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ANAPLASMATACEAE AS HUMAN PATHOGENS : BIOLOGY, ECOLOGY AND EPIDEMIOLOGY

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Abstract:

This review describes the biology, ecology, and epidemiology of known human pathogens in the family Anaplasmataceae that are transmitted by ticks and belong to the genus Anaplasma and genus Ehrlichia. We discuss the current status of diagnosis and surveillance of the diseases they cause, and address the challenges and new perspectives raised due to continuous recognition of new emerging human pathogens in the family Anaplasmataceae.

Key words : Anaplasma phacocytophilum, Ehrlichia chaffeensis, anaplasmosis, ehrlichiosis, tick-borne diseases.

INTRODUCTION

The family Anaplasmataceae is comprised of increasingly numerous genera of obligate intracellular Gram negative alphaproteobacteria. The current description of the family was formally proposed in 2001 based on their nucleotide sequence similarity, phenotypic and eco-biological features [1]. These invertebrate-transmitted bacteria possess specialized and diverse evolutionary adaptations that allow them to parasitize a variety of mammalian cells of hematopoietic and bone marrow origin or endothelium. Different species of Anaplasmataceae cause a number of pathological conditions in humans and animals with clinical manifestations ranging from asymptomatic chronic persistence to acute illness and life threatening diseases. Both the veterinary pathogens and the invertebrate pathogens of the family Anaplasmataceae have been discussed elsewhere in several outstanding reviews [2-8]. In this review, we highlight the biology, ecology, and epidemiology of known human pathogens in the Anaplasmataceae that are transmitted by ticks and belong to the genus Anaplasma and genus *Ehrlichia*, and discuss the difficulties and challenges of diagnosis and surveillance of the diseases they cause. Finally, we describe several newly emerging human pathogens in the family Anaplasmataceae.

TAXONOMY AND PRIMARY CELL ASSOCIATIONS

Dumler et al. 2001 described and defined four genera, Anaplasma, *Ehrlichia*, *Neorickettsia* and *Wolbachia* in the family Anaplasmataceae while several other genotypes were recognized as potential members that might comprise a new genus [1]. Only the first three genera contain both pathogenic and non-pathogenic species found in associations with different mammals. Bergey's Systematic Bacteriology added the genus *Aegyptionella* to the family Anaplasmataceae [9]. More recently, the genus *Neoehrlichia* was proposed; it currently consists of two *Candidatus* species, *Candidatus Neoehrlichia mikurensis* and *Candidatus Neoehrlichia* lotoris (Figure 1) [10, 11].

The genus Anaplasma includes five species of veterinary significance only, *Anaplasma marginale*, *A. bovis*, *A. centrale*, *A. ovis*, and *A. platys*. While *A. phagocytophilum* was long recognized for its detrimental impact on ruminants as the etiological agent of tick-borne fever, it is also responsible for causing illness in humans, horses and dogs (Table 1). Anaplasma phagocytophilum infects circulating white blood cells of different mammals, *A. platys* targets platelets, and *Anaplasma marginale*, *A. bovis*, *A. ovis*, and *A. centrale* each have unique adaptations for infecting mammalian red blood cells [1, 9]. *Aegyptianella pullorum* is most closely related to

the genus Anaplasma; it is also a tick-transmitted bacterium but infects and replicates only in avian red blood cells [12]. The genus *Ehrlichia* includes several species that appear to be pathogenic for both humans and animals, *Ehrlichia* (formerly Cowdria) ruminantium, *E. canis, E. chaffeensis, E. ewingii*, and *E. muris* (Table 1), and a number of propagated or molecular isolates whose pathogenic potential or taxonomic status are not yet defined. *Ehrlichia* ruminantium infects primarily endothelial cells; *E. canis*, *E. chaffeensis* and *E. muris* invade

BIOLOGY AND HOST CELL INTERACTIONS

Anaplasma and *Ehrlichia* grow as clusters of small cocci or pleomorphic coccobacilli in the cytoplasm of neutrophils, granulocytes or monocytes [16, 17]. Within infected cells Anaplasma and *Ehrlichia* reside inside intracytoplasmic vacuoles where they form micro-colonies called morulae and multiply by binary fission. The bacteria have a Gram-negative cell wall ultra-structure; however, they do not stain well with
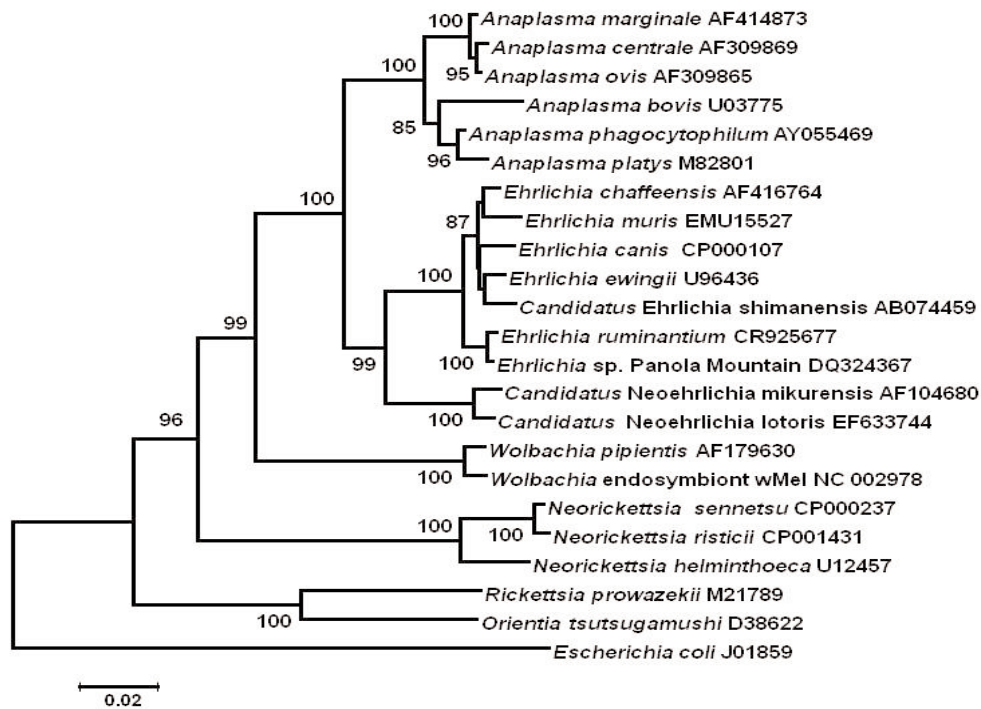
Figure 1 : Genetic relationships of different representatives of the family Anaplsmataceae based on their 16S rRNA gene sequences. The sequences of Rickettsia, Orientia and Escherichia coli were used as out-groups. Scale bar correspond to the number of nucleotide substitutions per site, number at each branch is the GenBank accession number.

and multiply in monocytes and macrophages, while E. ewingii grows in granulocytes and neutrophils.

The genus *Neorickettsia* includes *Neorickettsia* sennetsu (formerly called *Ehrlichia* sennetsu), N. helminthoeca and N. risticii (formerly E. risticii); these three agents are found in association either with flukes, trematodes, snails or metacercaria-containing aquatic insects [13, 14]. In humans or animals infected by ingestion of infested flukes, contaminated fish, or insects *Neorickettsia* grows within monocytes and macrophages.

In contrast, the members of the genus *Wolbachia* are found widely in a tremendous variety of arthropods, including insects, mites, termites, ticks, spiders, isopods and crustaceans and in filarial nematodes [15]. *Wolbachia* pipientis is the only current species formally designated within the genus *Wolbachia* although there are at least six distinct bushy clades of these diverse bacteria. In invertebrate hosts, *Wolbachia* infection is often associated with alteration of host reproduction through killing of male embryos, feminization of genetic males, induction of parthenogenesis or cytoplasmic incompatibility. In nematodes, *Wolbachia* is found in a mutualistic symbiotic relationship.

the Gram stain and are better visualized using Wright or Giemsa staining. The poor retention of safronin is likely due to other unusual characteristics of Anaplasma and *Ehrlichia*. Their cell wall lacks lipopolysaccharide (LPS) and peptidoglycan and it is rich in cholesterol acquired from the host cell [16-18]. The bacteria are pleomorphic with their length ranging from 0.2 to 2.0 ?m; they can be detected in two morphologically distinct forms, dense-cored cells and reticulate cells, most frequently seen in cultured cells, but they can also be observed in infected blood and tick midgut cells [16, 19-22].

Invasion and survival strategies are most well studied for *Anaplasma marginale*, *A. phagocytophilum* and *Ehrlichia chaffeensis*; however, more attention has been paid recently to other members of these genera as well [23-25]. Bacteria invade their target cells by binding to surface proteins (such as selectins) that are often glycosylated, and they are internalized within the endosomes via receptor-mediated endocytosis that is regulated by cyclic di-GMP signaling [23, 26-28]. Endosomes containing live bacteria are modified to avoid fusion with lysosomal vesicles and do not form mature acidified phagolysosomes. Replicative intracellular inclusions of *E. chaffeensis* are early endosomes that do not mature into late

Table I : Characteristic features of Anaplasmataceae pathogenic for humans

Species	Veterinary Disease	Human Disease	Target cell	Primary Vector	Reservoir	Geography	Genome Size, bp
Anaplasma phagocytophilum	Tick-borne fever, Equine anaplsmosis, Canine anaplasmosis	Human anaplasmosis	Neutrophils, granulocytes	Ticks, *Ixodes* spp.	Small to middle and large size mammals	Worldwide	1,471,282
Ehrlichia chaffeensis	Canine monocytic ehrlichiosis	Human monocytic ehrlichiosis	Monocytes, macrophages	Tick, *Amblyomma americanum*	Small rodents, cervids, canids	Primary in western hemisphere	1,005,812 1,176,248
Ehrlichia ewingii	Canine ehrlichiosis	Ewingii ehrlichiosis	Granulocytes, neutrophils	Tick, *Amblyomma americanum*	Small rodents, cervids, dogs	Western hemisphere	Not determined
Ehrlichia muris	Murine monocytic ehrlichiosis	Human monocytic ehrlichiosis	Monocytes, macrophages	*Ticks, Ixodes* spp.	Rodents, deer	Eurasia, North-western USA	In progress
Ehrlichia canis	Canine monocytic ehrlichiosis	Human monocytic ehrlichiosis	Monocytes, macrophages	Tick, *Rhipicephalus sanguineus*	Dogs, canids	Worldwide	1,315,030
Ehrlichia ruminantium	Heartwater	Unnamed disease	Endothelium, monocytes, macrophages	Tick, *Amblyomma variegatum*, *A. hebraeum*	Cattle, sheep, goat	Africa and West Indies	1,499,920-1,516,355
Ehrlichia sp. Panola Mountain agent	Unnamed disease	Unnamed disease	Not determined	Tick, *Amblyomma americanum*	Deer	United States, West Indies	Not determined
Candidatus Neoehrlichia mikurensis	Yes	Unnamed disease	Endothelium	Tick, *Ixodes* spp.	Rodents	Eurasia	Not determined
Neorickettsia sennetsu	Unknown	Sennetsu ehrlichiosis	Macrophages	Trematode	Trematode	Asia	859,006

endosomes; they are often surrounded by mitochondria and appear to inhibit mitochondrial activity [29, 30]. In contrast, *A. phagocytophilum* replicative inclusions are more characteristic of autophagosomal pathway and do not exhibit endosomal markers [31]. These processes are manipulated by bacterial protein synthesis and result in repression of NADPH oxidase and scavenging of superoxide and peroxide radical in the case of *A. phagocytophilum*, or selective accumulation of transferrin receptors induced by *E. chaffeensis*. Due to a highly orchestrated inhibition of host cell apoptosis, intracellular *A. phagocytophilum* and *E. chaffeensis* are able to exploit intracellular nutrients and to divide until the host cell bursts to liberate bacteria which then spread and infect other white blood cells [32]. The details of exit mechanisms have been recently described for *E. chaffeensis* and *E. muris* [33]. Actin-mediated formation of filopodia by infected macrophages may be crucial for targeted delivery of *Ehrlichia* to neighboring cells, another effective adaption for successful survival and evasion of host immune systems by these bacteria.

GENOME AND GENETIC CHARACTERISTICS

Complete genome sequences have been obtained for most species of the family Anaplasmataceae, including *Neorickettsia* spp. and *A. phagocytophilum*, as well as several isolates each of A. marginale, *E. chaffeensis,, E. ruminantium, E. canis* and Wolbacia spp. (table 1); furthermore, sequencing of the *E. muris* genome is in the last phase of its completion [34-37]. All

of these genomes consist of a single circular chromosome, which is largely syntenic in *A. phagocytophilum* and *E. chaffeensis*, and are of a relatively small size and coding capacity compared to most free-living bacteria. Members of the family Anaplasmataceae share a number of genes whose homologues are also found in other bacteria, while many of their unique genes encode proteins of unknown functions and are found only in that particular organism. Genomes of Anaplasma or *Ehrlichia* spp. encode only 1,100 to 1,300 proteins [34]. Comparative analyses reveal irreversible loss of numerous metabolic pathways including those for synthesis of at least a dozen amino acids and many metabolites which must be acquired from host cell pools. Genomes of *A. phagocytophilum* and *Ehrlichia* spp. also lack the genes for enzymes involved in glucose utilization (consistent with the observation that these bacteria cannot utilize glucose as a source of carbon energy) and do not contain genes that are required for the biosynthesis of lipopolysacharide and peptidoglycan. On the other hand, being aerobic bacteria, Anaplasma and *Ehrlichia* possess conserved sets of genes enabling pyruvate catabolism, and a complete tricarboxylic acid cycle and electron transport chain. Anaplasma and *Ehrlichia* also have genes for the biosynthesis of nucleotides and most vitamins and cofactors, including FAD, NAD, CoA, biotin, folate, thiamine and protohaem.

Genomes of Anaplasma and *Ehrlichia* spp. encode proteins of the type four secretion apparatus (T4SS) and two-component systems which are differentially expressed during host cell

entry and intracellular development; many genes encoding T4SS proteins are duplicated. Several genes encode proteins containing tandem repeats (TRP32, TRP47 and TRP120 proteins in *E. chaffeensis*) and ankyrin domains (AnkA) which are associated with host cell interactions and are often referred to as virulence factors for these bacteria.

SURFACE PROTEINS AND THEIR FUNCTIONS

The presence and expansion of genes encoding proteins belonging to the outer membrane species-specific super-family is another unique feature of bacteria of the family Anaplasmataceae. Differential expression of the major surface proteins, particularly proteins in the OMP1/MSP2/P44-P28 super-family, is the characteristic of Anaplasma and *Ehrlichia* [34, 35, 38]. In *A. phagocytophilum*, 3 copies of omp1, 1 copy of msp2, 2 copies of msp2 homologues, one of msp4, and 113 loci of p44 have been identified [34]. It is believed that P44 plays a central role in binding of *A. phagocytophilum* to the surface of neutrophils and granulocytes. It has been suggested, that continuing recombination of the multiple genetic copies of p44 at a single expression site may be responsible for P44 antigenic diversity and evasion of host immune responses by *A. phagocytophilum* infecting human, horses and sheep (reviewed in [32]). Furthermore, diverse P44 genes are expressed during different developmental stages of I. scapularis and in mice, and in different types of experimentally infected cells, suggesting that variable antigenic environmental adaptation occurs in *A. phagocytophilum*. It appears that among the diverse antigenic variants of P44, specific genetic loci associated with expression in human, canine or equine host can be identified [39]; divergence of p44 sequences of the Ap-1 variant has been clearly demonstrated [40]. The latter observation is particularly important since Ap-1 variants of *A. phagocytophilum* are found exclusively in tick and deer while other genetic variants that are associated with human illness are found in ticks and wild mice [20, 41]. Similarly, *A. phagocytophilum* strains circulating in California exhibit significant genetic diversity and unique host adaptations demonstrated by the susceptibility of wood-rats to strains of different origin, and such wood-rat strains do not cause clinical illness in horses [42].

In *E. chaffeensis*, 22 copies of OMP1/P28 genes are found, while its homologue in *E. canis* has 25 copies [34]. Significant genetic variations in p28 have been described among *E. chaffeensis* isolates by several investigators [43-45]. Differential expression of P28 has also been described in infected animals, macrophages and tick cell cultures; however, it is not yet demonstrated if a recombination mechanism is responsible for selective adaptation to eukaryotic hosts as has been described for *A. phagocytophilum* [46, 47]. Other *Ehrlichia*, including *E. canis*, E. ewingii, and *E. ruminantium*, possess genomic loci with similarly arranged sets of genes encoding for variably-expressed outer membrane surface proteins [46, 48, 49]. Recent observations also indicate that P44-P28 proteins can function as porins [50].

ECOLOGY AND NATURAL HISTORY

Anaplasma and *Ehrlichia* spp. are tick-borne agents with a world-wide distribution. Anaplasma phagocytophilum is maintained in zoonotic cycles between infected mammalian host reservoirs and ticks of the Ixodes ricinus/persulcatus complex. In the USA it is found in Ixodes scapularis in the eastern and mid-western states, and I. spinipalpis and I. pacificus in mountain and western coastal areas, respectively [51]. Ixodes ricinus, I. persulcatus and I. ovatuss are the primary vectors transmitting *A. phagocytophilum* in Europe and Asia [7, 52, 53]. Other nidicolous species of Ixodes ticks were found to be PCR positive for *A. phagocytophilum* l [54, 55]; however, the role of these ticks in the natural maintenance cycle and transmission of *A. phagocytophilum* need further evaluation as many of them are not known to feed on humans. In North Africa (Morocco, Algeria and Tunisia), I. ricinus is the main vector but Hyalomma detritum may also play a role in transmitting *A. phagocytophilum* to cattle [56, 57]. Furthermore, *A. phagocytophilum* is likely to circulate in a variety of ticks feeding on dogs or reptiles as observed in Tunisia, South Africa and Ghana [57-59]. A report from Israel indicates that *A. phagocytophilum* can be also detected in other ticks, such as H. marginatum, Rhipicephalus turanicus and Boophilus kohlsi feeding on large animals [60]. Many species of domestic and wild animals are important reservoirs of *A. phagocytophilum*. Beside cattle, sheep, , horses and dogs, potential reservoirs include small rodents, shrews, bears, opossums, rabbits, cervids, foxes, raccoons, wild boars, squirrels and lizards [7, 61]. The white-footed mouse, Peromyscus leucopus, is the primary mammalian reservoir for *A. phagocytophilum* variants pathogenic for humans in the USA [62, 63].

Both *Ehrlichia chaffeensis* and E. ewingii are mainly transmitted by the lone star tick, Amblyomma americanum in North America [64]. However, DNA of *E. chaffeensis* has also been detected in different parts of the world in association with D. variabilis and I. pacificus in the USA, I. ricinus and I. persulcatus in Russia, A. testudinarium and Haemaphysalis yeni in China, H. longicornis in South Korea and Rh. sanguineus in Cameroon [65, 66]. However, whether any of these tick species serve as competent vectors and amplification hosts or this simply represents detection of *ehrlichial* DNA that has been passively acquired in blood meals from infected host animals needs further determination. The white-tailed deer is the primary host for A. americanum and a natural reservoir of *E. chaffeensis* in America [67]. Dogs are highly susceptible to *E. chaffeensis* and can establish a persistent infection; however, coyotes and raccoons are thought to be important natural reservoirs of *E. chaffeensis* in the USA. Other mammals and tick species may be responsible for its maintenance in nature elsewhere [68]. Some reports have implicated migratory birds as potential vehicles for dissemination of ticks infected with *ehrlichia*e. Molecular evidence for E. ewingii outside the USA has been only reported in Cameroonian Rh. sanguineus and dogs and in a dog from Brazil [66, 69].

Because Anaplasmataceae are rarely and inefficiently passed transovarially in ticks, it is commonly accepted that perpetuation of Anaplasma and *Ehrlichia* primarily depends on chronic persistence in mammalian reservoirs and horizontal transmission between ticks and animals during every generation of ticks [70-72]. Immature ticks acquire Anaplasma and *Ehrlichia* while feeding on bacteremic animals and then the ticks efficiently pass bacteria transstadially to the next life stage which then can transmit to a naive mammalian host.

As humans are infected with Anaplasma and *Ehrlichia* by ticks, the geography of human cases overlaps the known endemic areas of infected ticks and their animal hosts. Most cases are diagnosed during the spring to early summer or fall months and this correlates with the biological cycles of feeding of overwintering infected adult ticks and nymphs infected as larvae. Human anaplasmosis and ehrlichioses have been

reportable in the USA since the early days of their recognition (1987-1993) possibly due to the significant mortality and morbidity observed in the first recognized cases [73]. Passively collected surveillance data show a steadily increasing trend in the number of annual cases which has reached 1,026 cases of human granulocytic anaplasmosis (HGA) and 1,137 cases of human monocytic ehrlichiosis (HME) in 2008 but no associated increases in the rates of mortality due to effective recognition and treatment of most cases [74]. Furthermore, it is probable that the actual incidence and distribution of diseases is significantly greater than that reported in passive surveillance data.

Outside the USA, human anaplasmosis has recently become formally reportable only in China; therefore extrapolations regarding the incidence and prevalence of anaplasmosis and ehrlichioses in other regions of the world depend upon a limited number of laboratory-confirmed cases and the geographically restricted serosurveys reported in the literature [75-79]. Based on this information HGA appear to be infrequent infection in most countries. The temporal distribution and demographic characteristics of the populations infected are similar in the USA and other countries.

Rarely infections with *A. phagocytophilum* may also occur through blood transfusion and organ transplantation, and nosocomial and perinatal transmission [80-82]. Inhalation of aerosolized blood has been implicated as another route of infectious exposure to *A. phagocytophilum* [83].

CLINICAL DISEASE, DIAGNOSIS AND TREATMENT

Human anaplasmosis and ehrlichioses are acute illnesses that occur 5 to 21 days (average 10 days) after the bite of an infected tick [63, 73, 84]. The severity ranges from asymptomatic seroconversion to a mild or severe febrile illness to multisystem organ failure in the case of human monocytic ehrlichiosis. The triad of fever (>101oF/38oC), headache and myalgia are the most common clinical manifestations. Other symptoms include nausea, vomiting, abdominal pain, diarrhea, arthralgias and respiratory symptoms. Involvement of the central nervous system is variable being reported in 2 to 29% of patients suffering with HME; however it is rare during the course of HGA [84-86]. Rash is not typically associated with anaplasmosis; however, published data indicate that it is observed in children suffering from HME and may appear in 17 to 67% of diagnosed cases [87, 88]. Basic laboratory findings include thrombocytopenia, leucopenia, and moderately increased concentrations of hepatic alanine and aspartate aminotransferases and lactic dehydrogenase without increase of bilirubin; elevation of the C-reactive protein concentration is typical for HGA patients [87-89].

Fatalities due to *A. phagocytophilum* infections are rare (0.7%) and typically arise from complicating opportunistic viral or fungal infections, advanced age, immunosuppression, or severe preexisting medical conditions [63, 84]. Furthermore, it has been suggested that elevated blood cholesterol levels may also increase the severity of this infection [90]. Many of HGA patients in the USA are hospitalized, and some of those require treatment in an intensive care unit [63, 84]. The overall case-fatality rate is 2-3% in patients infected with *E. chaffeensis*; life-threatening complications and death are most frequent in patients who are immune-compromised and elderly people [91-93]. It was originally believed that patients with underlying immunodeficiency are at primary risk of acquiring E. ewingii ehrlichiosis; however, immune-competent individuals can experience the disease with similar course and clinical manifestations [94]. The clinical features of HGA outside the USA are harder to assess because of the limited number of reported cases; however, it is typically described as a low morbidity illness [76, 95]. Similarly, *E. chaffeensis* is rarely implicated as an etiological agent outside the USA. Indeed, suspected cases of HME may be due to infections caused by *E. muris* or antigenically related agents but mistakenly interpreted as *E. chaffeensis* ehrlichiosis due to their serologic cross-reactivity [96].

Accurate diagnosis of anaplasmosis and ehrlichioses requires laboratory confirmation that should be attempted during the acute stage of illness to avoid possible subsequent clinical complications [92, 97-99]. Presumptive etiological diagnosis can be established by detection of morulae within the cytoplasmic vacuoles in peripheral blood neutrophils or monocytes. Confirmatory diagnosis requires immunohistochemical demonstration of bacteria in tissues or peripheral blood, or sequence confirmed PCR detection of bacterial nucleic acids in peripheral blood. Serologic detection of immunoglobulin G (IgG) and IgM antibodies reactive with *A. phagocytophilum* or *E. chaffeensis* and a 4-fold titer increase between acute and convalescent serum allows only retrospective diagnosis of exposure because of the time required for this seroconversion. Similarly, culture isolation from the peripheral blood in a suitable cell culture system allows definitive confirmation; however, its retrospective character limits its practical utility in clinical practice. Each method has its benefits and disadvantages, and guidance for their use has been discussed in detail by several authors [97, 99, 100]. A combination of results obtained using different acute tests increases the probability of accurate diagnosis of anaplasmosis and the ehrlichioses thus benefiting proper patient management and timely recovery. Retrospective confirmation is essential for improved discrimination of its acute differential clinical diagnosis particularly when PCR diagnosis is not available. However, species-specific serologic confirmatory testing is not available for E. ewingii due to the lack of an established isolate.

For optimal diagnostic results with tests performed during the acute stage of illness, samples must be collected prior to the initiation of antibiotic therapy and no later than 24-48 hr after appropriate treatment has begun. Common requirements for laboratory confirmed and probable cases of human granulocytic anaplasmosis and ehrlichioses and their applicability to surveillance of these diseases are described by the USA Council of State and Territorial Epidemiologists (http://www.cste.org/ps2009/09-ID-15.pdf). In Europe, similar case definitions and recommendations were developed by the European Society of Clinical Microbiology and Infectious Diseases study group for Coxiella, Anaplasma, Rickettsia and Bartonella (ESCAR-ESCMID) and European Network for Surveillance of Tick-Borne Diseases [101].

Doxycycline is the primary drug of choice for therapeutic treatment of anaplasmosis and ehrlichioses in all categories of patients [73]. These recommendations are primarily based on in vitro antibiotic susceptibility testing and empirical retrospective clinical data. Antibiotic treatment should be continued for 7 to 10 days (for at least 3 to 5 days after defervescence); however, the optimal duration of therapy has not been determined yet. There are limited data on successful use of rifampin in pediatric patients and pregnant women. Unfortunately, the effectiveness of rifampin therapy or other antibiotic regimens for treatment of anaplasmosis and

ehrlichioses have not been evaluated in controlled prospective studies (reviewed in [102]). Use of sulfa-containing drugs is responsible for severe forms of illness; likely due to unknown interactions beyond the established in vitro resistance of *A. phagocytophilum* and E.chaffeensis to cotrimoxazole [103, 104]. Anaplasma and *Ehrlichia* are also resistant to beta-lactams, macrolides and ketolides [103].

EMERGING PATHOGENS

Anaplasma and *Ehrlichia* emerged as human pathogens in the late 1980s and early 1990s. The resultant increased attention given to all bacteria in the family Anaplasmataceae and the diseases they cause, the continuous alert mounted by the medical community, extended surveillance of wildlife, and development of novel diagnostic tools has resulted in expansion of the number of known human pathogens in the last several years [73, 101, 105]. This trend has coincided with an increasing incidence of other tick-borne diseases and the recognition of new emerging tick-borne etiological agents [79, 105, 106]. At least four new species from the family Anaplasmataceae have been implicated as causes of human illness in different geographic regions. *Ehrlichia* canis has been recognized as the etiologic agent of canine monocytic ehrlichiosis since 1935, and it occurs worldwide. Recently, human cases due to *E. canis* with positive PCR identification were reported in Venezuela; these cases were all associated with dog handling [107]. The variant of *E. canis* implicated as the human etiological agent appears to be genetically different from the type strain of *E. canis* typically causing canine illness. Furthermore, the patients did not mount any significant antibody response as typically observed for human or canine monocytic ehrlichiosis.

Ehrlichia muris and related *Ehrlichia* agents found in Ixodes ticks from Japan and Russia have been long referred to as likely etiological causes of murine ehrlichiosis [108-111]. PCR diagnosis has sporadically implicated *E. muris* as a cause of febrile illness in Russian patients [96, 112], and more recently, a genetically similar *Ehrlichia* sp. has been found to infect people in the Mid-Western USA states [113], a region which is one of the two primary USA areas endemic for human granulocytic anaplasmosis. These findings suggest that human ehrlichiosis due to *E. muris* may occur widely in Eurasia, since this *ehrlichia* is found in ticks and animals from Slovakia to China and Japan [114, 115]. Similarly, 1-2% of healthy donors in Japan were seropositive to *E. muris* or an antigenically related bacterium; while 10% of febrile patients from the Perm region of Russia, an endemic area of I. persulcatus had antibodies reactive with *E. chaffeensis* antigen [96, 116].

Another enigmatic *Ehrlichia* sp. was originally found in I. ricinus ticks from the Netherlands and referred to as Schotti variant. Later it was described as a novel genus and species, *Candidatus Neoehrlichia mikurensis* and first isolated from a rodent [10]. Different genetic types of this microorganism have been identified in European and Asian populations of ticks based on its GroEL gene sequence [117]. Three independent 2010 publications from Germany, Sweden and Switzerland diagnosed severe illness due to this agent by PCR in 10 patients [117-119]. In contrast to the characteristic clinical manifestations and clinical findings of HGA or HME, these patients experienced severe coagulative disorder and thrombosis likely due to involvement of the endothelium affected by invasion, growth and spread of this *Neoehrlichia*. Finally, the important heart-water agent, *E. ruminantium*, was

identified as an etiologic agent in several human cases from South Africa [120]. Unfortunately, none of these reports from Africa have yet been published in the peer-reviewed literature, so additional investigations should be forthcoming. Loftis et al. reported detection of the so-called Panola Mountain *Ehrlichia* agent in Amblyomma spp. ticks from the United States, Caribbean islands and Africa [121, 122]. This organism appears to cause a mild febrile illness in humans and cervids [123, 124]. The most striking feature of this yet unnamed bacterium is its genetic and antigenic similarity to rodent-tropic isolates of *E. ruminantium* [125].

While new Anaplasma species causing human disease have not been described, the tremendous diversity and adaptability of *A. phagocytophilum* to different animal hosts is now well documented. Precisely what distinguishes the human pathogenic variants from those that are non-pathogenic remains elusive.

FUTURE PERSPECTIVES

Our expanding knowledge of the emerging human pathogens in the family Anaplasmataceae has not only raised questions about the true prevalence of these under-reported diseases but it has also highlighted significant unresolved problems in the appropriate diagnosis, surveillance, and treatment of these diseases. The clinical manifestations of known anaplasmosis and different ehrlichioses in humans are overlapping and non-specific so accurate clinical diagnosis, especially in the acute phase, is difficult without proper laboratory methods. However, even the most efficient diagnostic methods available are either useful only during a very short period of acute disease (such as PCR) or effective only retrospectively. Serologic testing often provides the sole data available but it is ineffective for differentiation of Anaplasma and *Ehrlichia* species due to their significant antigenic cross-reactivity within a genus and between genera. Little cross-reactivity exists between *Neoehrlichia* and these genera but antigenic cross-reactivity of other emerging agents of Anaplasmataceae is unknown. There is a need for development of more rapid and sensitive point-of-care diagnostic methods, particularly new DNA detection tools that may be used during the acute stage of infection. There is also room for development of more sensitive and reliable serological assays than IFA testing. Since several tick-borne agents may be present in the same tick, possible mixed infections with unusual clinical manifestations might require access to immediate assays for detection of Anaplasma, *Ehrlichia*, Rickettsia, Bartonella, Borrelia and Babesia, or multiplexed assays that may be more clinically useful by reducing costs and increasing physician interest in addressing ambiguous clinical findings. This approach may lead to discovery of new tick-borne disease agents that will pose yet another set of challenges for veterinary, medical and public health specialists, and for fundamental studies of the evolution of this fascinating family of intracellular bacteria.

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