been described in Cameroon where confirmation of the diagnosis was based on PCR detection of *E. chaffeensis* DNA from ill patients and dogs (Ndip et al., 2009a; Ndip et al., 2009b). *E. chaffeensis* has been found in 5 to 15% of *A. americanum* ticks collected from at least 15 states in endemic areas in the eastern US (Ijdo et al., 2000; Stromdahl et al., 2001; Whitlock et al., 2000). Human infections with *E. chaffeensis* or antigenically related ehrlichiae have been reported in Europe (Nutl et al., 1998), Asia (Heppner et al., 1997), South America (Ripoll et al., 1999), and Africa (Uhaa et al., 1992). Most reports of HME from other countries are based on serological studies and therefore cannot be confirmed as *E. chaffeensis* infections.

The epidemiology of HEE remains poorly defined due to the lack of a specific serologic assay for this organism and absence of a dedicated reporting system for this disease. Laboratory diagnosis relies on nucleic acid amplification, but new serologic assays to detect *E. ewingii* antibodies have been recently developed (Zhang et al., 2008). 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 (74). All cases involving tick transmission have been described in immunocompromised patients (Buller et al., 1999; Paddock et al., 2001).

**Clinical spectrum and treatment**

HME and HEE manifest as undifferentiated febrile illnesses 1 to 3 weeks after the bite of an infected tick. For HME, the most frequent clinical findings reported anytime during the acute illness are fever, malaise, headache, dizziness, chills, and myalgias (Eng et al., 1990; Everett et al., 1994; Fishbein et al., 1989; Fishbein et al., 1994; Olano et al., 2003a; Olano et al., 2003b; Schutze and Jacobs, 1997). HME is more common in male (>2:1) patients >40 years of age; the majority (>80%) report a tick bite (Fishbein et al., 1994; Olano et al., 2003b). Many HME cases are associated with recreational or occupational activities that increase exposure of humans to tick infested environments (Petersen et al., 1989; Standaert et al., 1995). HME presents as a more severe disease in patients >60 years of age and in immunocompromised patients including persons with HIV/AIDS in whom severe complications can arise such as adult respiratory distress syndrome, acute renal failure, shock and CNS involvement (Paddock et al., 2001). Patients with HEE present with a milder disease with few complications suggesting that *E. ewingii* is less pathogenic (Buller et al., 1999). Hematologic and biochemical abnormalities usually include leukopenia, thrombocytopenia, anemia, mildly elevated serum hepatic transaminase activities, and hyponatremia (Fishbein et al., 1994; Olano et al., 2003b; Paddock et al., 2001). A high proportion of immunocompetent (41 to 62%) and immunocompromised patients (86%) require hospitalization (Fishbein et al., 1994; Olano et al., 2003b; Paddock et al., 2001) and delays in antibiotic treatment are associated with more pulmonary complications, increased transfer to intensive care, and longer duration of illness (Hamburg et al., 2008). Immunocompromised patients (human immunodeficiency virus-infected persons, transplant recipients, corticosteroid-treated patients) have a high risk of fatal infection associated with overwhelming infection not typically observed in immunocompetent patients (Paddock et al., 2001). No deaths have been reported as a result of infection with *E. ewingii* (Buller et al., 1999; Paddock et al., 2001).

*In vitro* susceptibility testing has shown that *E. chaffeensis* is resistant to representatives of most classes of antibiotics including aminoglycosides (gentamicin), fluoroquinolones (ciprofloxacin), β-lactams (penicillin), macrolides and ketolides (erythromycin and telithromycin), and sulfa-containing drugs (co-trimoxazole) (Brouqui and Raoult, 1992). Patients with HME or HEE respond well to tetracyclines, which have bacteriostatic activities against *Ehrlichia* spp. and other rickettsial agents (Brouqui and Raoult, 1992; Horowitz et al., 2001). Doxycycline is preferred over tetracycline because of its pharmacokinetics and negligible staining of immature teeth. After the first trimester of pregnancy, doxycycline is contraindicated, and successful treatment with rifampin has been reported as an effective alternative (Buitrago et al., 1998).

5. Overview of protective immune mechanisms

Numerous studies with multiple *Ehrlichia* spp. indicate that IFN-γ is an essential mediator of protection (Mahan et al., 1994b; Mahan et al., 1996; Mutunga et al., 1998; Totte et al., 1993; Totte et al., 1996). Moreover, CD4+ and CD8+ T cells both contribute to IFN-γ production (Bitsaktsis et al., 2004; Esteves et al., 2004a; Esteves et al., 2004b; Ismail et al., 2004). Notably,
similar conclusions regarding the importance of MHC class I, CD4+ and CD8+ T cells, and the synergistic roles of IFN-γ and TNF-α have been reported in mice infected with *E. muris* (Feng and Walker, 2004). An important role for CD4+ T cells in immunity to *E. ruminantium* and IOE has been suggested (Bitsaktsis et al., 2004; Byrom et al., 2000; Totte et al., 1997). Similarly, mice lacking functional MHC class II genes are unable to clear *E. chaffeensis* infection, suggesting that CD4+ T cells are essential for ehrlichial clearance (Ganta et al., 2002). The intradermal environment (natural route of inoculation) appears to promote the induction of protective type-1 responses characterized by increased CD4+ and CD8+ T cells and IFN-γ producing CD4+ T cells (Stevenson et al., 2006).

Antibody mediated immunity appears to play a significant role in protection against *E. chaffeensis* infection. Infection of SCID mice (B and T cell deficient) with *E. chaffeensis* results in an overwhelming infection (Li et al., 2001; Li et al., 2002; Winslow et al., 2000). Furthermore, mice lacking B cells or FcγRI are unable to resolve an ordinarily sublethal infection by IOE, and passive transfer of antibodies in these mice results in significant reduction in bacterial load (Yager et al., 2005). Similarly, passive transfer of antibodies, but not Fab fragments, also protects mice against lethal infection (Feng and Walker, 2004). The specific anti-ehrlichial antibody-mediated mechanism is not fully understood, but appears to involve binding of antibody to the Fc receptor (Lee and Rikihisa, 1997; Yager et al., 2005) and subsequent generation of a proinflammatory cytokine response (Lee and Rikihisa, 1997) and generation of oxidative defenses (Yager et al., 2005).

**Vaccines: Current status and feasibility**

Although *Ehrlichia* are responsible for serious diseases of livestock, companion animals and humans, there are no vaccines available for human ehrlichioses and only one infection-treatment immunization regimen is available for the veterinary ehrlichial disease heartwater, caused by *E. ruminantium*. *Ehrlichia* are maintained in nature through subclinical infections of vertebrate hosts (carriers) as well as ticks and have evolved mechanisms to persistently infect mammalian hosts by subverting the innate and adaptive immune responses (Harrus et al., 1998). Effective immune responses leading to the elimination of infections without treatment have been described in *E. canis*-infected dogs (Breitschwerdt et al., 1998a; Harrus et al., 1998), and infection and treatment strategies in Africa for *E. ruminantium* have been used for decades to provide protection against challenge (van der Merwe L., 1987). Furthermore, experimental *E. ruminantium* vaccines (inactivated, live attenuated and recombinant) have demonstrated protection against homologous challenge, although less protection has been achieved against natural field challenge (Allsopp, 2009). Cell mediated immune responses and IFN-γ production correlate with protection against *Ehrlichia* spp. (Bitsaktsis et al., 2004; Totte et al., 1997), and antibodies also play an important role in immunity (Feng and Walker, 2004; Winslow et al., 2000; Yager et al., 2005). Thus, vaccines that stimulate humoral and cell mediated immune responses, prevent disease or minimize clinical signs, shorten duration of illness and/or prevent progression to a chronic infection appear to be feasible.

**Experimental Vaccines**

The emergence of human ehrlichioses in the last decade and the risk to public health has elicited interest in the development of vaccines for HME. The use of vaccines to prevent HME may be especially useful for persons who are active outdoors and are at an increased risk level.
for acquiring the disease. Ehrlichioses, such as heartwater and CME, are important veterinary diseases. Thus, considerable effort has been made to develop vaccines for \textit{E. ruminantium}, which causes large economic losses to the livestock industry in sub-Saharan Africa, and creates limitations on livestock production and export. Consequently, much of the knowledge base for ehrlichial disease vaccine development has involved \textit{E. ruminantium}, where experimental vaccine compositions have been tested, including live, attenuated, nucleic acid and recombinant subunit candidates.

A small group of major immunoreactive proteins of \textit{E. chaffeensis} and \textit{E. canis} has been identified on the basis of immunoblot reactivity, and most of these proteins contain tandem repeats or ankyrin repeats, and most have been molecularly defined (Doyle et al., 2006; Luo et al., 2008; McBride et al., 2003; McBride et al., 2006; Yu et al., 1996). Moreover, many of these proteins are secreted effector proteins that have major species-specific antibody epitopes (Doyle et al., 2006; Luo et al., 2008; Luo et al., 2009; Luo et al., 2010; McBride et al., 2006; Nethery et al., 2007). However, there is relatively little information regarding the protective efficacy of specific immunoreactive proteins. Major immunoreactive \textit{E. chaffeensis} proteins are 200-, 120-, 88-, 55-, 47-, 40-, 28- and 23-kDa (Chen et al., 1994; Rikihisa et al., 1994); \textit{E. canis}, 200-, 140-, 95-, 75-, 47-, 36-, 28-, and 19-kDa (McBride JW et al., 2003); and \textit{E. ruminantium}, 160-, 85-, 58-, 46-, 40-, 32- and 21-kDa (Mahan et al., 1994a). \textit{E. chaffeensis} immunoreactive proteins (Ank200, TRP120, TRP47, TRP32 [VLPT], OMP-1 family [22 genes], and MAP2) have been molecularly characterized as well as the corresponding orthologs in \textit{E. canis} (Ank200, TRP140, TRP36, TRP19 [VLPT], OMP-1 family [25 genes] and MAP2, respectively). Some of these immunoreactive orthologs have been molecularly identified and characterized in \textit{E. ruminantium} including (MAP1 family [16 genes], MAP2, and mucin-like protein [clone hw26; TRP36/47 ortholog]) (Jongejan and Thielemans, 1989; Mahan et al., 1994a; Sulsona et al., 1999).

Major immunoreactive proteins identified in \textit{Ehrlichia} spp. that have been the primary targets for experimental subunit vaccines include the major outer membrane proteins. Recombinant subunit and nucleic acid vaccines that contain a major surface protein ortholog of \textit{Ehrlichia} spp. (designated MAP1 in \textit{E. ruminantium}; p28 in \textit{E. chaffeensis} and p28/p30 in \textit{E. canis}), which is a member of a paralogous nonidentical multigene family of outer membrane protein genes (16 to 25 genes) in each respective \textit{Ehrlichia} species. Partial protection using a recombinant version of the \textit{E. chaffeensis} P28 protein has been demonstrated in mice after homologous challenge (Ohashi et al., 1998). Moreover, significant protection against homologous challenge using \textit{E. ruminantium} MAP1 DNA vaccination and recombinant protein boost was demonstrated in a mouse model (Nyika et al., 2002). There is substantial divergence in \textit{map1/p28} genes among different isolates of \textit{E. ruminantium} and \textit{E. chaffeensis}, and therefore this diversity may complicate development and implementation of vaccines utilizing this protein. Conversely, the \textit{p28/p30} genes of \textit{E. canis} appear to be highly conserved among geographically dispersed strains, and thus, may facilitate more rapid development of effective vaccines utilizing this antigen. Most recently, several new vaccine candidates have been identified and molecularly characterized in \textit{E. chaffeensis} that have major species specific epitopes within tandem repeat regions. These tandem repeat proteins (TRP120, TRP47 and TRP32) from \textit{E. chaffeensis} are consistently recognized by antibodies in convalescent antisera. The ability of these proteins to protect against homologous challenge is currently under active investigation.
Future Prospects

The emergence of human ehrlichioses in the late 20th century has focused new resources and efforts to improve diagnosis and treatment, and to understand pathogenic and protective immune mechanisms that will facilitate vaccine development. The completion of several *Ehrlichia* genome sequences has provided insight into their evolution, virulence mechanisms, clues to the unique strategies that they utilize to survive in both invertebrate and vertebrate hosts, and their interaction with and dependence on the host cell for survival. Molecular identification and characterization of the majority of the major immunoreactive proteins has been accomplished. These new vaccine prospects coupled with a more complete understanding of ehrlichial pathobiology and interaction with the innate and adaptive host immune responses, and useful animal models will undoubtedly stimulate the development of new and more effective nucleic acid or subunit vaccines for human and veterinary use in the future. New technologies including next-generation sequencing will provide researchers with the capability to rapidly and fully explore pathogen gene expression in order to define the dynamics of pathogen phenotype in invertebrate and vertebrate hosts, and new vaccine strategies will be identified through this exploration. New insights into immunoprotective mechanisms and molecular pathogen-host interactions have marked new areas of progress that have addressed key gaps in our knowledge that are required to make effective vaccines.

Key Points

- Vaccination is the most cost effective long term means of controlling human ehrlichioses, and commercial interest and progress in vaccine development for heartwater and canine ehrlichiosis will enhance prospects for a human ehrlichiosis vaccine.
- Immunologically well characterized murine models are available for determining vaccine candidate efficacy and defining protective and pathologic immune mechanisms.
- Defining *Ehrlichia* proteins that elicit protective humoral and cell mediated immune responses is needed and is an area of active investigation.
- Understanding innate and adaptive immune evasion strategies of *Ehrlichia* will improve prospects of effective vaccine development.
- Effective vaccine development will depend on understanding ehrlichial biology and phenotype in mammalian and arthropod host environments.
- Major immunoreactive tandem and ankyrin repeat proteins of *Ehrlichia* have been recently molecularly characterized that contain major continuous species-specific antibody epitopes. Some of these proteins are known to be involved in complex molecular host interactions that may contribute to pathogen survival and may be blocked by the host immune response.

References


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**RICKETTTSIOSIS**

**Introduction**

*Rickettsia* are arthropod-borne, gram-negative, obligately intracellular bacteria that reside in the cytosol of host cells. Tick-borne *Rickettsia* include the most deadly bacterial organism, *R. rickettsii*, low pathogenic organisms such as *R. parkeri* and *R. sibirica*, and nonpathogenic organisms such as *R. peacockii*, *R. bellii*, and *R. montanensis*. Lethality of Rocky Mountain spotted fever (RMSF) caused by *R. rickettsii* in the pre-antibiotic era was as high as 80%. Even
with the availability of effective rickettsiostatic antibiotic treatment, the mortality rate is around 3-5% for RMSF because of late diagnosis and delay in starting appropriate therapy (Paddock et al., 2002; Raoult et al., 2004). *R. rickettsii* not only causes severe disease naturally, it is also a potential terrorism agent because it is highly infectious at a very low dose (Wike et al., 1972).

**Etiologic agents**

*Rickettsiae* are small gram negative bacteria (0.3 – 0.5 x 0.8 – 1.0 µm). Rickettsial diseases are transmitted by arthropods including ticks, mites, lice, and fleas. Based on LPS antigens rickettsiae are classified into typhus group (TG) and spotted fever group (SFG). TG rickettsiae are transmitted by lice and fleas. SFG rickettsiae include more than 20 species and most of them are tick-borne except for mite-borne *R. akari* and flea-borne *R. felis*. The non-tick borne *Rickettsia* will not be discussed further. All *Rickettsia* multiply in the cytoplasm of host cells, but SFG rickettsiae can also multiply in the nuclei of host cells. *Rickettsia* organisms have undergone genome reduction resulting in a smaller genome (approximately 1Mb), and have lost genes encoding enzymes for sugar metabolism, lipid biosynthesis, nucleotide synthesis and amino acid synthesis (Andersson et al., 1998). SFG rickettsiae spread from cell to cell via actin-based mobility (Teyssseire et al., 1992).

**Epidemiology/Public health importance**

The distribution of tick borne SFG rickettsioses is restricted to areas where their tick reservoirs are present such as Rocky Mountain spotted fever in the Americas, Mediterranean spotted fever in Europe, Africa and Asia, Japanese spotted fever in Japan and Korea (Table 1).

**TABLE 1** Distribution of tick borne rickettsioses

<table>
<thead>
<tr>
<th>Disease</th>
<th>Rickettsia agent</th>
<th>Geographic Distribution</th>
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<tbody>
<tr>
<td>African tick-bite fever</td>
<td><em>Rickettsia africai</em></td>
<td>Sub-Saharan Africa, Caribbean islands</td>
</tr>
<tr>
<td>Far eastern spotted fever</td>
<td><em>Rickettsia heilongiangensis</em></td>
<td>Far East of Russia and China</td>
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<tr>
<td>Flinders Island spotted fever</td>
<td><em>Rickettsia honei</em></td>
<td>Australia and southeastern Asia</td>
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<tr>
<td>Mediterranean spotted fever</td>
<td><em>Rickettsia conorii</em></td>
<td>Southern Europe, southern and western</td>
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<td></td>
<td></td>
<td>Asia, and Africa</td>
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<tr>
<td>North Asian tick typhus</td>
<td><em>Rickettsia sibirica</em></td>
<td>Asia, Europe, and Africa</td>
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<tr>
<td>Lymphangitis-Associated Rickettsiosis</td>
<td><em>Rickettsia sibirica</em></td>
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<td></td>
<td><em>mongolotimonae</em></td>
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<tr>
<td>Oriental spotted fever</td>
<td><em>Rickettsia japonica</em></td>
<td>Japan and Korea</td>
</tr>
<tr>
<td>Queensland tick typhus</td>
<td><em>Rickettsia australis</em></td>
<td>Australia</td>
</tr>
<tr>
<td>Rocky Mountain spotted fever</td>
<td><em>Rickettsia rickettsii</em></td>
<td>North, Central and South America</td>
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<tr>
<td>Tick-borne lymphadenopathy</td>
<td><em>Rickettsia slovaca</em></td>
<td>Europe</td>
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<tr>
<td>Unnamed</td>
<td><em>Rickettsia parkeri</em></td>
<td>North and south America</td>
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<tr>
<td>Unnamed</td>
<td><em>Rickettsia massilae</em></td>
<td>Europe and North and South America</td>
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<tr>
<td>Unnamed</td>
<td><em>Rickettsia aeschlimannii</em></td>
<td>Europe and Africa</td>
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<tr>
<td>Unnamed</td>
<td><em>Rickettsia monacensis</em></td>
<td>Europe</td>
</tr>
<tr>
<td>Unknown</td>
<td><em>Rickettsia helvetica</em></td>
<td>Europe and Asia</td>
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Tick-borne rickettsiae are maintained in nature largely via transovarian transmission in ticks. Nonvirulent and low virulent tick-borne *Rickettsia* do not cause adverse effects on their...
In the United States, tick vector, but virulent *Rickettsia* such as *R. rickettsii* and *R. conorii* are pathogenic for *Dermacentor* and *Rhipicephalus* ticks, respectively (Niebylski et al., 1999; Santos et al., 2002). Thus, virulent rickettsiae such as *R. rickettsii* need an animal host to amplify the organisms to establish new lines of transovarian rickettsial maintenance (e.g., *D. variabilis* ticks acquire *R. rickettsii* while feeding on rickettsemic cotton rats) (Niebylski et al., 1999). The vectors of Rocky Mountain spotted fever are *D. variabilis* (American dog tick) in the eastern two-thirds of the US and regions of the Pacific coast states, *D. andersoni* (wood tick) in the Rocky Mountain states, *Rhipicephalus sanguineus* (brown dog tick) in the southwestern US and northern Mexico, and *Amblyomma cajennense* and *A. aureolatum* in South America.

The seasonal and geographic distribution of each rickettsiosis reflects the months of activity of the vector and its contact with humans. Over 90% of cases of Rocky Mountain spotted fever occur during April through September. Approximately 250-1200 cases of Rocky Mountain spotted fever have been reported annually in the past (http://www.cdc.gov/ncidod/dvrd/rmsf/epidemiology.htm) and currently more than 2000 cases are reported each year.

**Clinical spectrum and treatment**

Rickettsial diseases are characterized at onset by fever, severe headache, and muscle aches. In Rocky Mountain spotted fever, the characteristic maculopapular rash typically appears 3 to 5 days later. In severe disease, petechiae may appear in the center of the maculopapules. However, up to 10% to 15% of people with RMSF never develop a rash, a condition often referred to as "Rocky Mountain spotless fever" (Sexton and Corey, 1992). Rashes are less frequent in less severe rickettsioses such as African tick bite fever and *R. parkeri* infection. Focal skin necrosis with a dark scab (an eschar) at the site of tick feeding is a common feature of boutonneuse fever, African tick bite fever, North Asian tick typhus, Queensland tick typhus, Japanese spotted fever, Flinders Island spotted fever, tick-borne lymphadenopathy, and the recently described infections in the US caused by *R. parkeri* and a novel strain 364 D, but is rare in Rocky Mountain spotted fever.

Tetracyclines are first-line treatment, and doxycycline may be used to avoid tooth staining in children. Tetracyclines are rickettsiostatic, not rickettsicidal. Ciprofloxacin and other fluoroquinolones are effective against certain rickettsiae. Because diagnostic tests can take time and may be insensitive, antibiotics are usually begun presumptively to prevent significant deterioration, death, and prolonged recovery.

**Overview of protective immune mechanisms**

Most of our understanding of the immune response against *Rickettsia* is derived from in vitro studies as well as the murine models of rickettsioses. Proinflammatory cytokines such as IFN-γ and TNF-α are essential for primary defense against rickettsial infection. These cytokines act in concert to activate endothelial cells, the major target cells of rickettsial infections, as well as other minor target cells to kill intracellular organisms via a nitric oxide synthesis-dependent mechanism. The sources of these protective cytokines are hypothesized to be the T lymphocytes and macrophages that infiltrate the perivascular space surrounding the vessels with infected endothelium.

Cell mediated immunity plays a critical role in host defenses against rickettsial infections (Walker et al., 2001). There are two important effector components of acquired
immune response against *Rickettsia*, namely IFN-γ production by CD4+ and CD8+ type-1 cells, which activates intracellular bactericidal mechanisms of endothelial cells and macrophages, and the generation of *Rickettsia*-specific cytotoxic CD8+ T cells that lyse infected target cells via pathways involving perforin and/or granzymes. CD8+ T cells are more important in clearance of rickettsial infection against rickettsiae than CD4+ T cells (Walker et al., 2001). Although adoptive transfer of either CD4 or CD8 immune T lymphocytes control the infection and lead to survival, only depletion of CD8 T lymphocytes altered the outcome of infection, and depletion of CD4 cells had no observed effect on the course or outcome of infection (Walker et al., 2001).

Humoral response may play an important role in protection against infection and antibodies against surface protein antigens are very likely critical effectors of vaccines-induce protective immunity. In animal experiments, antibodies to *Rickettsia* or rickettsial outer membrane proteins can neutralize rickettsial infection (Anacker et al., 1987; Li et al., 1988). However, natural infection does not result in the production of protective antibodies prior to clearance of rickettsiae. Thus, humoral immunity may be more important in preventing reinfection as in vaccine-induced immunity than in clearance of primary infection.

**Vaccine feasibility and current status.**

Currently no commercial vaccine is available for any rickettsial disease. Infection with *R. rickettsii* and *R. conorii* is thought to provide long lasting immunity against re-infection. Thus, it feasible to develop a vaccine against rickettsial diseases. In theory, a subunit vaccine targeting a conserved rickettsial protein such as OmpB may be developed to prevent all rickettsial diseases. The best rickettsial vaccine may be an attenuated organism that can multiply but does not cause disease in the host. Attenuated *Rickettsia* has been achieved with gene knockout technology, and more attenuated strains of *Rickettsia* will become available for vaccine evaluation.

**Experimental vaccines and other potential vaccine prospects**

**Inactivated rickettsial vaccine.** The history of development of vaccines against Rocky Mountain spotted fever contains numerous failures and limited success in preventing or ameliorating disease. The first rickettsial vaccine was a killed *R. rickettsii* preparation from infected ticks (Spencer and Parker, 1925). The method of propagating *R. rickettsii* in yolk sac of embryonated chicken eggs was adapted soon after the development of this method in 1938 (Cox, 1939). A third killed Rocky Mountain spotted fever vaccine was prepared from cell culture-propagated *R. rickettsii* by the U.S. Army in the 1970s (Kenyon et al., 1972).

A challenge trial in human volunteers was conducted in 1973. Neither the yolk sac vaccine nor the tick vaccine prevented the illness, which, of course, was treated promptly to prevent severe illness or death. The yolk sac vaccine was withdrawn from the market in 1978. Subsequent challenge trial of the killed *R. rickettsii* vaccine prepared from cell culture yielded protection of 25% of the volunteers who received it. Evaluation of the recipients’ immune responses revealed failure to stimulate sustained cellular immunity (Clements et al., 1983).

**Subunit vaccine for Rickettsia.** Two surface protein antigens of *R. rickettsii*, OmpA and OmpB, have been identified as major protective antigens and are candidates for use as subunit vaccines. The first evidence that OmpA and OmpB contain protective epitopes came from the studies of monoclonal antibodies to heat sensitive epitopes of OmpA and OmpB, which neutralized *R. rickettsii* toxicity in mice and infection in guinea pigs (Anacker et al., 1987; Li et
al., 1988). Immunization with the *E. coli*-expressed OmpA N-terminal fragment partially protects guinea pigs against a lethal challenge dose of *R. rickettsii* (McDonald et al., 1988). A fragment from the N-terminus of *R. conorii* OmpA protects guinea pigs against experimental infection with *R. conorii* and partially protects guinea pigs from challenge with the heterologous *R. rickettsii* (Vishwanath et al., 1990). Fragments of the *ompA* and *ompB* genes have been tested as DNA vaccines. In a regime of DNA immunization followed by boosters of the corresponding peptide, mice immunized with one of several *R. rickettsii* *ompA* or *ompB* fragments are partially protected against a lethal challenge with heterologous *R. conorii* (Diaz-Montero et al., 2001). It is not known whether the incomplete protection of OmpA and OmpB to the heterologous *Rickettsia* species challenge in these experiments is caused by the antigenic differences between the rickettsial species, the immunization regime, or the antigen composition.

**Attenuation of Rickettsia by gene knockout.** Because of the difficulty of transforming *Rickettsia*, scientists have been unable to knock out rickettsial genes to test their function and to create an attenuated rickettsial vaccine until recently. The phospholipase D (*pld*) was the first rickettsial gene that was genetically knocked out. The *pld*-knockout Evir strain is avirulent for guinea pigs at the doses for which the Evir strain is virulent (Driskell et al., 2009). A Sca2 knockout strain of *R. rickettsii* has lost actin-based mobility in cell culture, and in a guinea pig model of infection, the Sca2 mutant did not elicit fever, suggesting that Sca2 is a virulence factor of spotted fever group rickettsiae (Kleba et al., 2010).

**Future prospects**

The protective immune response to rickettsial infection involves both innate and adaptive immune responses. A concerted action of CD8+ T cells, and CD4+ T cells producing IFNγ, and antibodies is required to clear infection and to prevent reinfection. Live attenuated vaccine mimics the natural infection, thus *Rickettsia* that are genetically attenuated by gene knockout are the strongest future direction for developing a rickettsial vaccine.

**Key issues**

*Rickettsia* are obligately intracellular bacteria and are transmitted by arthropods, including ticks. *R. rickettsii* cause fatal disease that can be prevented by avoiding tick bites and removing attached ticks promptly.

There is no vaccine for rickettsial diseases despite the fact rickettsial infection stimulates long term immunity. Vacccines are needed for Rocky Mountain spotted fever.

Because diagnostic tests can take time and may be insensitive, antibiotic treatment should be initiated based on clinical and epidemiological information.

**References**


ANAPLASMOSIS

Introduction

FIGURE 1 Phylogenetic tree of order Rickettsiales.

[Genera in the families Anaplasmataceae (on yellow background) and Rickettsiaceae (on blue background) are shown. Species of interest are circled or boxed. The Rickettsia are subgrouped according to ancestral group (AG), transitional group (TrG), Typhus group (TG) and spotted fever group (SFG). The tree is based on a clustalW alignment of 16S ribosomal RNA gene sequences using POWER (http://power.nhri.org.tw/power/home.htm).]

Anaplasma are gram-negative α-Proteobacteria belonging to the order Rickettsiales, and family Anaplasmataceae (Figure 1) (Dumler et al., 2001). Like most Rickettsiales, organisms in the genus Anaplasma are small, ranging from 0.2-0.9 μm. Of the four genera in the family Anaplasmataceae, Anaplasma is most similar in lifestyle and evolutionary history to the closely related Ehrlichia spp. The genus Anaplasma contains five recognized species: A. bovis, A. ovis, A. platys, A. marginale and A. phagocytophilum, with the first four species infecting animals, and the last being a zoonotic agent. The type species for the genus is A. marginale, a cattle pathogen that was recognized in the early 1900s (Theiler, 1910). Of these species, by far the most research has been done on the latter two species, A. marginale which causes anaplasmosis, and the human
pathogen *A. phagocytophilum*, which causes human granulocytic anaplasmosis. All *Anaplasma* species are obligate intracellular organisms infecting mature or immature hematopoietic cells where they replicate within membrane bound vacuoles (Dumler et al., 2001). *Anaplasma* spp. are therefore blood-borne pathogens and are transmitted from host to host by Ixodid ticks. This manuscript will focus on the human pathogen, *A. phagocytophilum*.

**Etiologic agent**

*A. phagocytophilum* was previously classified as an *Ehrlichia* (the agent of human granulocytic ehrlichiosis). Accumulating genetic information on a number of pathogens originally named as *Ehrlichia* species drove the reorganization of the families Rickettsiaceae and Anaplasmataceae in 2001 wherein *Ehrlichia equi* and *Ehrlichia phagocytophila* were unified as a single species with the agent of human granulocytic ehrlichiosis, to create the new species *Anaplasma phagocytophilum* (Dumler et al., 2001). Because anaplasmosis is recognized as a cattle disease, *A. phagocytophilum* is said to cause human granulocytic anaplasmosis (HGA).

*A. phagocytophilum* infects polymorphonuclear leucocytes (neutrophils), where it replicates within a membrane bound vacuole forming a morula or microcolony (Chen et al., 1994; Rikihisa, 1991). *A. phagocytophilum* has a biphasic developmental cycle with two morphologically distinct forms referred to as dense cored cells (DC) and reticulate cells (RC) (Popov et al., 1998). DCs are small electron dense bodies that predominate in early infection, are thought to be metabolically inert and play a role in attachment and invasion of the host cell (Munderloh et al., 1999). RCs are electron lucent larger pleomorphic cells that undergo binary fission and are thought to be the metabolically active form of the organism (Ismail et al., 2010; Troese and Carlyon, 2009).

The *Rickettsiales* are the closest extant relatives of the bacterial lineage that led to the mitochondria, and have undergone extensive genome reduction with individual species displaying small genome sizes between 0.8 and 1.5 Mb (Sallstrom and Andersson, 2005; Andersson et al., 1998; Viale and Arakaki, 1994). The *A. phagocytophilum* genome, completed in 2006, is 1,471,282 bp and is reported to contain 1264 protein coding genes as well as 37 tRNA and 3 rRNA genes (Dunning Hotopp et al., 2006). While this number of protein coding genes is greater than many of the closely related organisms (range = 805-1264), this difference is largely due to differences in annotation style (many small open reading frames were annotated) rather than a real difference in coding capacity (Brayton et al., 2008).

**Epidemiology/Public health importance**

HGA was first identified as a human pathogen in 1990 when a patient in Wisconsin died after a short acute febrile illness (Dumler et al., 2005). HGA is increasingly recognized as a frequent cause of fever after tick bite in the Upper Midwest, New England, parts of the mid-Atlantic states, northern California (Figure 2), many parts of Europe and Asia (Dumler et al., 2005). In the United States, cases of HGA have increased since the CDC started tracking cases in 1999 (Figure 3) with 1009 cases reported in 2008 (Hall-Baker et al., 2010).
A. phagocytophilum infects many species including dogs, cats, horses, deer, cattle, sheep, mice, wood rats, bank voles, squirrels, opossums, skunks and raccoons (Foley et al., 2008; Hackett et al., 2006; Lester et al., 2005; Levin et al., 2002; Stuen and Bergstrom, 2001; Stuen et al., 2001a; Stuen et al., 2001b; Stuen et al., 2001c; Pusterla et al., 1999). Humans are accidental, dead end hosts for A. phagocytophilum, typically becoming infected when humans encroach on small mammal-tick habitats (Dumler et al., 2005). The major mammalian reservoir is the white-footed mouse, which typically has a transient bacteremia (1-4 weeks). While white tailed deer can be persistently infected, they do not appear to harbor strains of A. phagocytophilum that cause HGA (Massung et al., 2005). Strain definition for A. phagocytophilum is not well defined,
however 1-2 nucleotide differences in the 16S ribosomal RNA gene and 1-3 bp differences in the GroESL gene have been used to identify strain variants (Stuen et al., 2003; Massung et al., 2002).

In the Midwestern and Eastern US *Ixodes scapularis* is the vector, while *I. pacificus* vectors *A. phagocytophilum* in the Western US. *I. ricinus* is the major vector in Europe, and *I. persulcatus* transmits disease in Asia. Tick infection is established after an infectious blood meal, and the bacterium is transstadially but not transovarially passed (Dumler et al., 2005). These ticks also transmit agents that cause Lyme disease, babesiosis and tick-borne meningoencephalitis, with about 10% of HGA patients showing serological evidence of co-infection with one of these agents (Ismail et al., 2010; Dumler et al., 2007; Dumler et al., 2005).

**Clinical spectrum/treatment**

HGA presents with fever, headache, leukopenia, thrombocytopenia, absence of a skin rash 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 (Ismail et al., 2010; Dumler et al., 2005). Some individuals infected with *A. phagocytophilum* do not become ill or experience only very mild symptoms and do not seek medical treatment, however, at the other end of the spectrum, the disease can prove fatal, particularly in immunocompromised or elderly individuals (Thomas et al., 2009; Dumler et al., 2005).

Accurate diagnosis of HGA is often difficult due to the non-specific nature of the symptoms. An initial diagnosis is based on the patient's symptoms and a possible history of tick exposure. Diagnosis is confirmed by laboratory tests: 1) microscopic examination of Wright- or Giemsa-stained peripheral blood smears looking for dark staining morulae within neutrophils can be used early in infection, 2) a polymerase chain reaction (PCR) assay is the most sensitive test, provides rapid diagnostic evaluation and can discriminate between several tick-borne diseases that present with similar symptoms, 3) *A. phagocytophilum* can be cultivated within two weeks in the human promyelocytic leukemia cell line HL-60 by direct inoculation of cell cultures with peripheral blood from a potentially infected patient, and 4) serodiagnosis is most commonly used, but is not rapid, as it requires time for antibody to develop which typically takes longer than the onset of clinical symptoms; additionally, nonspecificity of this test may result from cross reactions with *Ehrlichia chaffeensis* (Ismail et al., 2010; Thomas et al., 2009).

The treatment of choice for both adults and children is a tetracycline antibiotic, usually doxycycline, which should be initiated promptly for improved outcomes and continued for 10-14 days (Hamburg et al., 2008; Chapman et al., 2006).

**Overview of protective immune mechanisms**

Relatively little is known about protective mechanisms of immunity to *A. phagocytophilum* infection except that protective immunity is mediated by cellular and humoral immune mechanisms. Development of high titer antibody responses is believed to be indicative of protective immunity (Ismail et al., 2010). Studies in sheep indicate that protection can last from a few months to > 1 year and that a measure of heterologous protection is afforded by some strains but not others (Stuen et al., 2003). Reinfection has been confirmed in some individuals which suggests that long term immunity was not engendered, however a lack of cross protection between different strains of *A. phagocytophilum* must also be considered (Levin et al., 2004; Horowitz et al., 1998).
Similar to *A. marginale*, *A. phagocytophilum* effects immune evasion through antigenic variation of the Msp2/p44 immunodominant surface protein (Granquist et al., 2010; Barbet et al., 2003). There is at least one expression site for *msp2/P44* and ~100 functional pseudogenes which can recombine into the expression site to generate variation (Dunning Hotopp et al., 2006; Barbet et al., 2003). The Msp2/P44 protein is characterized by conserved amino- and carboxy-termi flanking a central hypervariable region (Murphy et al., 1998). Msp2/P44 functional pseudogenes are typically truncated at the 5’ and 3’ ends, and recombine into the expression site through a RecF dependent gene conversion mechanism (Lin et al., 2006).

**Current vaccine status, experimental vaccines and potential vaccine prospects**

Vaccination against the cattle pathogen *A. marginale* is effected by infection with an avirulent strain, providing a measure of crossprotection without sterile immunity. This blood-based vaccine is not used in the U.S. due to the threat of transmitting emerging pathogens. There are no vaccines currently available for HGA. The search for vaccine candidates has focused on surface proteins as these are the interface for interactions with the host cell; however these studies are in their infancy. Little is known about the surface proteins of *A. phagocytophilum*, aside from Msp2/P44, which due to rapid antigenic variation does not constitute a good vaccine candidate (Ge and Rikihisa, 2007). The genome sequence provides a useful tool to facilitate research to identify vaccine candidates and was recently used in combination with a proteomic approach to identify surface exposed proteins. Two proteins were identified, Asp55 and Asp62, that were also recognized by immune serum from a patient with HGA. Peptide anti-sera for these two proteins were able to partially neutralize *A. phagocytophilum* infection in the human promyelocytic leukemia cell line, HL-60 cells (Ge and Rikihisa, 2007).

Preliminary studies aimed at understanding the pathogen-tick interface have the long term goal of developing transmission blocking vaccines, although this research has focused more on interrupting the transmission of the cattle pathogen *A. marginale* (de la Fuente et al., 2010; Ramabu et al., 2010; Noh et al., 2008). Novel approaches that could reduce the level of human contact with disease agents include the development anti-tick vaccines. An anti-tick vaccine based on the *Rhipicephalus microplus* gut protein Bm86 has been successful in field trials in Cuba, however, implementation of a similar vaccine was not successful in Australia (de la Fuente et al., 2007; Rodriguez Valle et al., 2004). A successful anti-tick vaccine would be introduced orally to the wildlife reservoirs in a similar fashion to rabies vaccine, and could eliminate the need for broad scale human vaccination (Slate et al., 2009). This strategy would reduce, but eliminate risk of transmission. As yet, there are no candidates ready for testing for either of these strategies for ticks that transmit HGA.

**Theraupeutics and other biologics for prevention**

Preventative antibiotic therapy is contraindicated for individuals who have had recent tick bites but are not ill. Prevention is effected by avoidance of tick bites through the following strategies: 1) avoidance of tick dense areas, 2) wearing of light colored protective clothing (i.e. long pants, closed toed shoes, etc.), 3) frequent checks for crawling and attached ticks, and 4) application of a repellant such as DEET (N,N-diethyl-m-toluamide). Should a person find an attached tick prompt removal reduces the threat of transmission, as studies have shown that a period of 4 to 24 hours may be necessary before successful transmission from tick to host takes place (Ismail et al., 2010; Bakken and Dumler, 2008; Katavolos et al., 1998).
Key issues

- Additional effort in identifying surface exposed targets that could illicit protective immune responses is needed. The genome is a key tool in this endeavor and the advent of affordable, widely available proteomic tools should facilitate these efforts. Understanding mechanisms of immune evasion will also help in assessment of vaccine candidates.
- Understanding the strain composition that makes up the *A. phagocytophilum* species will facilitate development of appropriate vaccine candidates; i.e. variation in leading vaccine candidate antigens may not be relevant if the variant strain does not infect humans. Representative *A. phagocytophilum* genome sequences from an array of hosts could assist in exploring the strain composition but this organism does not lend itself to high throughput, next generation sequencing strategies due to the highly repetitive nature of the genome sequence.
- Understanding of the transmission biology of *A. phagocytophilum* would facilitate the development of transmission blocking vaccines and potentially help elucidate targets for anti-tick vaccines as well.
- Understanding host differences in pathogenesis would aid in determining if vaccines tested in sheep, for example, would be relevant for humans.

References


EHRlichioSES, RICKETTSIOSES AND ANAPLASMOSIS IN THE UNITED STATES


