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Current management of human granulocytic anaplasmosis, human monocytic ehrlichiosis and *Ehrlichia ewingii* ehrlichiosis

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Abstract

Anaplasma phagocytophilum, *Ehrlichia chaffeensis* and *Ehrlichia ewingii* are emerging tick-borne pathogens and are the causative agents of human granulocytic anaplasmosis, human monocytic ehrlichiosis and *E. ewingii* ehrlichiosis, respectively. Collectively, these are referred to as human ehrlichioses. These obligate intracellular bacterial pathogens of the family Anaplasmataceae are transmitted by *Ixodes* spp. or *Amblyomma americanum* ticks and infect peripherally circulating leukocytes to cause infections that range in clinical spectra from asymptomatic seroconversion to mild, severe or, in rare instances, fatal disease. This review describes: the ecology of each pathogen; the epidemiology, clinical signs and symptoms of the human diseases that each causes; the choice methods for diagnosing and treating human ehrlichioses; recommendations for patient management; and is concluded with suggestions for potential future research.

Keywords

Anaplasma phagocytophilum; anaplasmosis; diagnosis; *Ehrlichia chaffeensis*; *Ehrlichia ewingii*; human ehrlichiosis; human granulocytic anaplasmosis; human monocytic ehrlichiosis; treatment

Participation in outdoor activities during months when tick activity is high and human encroachment on areas endemic for ticks have increased the potential for exposure to zoonotic pathogens that cycle between ticks and mammalian reservoir hosts, and have led to the emergence of several tick-borne diseases, including ehrlichioses, in the human population. The

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term 'ehrlichiosis' is a generic name ascribed to infections caused by members of the family Anaplasmataceae, which consists of obligate intracellular bacteria that replicate within the confines of host cell-derived vacuoles in their mammalian host cells. Five Anaplasmataceae members can infect humans, but only three of them – *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum* and *Ehrlichia ewingii* – have been sufficiently investigated and are the subjects of this review. In the 1990s, each of these bacterial pathogens was first recognized as an etiologic agent of human disease. *E. chaffeensis* infects peripherally circulating monocytes to cause human monocytic ehrlichiosis (HME; Table 1) [1]. *E. ewingii*, which was originally shown to infect peripheral blood neutrophils of dogs to cause canine granulocytic ehrlichiosis, causes a similar disease in humans referred to as *E. ewingii* ehrlichiosis [2]. *A. phagocytophilum* also infects neutrophils to cause human granulocytic anaplasmosis (HGA) [3]. *A. phagocytophilum* has long been recognized as a veterinary pathogen and was originally ascribed to two separate *Ehrlichia* species. The bacterium infects ruminant neutrophils to cause tick-borne fever and was referred to as *Ehrlichia phagocytophila* [4]. Likewise, it infects horses to cause equine granulocytic ehrlichiosis and was designated as *Ehrlichia equi* [5]. The first human infection was deemed 'human granulocytic ehrlichiosis' (HGE) and the bacterium was generically named the 'agent of HGE' [3]. In 2001, taxonomic reclassification grouped *E. equi*, *E. phagocytophila* and the HGE agent into the single species, *A. phagocytophilum* [6]. As the revised nomenclature continues to be accepted, HGA is the preferred name to describe human *A. phagocytophilum* infection. *Ehrlichia canis*, which is a bacterium that causes chronic and life-threatening infections of dogs, has been shown to infect humans [7,8]. Very little is known regarding interactions of this pathogen with human hosts. Indeed, the patient for whom *E. canis* infection was first confirmed showed no clinical or laboratory evidence of disease [8], and only recently has *E. canis* infection been linked to disease in humans [9]. Because the role of *E. canis* as a pathogen of humans remains relatively unexplored, it will not be discussed further. Since human ehrlichioses can be debilitating or even fatal, early diagnosis is paramount for effective treatment. This review summarizes the ecology of *A. phagocytophilum*, *E. chaffeensis* and *E. ewingii*, as well as the epidemiology of the human diseases that they cause, and the clinical signs and symptoms associated with each infection. It also highlights the advantages and disadvantages of each diagnostic method and treatment regimen applied to ehrlichial diseases. Guidelines for obtaining clinical clues to properly diagnose human ehrlichioses and patient management are discussed. The bulk of the review focuses on *A. phagocytophilum* and *E. chaffeensis* as they and the diseases they cause have been studied considerably more than *E. ewingii* and *E. ewingii* ehrlichiosis.

Ecology & epidemiology

Ehrlichia chaffeensis resides primarily in the southeastern, south-central and mid-Atlantic USA where its vector, the lone-star tick *Amblyomma americanum*, is endemic [10]. Most cases reported to the US Centers for Disease Control and Prevention (CDC) are from Missouri, Oklahoma, Tennessee, Arkansas and Maryland [11]. *E. chaffeensis* has been found in 5–15% of *A. americanum* ticks in 14 American states [12]. There is a lower prevalence of *E. chaffeensis* in nymphal ticks than adults [13]. There is no evidence for transovarial transmission of the pathogen, but trans-stadial transmission has been demonstrated in the laboratory [14]. The white-tailed deer (*Odocoileus virginianus*) is the principal reservoir for *E. chaffeensis* [15] in the USA, although it is maintained by a diverse range of wild and domestic animals. *E. chaffeensis* DNA has been detected in the peripheral blood of Brazilian marsh deer (*Blastocerus dichotomus*) in southeast Brazil and spotted deer (*Cervus nippon*) in Korea and Japan [16–18]. Otherwise, the worldwide distribution of *E. chaffeensis* in nature is relatively unknown.

In 1987, Maeda and colleagues published the index case of HME [7]. The patient was from Michigan and had travelled to Arkansas in late March 1986, where ticks had bitten him. In

mid-April, he became critically ill and was hospitalized. He presented with fever and confusion and developed anemia and thrombocytopenia. The symptoms resolved and hematologic parameters returned to normal following treatment with doxycycline. Intravacuolar inclusions of bacteria were identified in monocytes of a peripheral blood smear and the etiologic agent was presumed to be *E. canis*. Questions were raised as to whether *E. canis* was the causative agent [19]. In 1991, Anderson and colleagues isolated a monocytotropic bacterium from a soldier stationed at Fort Chaffee Army Base in northwestern Arkansas [1]. The bacterium's 16S rRNA gene sequence was 98.2% identical to that of *E. canis*. The agent was named *E. chaffeensis* and delineated as the etiologic agent of HME. A total of 3408 HME cases have been reported to the CDC over the past 6 years (2003 through to the end of December 2008; Figure 1) with the greatest number of cases (837) occurring in 2008 [20]. The majority (90–93%) of HME cases occur between April and September, which coincides with peak levels of *A. americanum* feeding activity on humans [21,22]. The median age for HME is 50 years with approximately 59% of patients being male [23]. While human ehrlichial infections ascribed to *E. chaffeensis* have been reported in other countries including Mali, Korea and Peru [24–26], these reports relied on either serological tests or indirect immunofluorescence assays that lacked the specificity to distinguish between *E. chaffeensis* and antigenically related bacterial species that would induce cross-reacting antibodies.

A. phagocytophilum is naturally maintained in a zoonotic cycle between tick vectors of the *Ixodes persulcatus* complex and mammalian reservoir hosts. In the USA, this vector–vertebrate host cycle includes *Ixodes scapularis* in the eastern and mid-western states, and *Ixodes pacificus* and *Ixodes spinipalpis* in western coastal and mountain areas, respectively [27]. Approximately 10–50% of *Ixodes scapularis* ticks in the USA are infected with *A. phagocytophilum* [28,29]. The vectors for transmitting the bacterium to humans in Europe and Asia are *I. ricinus* and *I. persulcatus*, respectively [30,31]. *A. phagocytophilum* has been detected in *I. ricinus* ticks from most European countries including Italy [32–34], the UK [35], France [36,37], Slovenia [38], Switzerland [39,40], The Netherlands [41,42], Germany [43,44], Bulgaria [45], Spain [46], Russia [47] and Poland [48–50], with the prevalence of infection ranging from 2 to 45%. The bacterium has been found in *Ixodes trianguliceps* in the UK and in *Ixodes ventralis* in Portugal [51–54]. However, *I. trianguliceps* probably does not play a role in transmitting *A. phagocytophilum* to humans or domestic animals, as its nidicolous activity is likely to restrict it to small rodent reservoirs. Likewise, *I. ventralis* infests a variety of small rodents and lizards, but has not been shown to feed on humans [53,54]. The *Ixodes* spp. ticks that transmit HGA are also vectors for the causative agents of other human diseases, including *Borrelia burgdorferi* (Lyme disease) [55], *Babesia microti* (babesiosis) [56] and tick-borne encephalitis virus [57]. Coinfection by *A. phagocytophilum* and one or more of these agents has been confirmed in both humans and laboratory animals [58–60]. *A. phagocytophilum* is not transmitted transovarially from an adult female tick to her progeny [14] and ticks must therefore acquire the bacterium through a blood meal. Adult *Ixodes* spp. ticks are active from March through June and October through December in the northern USA. Nymph activity is highest from May through September, while larval activity is highest June through October. The southern USA sees all stages active from November through May [13]. In Europe and Asia, all stages of *I. ricinus* are active April through June and in the winter months [13]. The white-footed mouse (*Peromyscus leucopus*) is a prominent reservoir of *A. phagocytophilum* in the USA [61]. Non-*Peromyscus* reservoirs in the USA include white-tailed deer, (*O. virginianus*), raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*), *Sciurus* spp. (squirrels), striped skunks (*Mephitis mephitis*), dusky-footed woodrats (*Neotoma fuscipes*) and chipmunks (*Tamias senex*) [62–69]. Evidence is emerging that *A. phagocytophilum* strains carried by some of these reservoirs are nonpathogenic for humans [66,70]; and a definitive role for white-tailed deer in maintaining human-infective *A. phagocytophilum* is being re-examined [71]. In Europe, small mammals, including the wood mouse (*Apodemus sylvaticus*), bank vole (*Clethrionomys glareolus*), common shrew (*Sorex*

aratus), roe deer (*Capreolus capreolus*), foxes (*Vulpes vulpes*), wild boar (*Sus scrofa*), as well as horses, cattle, sheep and dogs are important reservoirs [39,72,73].

Human granulocytic anaplasmosis was first defined in the USA in 1994 [3] when it was determined that 12 patients presented with peripheral granulocytes harboring vacuolar inclusions of organisms resembling *E. chaffeensis*. As such, the disease was originally deemed HGE [74]. A total of 3637 HGA cases in the USA were reported to the CDC from 2003 through 2008, with 2007 seeing the most cases (834 cases; Figure 1). Despite the steady increases in reported HGA and HME cases, the true incidence and prevalence of each disease is probably under-represented. Indeed, active surveillance rates are as high as 24–58 HGA cases per 100,000 individuals in Connecticut [75] and the upper midwest [76] and 330–414 HME cases per 100,000 individuals in Tennessee [77] and southeastern Missouri [78]. Seroprevalence studies show rates of 14.9% for HGA in northwestern Wisconsin [79] and 12.5% for HME in Tennessee [80].

As HGA is not as yet a reported disease in Europe, data regarding incidence and prevalence are dependent upon laboratory-confirmed cases reported in clinical publications. The first serologic evidence of human exposure to *A. phagocytophilum* was reported in Switzerland in 1995 [81]. The first confirmed HGA case was diagnosed in Slovenia in 1997 [82]. Subsequent cases of HGA or *A. phagocytophilum* seropositivity have since been reported in The Netherlands [83], Spain [84], Sweden [85], Norway [86], Croatia [87], Poland [88], Slovenia [58] and Greece [89], with the majority of cases occurring June through August (73%) [30]. Just over half of the patients were males (53%), which is similar to the findings in the USA [90,91]. Several of these studies assessed either the percentage of humans that carry *A. phagocytophilum* antibodies or *I. ricinus* ticks positive for *A. phagocytophilum* DNA and collectively indicate that seroprevalence of anti-*A. phagocytophilum* antibodies in Europe is relatively high (up to 15.4%) considering the low infection rates of *I. ricinus* ticks (0.6–6%) with *A. phagocytophilum* in the same regions. This could be due to a large number of humans being frequently bitten by infected ticks and remaining seropositive for a number of years or problems with the detection methods used [92]. An *A. phagocytophilum* strain that is putatively nonpathogenic for humans was recently identified in *I. ricinus* ticks in Spain [93]. Thus, *I. ricinus*-mediated inoculation of such nonpathogenic strains into humans could conceivably elicit production of antibodies that would confirm seropositivity for exposure to *A. phagocytophilum*. The majority of the above European studies [30,81–89] calculated no significant difference in seroprevalence between males and females or in age ranges. Some studies reported regional differences in human seroprevalence between rural and urban areas [89]. As would be expected, the majority of the studies determined that people living in rural areas in close contact with habitats favorable to ticks or individuals working closely with animals were more likely to be seropositive for *A. phagocytophilum* antibodies. However, a study conducted on the island of Crete, Greece, determined that not only was the human seroprevalence higher than in most other European countries (24.1%), but also there was no significant difference in the seroprevalences of people living in rural and urban areas. This was attributed to the Cretan culture where livestock farming is embraced as a way of life for both rural and urban dwellers [89].

Ehrlichia ewingii was first described in 1992 when it was attributed to be the etiologic agent of canine granulocytic ehrlichiosis [94]. In 1999, PCR amplification and subsequent sequence analysis of peripheral blood leukocytes of patients with suspected ehrlichiosis revealed the causative agent to be *E. ewingii* for four cases that occurred in Missouri between May and August [2]. Similar to *E. chaffeensis*, *E. ewingii* is transmitted by the lone-star tick [95]. In 2000, a field study in North Carolina put the prevalence of tick infection with *E. ewingii* at less than 1% for adults and 0.4% for nymphs [96]. DNA has also been amplified from lone-star ticks collected in Florida and Missouri where prevalence in adult ticks is 1.6 and 5.4%,

respectively [95]. Transstadial passage of *E. ewingii* and successful transmission of this pathogen among dogs by *A. americanum* has been demonstrated experimentally [97]. *E. ewingii* DNA has been isolated from wild white-tailed deer in Arkansas, Georgia, North Carolina and South Carolina [98]. As the white-tailed deer is a key host to all stages of the lone-star tick and is also the principal reservoir for *E. chaffeensis* [10,15], *E. ewingii* probably exhibits similar ecological dynamics to those of *E. chaffeensis*. Since becoming a reported disease in the USA in 2008, only nine cases of *E. ewingii* ehrlichiosis have been reported to the CDC [20], partly owing to the difficulty in definitive identification of this infection. Despite the fact that the numbers of *E. ewingii* and *E. chaffeensis* infections in white-tailed deer and domestic dogs are comparable, confirmed cases of HME far out number confirmed cases of human illness due to *E. ewingii*. This may be attributed to *E. ewingii* possibly causing a milder illness than *E. chaffeensis*, particularly in individuals with no underlying immune-suppressing conditions [95].

In 2008, there were 69 cases of ehrlichiosis of undetermined etiology in the USA for which clinical criteria supporting either *Ehrlichia* spp. or *A. phagocytophilum* infection had been met, and for which there were insufficient diagnostic data to definitively classify the causative agent. For example, while morulae may have been identified in white blood cells, other supportive laboratory results would have been absent, or serologic tests could not discern between *E. chaffeensis* and *A. phagocytophilum* infection [20].

The past few years have seen evidence of blood transfusion, nosocomial and perinatal transmission of *A. phagocytophilum*. In November 2007, the Minnesota State Department of Health was contacted regarding a patient hospitalized with HGA who had previously undergone multiple blood transfusions [99]. The patient acknowledged that he had travelled to an area endemic for *Ixodes* spp. ticks 3 weeks prior, but neither spent a lot of time outside nor recalled having a tick bite. Upon screening samples of the 59 blood donors to this patient for evidence of *A. phagocytophilum* infection, a sample from a 64-year-old female donor tested positive. The donor had spent time in wooded areas of northeastern Minnesota where HGA is endemic, but did not recall having been bitten by a tick and had no history of fever in the month leading up to her blood donation or afterwards. In 2008, nine people were diagnosed with HGA based on PCR detection of *A. phagocytophilum* DNA in peripheral blood and maximum antibody titers less than or equal to 256 at a primary-care hospital in the Anhui province of China [100]. Neither blood smears nor blood cultures were performed for diagnosis of *A. phagocytophilum* infection. The nine cases were among 39 healthcare workers and relatives, none of whom suffered tick bites but had contact with an index patient within 12 h of her death from suspected fatal HGA while she experienced extensive hemorrhage and underwent endotracheal intubation. Because routine blood and body fluid precautions were probably not strictly adhered to in this case, it suggests that there is a risk of nosocomial transmission of HGA infection in situations where proper blood and body fluid precautions are not followed. This may be the first evidence of human-to-human transmission of HGA and the first case of HGA in China. However, Krause and Wormser caution that this report be considered preliminary because, even though it fulfils the case definition of HGA, it lacks diagnostic certainty as *A. phagocytophilum* cultures from blood were not attempted [101]. Transplacental transmission of *B. burgdorferi* is well documented [102]. Therefore, it is not surprising that Horowitz and colleagues consider this route of transmission of HGA in the case of a pregnant woman near term in which *A. phagocytophilum* was transmitted perinatally to her infant [103]. Because the placenta and umbilical cord blood had been discarded by the time the infant became ill, transplacental transmission could not be confirmed. It is uncertain whether more serious complications could have arisen had the infection occurred earlier in the pregnancy, as stillbirth and abortion are consequences of *A. phagocytophilum* infection in sheep and cattle [104,105].

Clinical manifestations & laboratory findings

Onset of both HGA and HME occurs 5–21 days after receiving a bite from an infected tick [91]. HGA ranges in severity from asymptomatic seroconversion to a mild or severe febrile illness. In rare instances, severe disease can result in organ failure or death. Nonspecific signs and symptoms including acute onset of fever, headache, malaise and myalgias are typical, even in severe infection [11,106,107]. Less common symptoms include nausea, abdominal pain, diarrhea and cough [106]. Laboratory findings can include leukopenia, lymphopenia, thrombocytopenia and elevated serum levels of hepatic enzymes [11,106,107]. Clinical manifestations and laboratory findings reported for HGA cases in Europe and the USA are similar except that the disease appears to be milder and to resolve sooner in European cases, even in the absence of antibiotic treatment [106]. Leukopenia and impaired neutrophil function with HGA could promote susceptibility to secondary and opportunistic infections [90]. Other possible contributing factors include underlying diseases and hospitalization [27]. Most patients' symptoms usually resolve within 30 days, even without antibiotic treatment [108]. However, various complications may be evident at the time of presentation or may appear several days or longer after onset. Such complications can include peripheral neuropathies and CNS complications and are mostly associated with increasing age, immunosuppression, chronic inflammatory illness, underlying malignant disease or clinical severity of HGA [109]. Nearly half of patients with severe HGA require hospitalization with 17% requiring admittance to intensive care [90].

Similar to HGA, HME and *E. ewingii* ehrlichiosis present as undifferentiated febrile illnesses characterized by fever, chills, headache, myalgias and arthralgia. The most common additional symptoms associated with HME are coughing, nausea, diarrhea, vomiting, anorexia and abdominal pain. Ataxia and dyspnea are less common [10]. A nonspecific skin rash is seen in less than 10% of adults at the time of presentation, though approximately 30% will develop a rash within the first week of illness. A skin rash is more frequent in children with HME [10]. There is a greater likelihood of rash and nausea with HME than *E. ewingii* infection [21]. Laboratory abnormalities for HME and *E. ewingii* ehrlichiosis include leukopenia, thrombocytopenia and elevated hepatic transaminase levels [21,80,110,111], although they are generally less severe for *E. ewingii* ehrlichiosis [95]. Among patients with HME, 60–85% are hospitalized and 15% develop moderate-to-severe illness. Complications with HME can include meningoencephalitis, acute renal failure, myocarditis, adult respiratory distress syndrome, gastrointestinal hemorrhage and disseminated intravascular coagulopathy [10,21]. Patients with HIV or other forms of immunocompromise can develop a toxic shock-like syndrome following *E. chaffeensis* or *E. ewingii* infection. Fewer complications arise in immunocompromised patients infected with *E. ewingii* when compared with those infected with *E. chaffeensis* [112].

Fatalities associated with HGA occur in less than 1% of infections and are usually due to opportunistic infections, whereas 3% of HME cases are fatal with the deaths occurring most commonly in immunosuppressed individuals who develop respiratory distress syndrome, hepatitis or opportunistic nosocomial infections [12,90,109,112]. No fatal cases have been reported for *E. ewingii* infection.

Diagnosis

Accurate diagnosis of many tick-borne diseases is hampered owing to similar clinical manifestations, overlapping geographic distributions and shared vectors. Also, patients are often unable to recall having been bitten by a tick. Therefore, after determining that a patient has been exposed to areas typical of high tick activity, a tick-borne infection should be

considered. Laboratory confirmation can be carried out using a number of molecular microbiological and serological techniques.

Examination of peripheral blood smears

The quickest diagnostic method after onset of disease is via microscopic examination of Wright- or Giemsa-stained peripheral blood smears. The trained eye should look for intravacuolar bacterial inclusions termed morulae (Latin for mulberry), which stain dark blue or purple and are stippled in appearance (Figure 2A & C). Morulae detected in neutrophils are indicative of infection by *A. phagocytophilum* or *E. ewingii*, while detection in monocytes delineates infection by *E. chaffeensis*. This test should be carried out within a week of disease onset, as sensitivity is highest at this time (Table 2) [113]. Blood samples must be taken prior to administering doxycycline therapy since morulae disappear from the blood within 24–72 h after treatment begins [10,27]. During the first week of infection, the sensitivity of this assay is 25–75% for detecting *A. phagocytophilum* and 2–38% for detecting *E. chaffeensis* and declines thereafter [114]. Sensitivity data for *E. ewingii* detection are not available. A limitation of this assay is that it requires a well-trained microscopist. For instance, prolonged examination is often required to accurately detect *A. phagocytophilum* morulae, as they can be present in less than 0.1% of neutrophils. Also, the trained eye should be able to distinguish common intracellular inclusions and artefacts on blood smears. Such phenomena include crystal stain artefacts (crystalloid deposits outside or on top of cells), bacterial contamination of the stain solutions (cocci or bacilli that are also present on control slides), Döhle bodies, toxic granulation and platelets overlying leukocytes [27].

Amplification of bacterial DNA

The polymerase chain reaction (PCR) is currently the most sensitive tool for detecting *A. phagocytophilum* or *E. chaffeensis* during acute infection. After the first week, the bacteremic phase of infection rapidly wanes, thereby limiting the effectiveness of PCR as a diagnostic technique (Table 2) [113]. Sensitivity is 60–85% for detecting *E. chaffeensis* DNA [77] and 67–90% for detecting *A. phagocytophilum* DNA [103]. At present, PCR is the only specific diagnostic test available for *E. ewingii* infection as culture isolation of this organism has yet to be achieved and *E. ewingii* and *A. phagocytophilum* morulae are indistinguishable. Multiplex or real-time PCR can reduce the time spent waiting for results by combining the detection of two or more ehrlichiosis and anaplasmosis agents into a single experiment or obviate the need for time consuming gel analysis steps, respectively [115,116].

In vitro cultivation of bacteria in cell culture

Recovery of *E. chaffeensis* and *A. phagocytophilum* in antibiotic-free mammalian cell culture can also be used to definitively diagnose infection. *A. phagocytophilum* is usually cultivated in the human promyelocytic leukemia cell line HL-60 [117,118] by direct inoculation of cell cultures with peripheral blood from a potentially infected patient. The bacteria develop within vacuoles to form morulae in the cytoplasm of infected cells, which can be detected using Wright or Giemsa staining (Figure 2B & D). Intracellular organisms can be visualized as early as 5 days postinoculation or can remain undetectable for more than 2 weeks. The canine histiocytic cell line DH82 is usually employed for culturing *E. chaffeensis* [77]. *E. chaffeensis* morulae can be visible in susceptible cells from 2 to 36 days postinoculation [10]. *E. ewingii* has yet to be successfully cultured in the laboratory. Owing to the antibiotic-free environment needed to grow these intracellular bacteria and requisite training in cell culture techniques, only a few specialized laboratories perform this task. Another limitation of this method is that it can take up to 2 weeks or longer for a positive result. As such, cultivation is not generally available or useful for clinical diagnosis at the time of active disease.

Serodiagnosis

Serological detection of antibodies in patient serum or plasma by the indirect immunofluorescence assay is the most frequently used assay for clinical diagnosis [119]. However, it requires sufficient time for antibodies to develop towards the infectious agent. Therefore, although the most sensitive and widely used diagnostic method, it is not the most rapid or practical, as an antibody response has not usually developed at the time a patient presents with active disease. The assay employs the detection of IgM and IgG antibodies, together or separately, that are reactive against *A. phagocytophilum*- or *E. chaffeensis*-infected tissue culture cells or purified bacteria fixed to glass slides [91]. Sensitivity is high 2–4 weeks following disease onset compared with a few days for PCR, blood smear microscopy and cell culture (Table 2). A diagnosis of *A. phagocytophilum* or *E. chaffeensis* infection is confirmed by a fourfold increase in antibody titer between acute and convalescent sera or a seroconversion to a titer of 128 or higher [108,120]. However, the Consensus Approach for Ehrlichiosis Task Force has suggested that patients with single titers of 64 and 128 be considered probable cases of HME and those with single titers greater than 256 be considered confirmed HME cases [120]. Moreover, seropositivity against *E. chaffeensis* or *A. phagocytophilum*, while waning in titer, often lasts for months and sometimes years after initial exposure [108]. Thus, an antibody titer must be considered in the context of other clinical evidence of infection and should not be the sole criteria for a diagnosis. *E. ewingii* has never been cultured *in vitro*; therefore, serological diagnosis is not presently feasible. Sensitivity ranges from 82–100% and 88–90%, respectively, for IgG antibodies against *A. phagocytophilum* and *E. chaffeensis* [119,121], and 27–37% and 44%, respectively, for IgM [78,119]. Specificity studies have only been conducted for *A. phagocytophilum*, for which the range is 83–100% [91]. Nonspecificity can occur owing to cross-reactivity among *A. phagocytophilum* and *E. chaffeensis* antibodies [91]. Likewise, indirect immunofluorescence assays cannot distinguish between *E. chaffeensis* and *E. ewingii* antibodies [2,122]. As such, it is likely that some patients diagnosed as having HME based on indirect immunofluorescence antibody results may have actually been afflicted with *E. ewingii* ehrlichiosis. Despite the efficacy of serologic diagnosis of HGA or HME, a number of conditions can cause false-positive serologic test results. These include Rocky Mountain spotted fever, typhus, Q fever, brucellosis, Lyme disease, Epstein–Barr virus and autoimmune disorders that produce autoantibodies such as rheumatoid factor, antinuclear antibodies, antineutrophil cytoplasmic antibodies and antiplatelet antibodies [91].

Patient history

Early clinical manifestations associated with HME, HGA and *E. ewingii* ehrlichiosis may resemble nonspecific symptoms and signs of other infectious and noninfectious diseases, which can obfuscate correct diagnosis. It is therefore important for primary care clinicians who will probably be first to evaluate such patients, such as general practitioners, pediatricians, internists or emergency room physicians, to obtain a thorough clinical history. Important clinical historical features include: recent tick bite or exposure; travel to endemic areas for tick-borne diseases; and the presentation of similar illness in family members, coworkers or pet dogs. A patient's history might reveal outdoor activities in areas likely to be tick-infested (high grass and/or low brush) or such areas that border roads, trails, yards, or fields between the months of April to September. In endemic areas, even recreation in grassy yards or contact with the family dog poses a risk. Most patients will not recall being bitten by a tick perhaps because of the tick's small size or because it attached at a nonobvious spot. Thus, the absence of confirmed tick exposure should not exclude diagnosis of HME, HGA or *E. ewingii* ehrlichiosis [11].

Knowledge of the endemic regions for human ehrlichioses is important, especially for clinicians practicing in areas where the incidence of these diseases is low. A history of recent

travel from an endemic area, especially if the patient participated in outdoor activities in an area likely to be tick-infested, should warrant suspicion of an ehrlichial or other tick-borne illness [11]. Temporal and geographically related clustering of human ehrlichioses can occur. Indeed, clusters of HME among residents of a golfing community and soldiers on field maneuvers have been reported [80,123]. Thus, it is prudent to consider a human ehrlichiosis in addition to a communicable infection as a possible diagnosis for family members, coworkers or individuals frequenting a common area that present with a similar illness, especially in areas endemic for, or for people that have recently travelled to areas endemic for, human ehrlichioses [11].

Patient management

Assessing clinical signs and symptoms, laboratory and diagnostic tests, and a thorough patient history will collectively aid clinicians in formulating a differential diagnosis and treatment plan. A patient presenting with acute febrile illness who otherwise feels well, has an unrevealing history and physical examination, and has normal laboratory findings might warrant reassessment in 24 h if the patient fails to improve [11]. If laboratory testing of a patient with a history of probable exposure to ticks in a region endemic for a human ehrlichiosis agent reveals leukopenia, thrombocytopenia and/or metabolic abnormalities, then the clinician should consider obtaining PCR and blood cultures for likely pathogens including specific serologic tests [11]. In addition, the clinician should initiate antibiotic therapy. The onset of HGA, HME or *E. ewingii* ehrlichiosis can be rapid and has the potential for severe debilitating or even fatal outcomes, particularly in those that are already immunosuppressed [10,11,107]. Accordingly, antibiotic therapy should be started as soon as either infection is suspected even before laboratory diagnosis has been confirmed [11,107]. Blood samples for diagnosis should be taken before treatment begins since doxycycline therapy rapidly reduces the detected quantities of infected cells and bacterial DNA. Certain HGA, HME or *E. ewingii* ehrlichiosis patients can be treated as outpatients with oral medication, particularly if a reliable caregiver will be present and the patient is compliant with follow-up assessment and treatment [11].

At least 50% of patients with HME or HGA are hospitalized to rule out life-threatening conditions and to manage the disease [10,91]. Clinical indications that would prompt hospital admittance include immunocompromise, pain management, confusion, abnormal spinal fluid findings, infiltrates on chest radiographs, hypotension/shock or acute organ failure [11]. Approximately 3% of HME and 0.5–1% of HGA patients who seek medical attention will die from the infection [10,91]. The severity of disease appears to be related in part to the patient's immune status [90,91,107,112]. Although the case fatality rate for HGA is lower than that for HME, complications such as respiratory failure, toxic shock-like syndrome, rhabdomyolysis, pancreatitis, acute renal failure and opportunistic viral or fungal pathogen infections can occur, especially among patients who have comorbid illnesses or are immuno-suppressed. Advanced age and delays in diagnosis and initiation of antibiotic therapy often lead to more severe HGA [11,90,91].

Antibiotic treatment

The current recommended therapeutic regimens for HME, HGA and *E. ewingii* ehrlichiosis is administration of doxycycline or tetracycline for 5–14 days [10,27,90,107]. Owing to its twice-daily dosage, compared with four doses for tetracycline and better patient tolerance, doxycycline is the antibiotic of choice. While repeated exposures to tetracycline increase the risk for dental staining [124], limited use of this drug in children under 8 years of age has a negligible effect [125,126]. Because HGA, HME and *E. ewingii* ehrlichiosis can be life-threatening and since the risk of dental staining with a short treatment course is minimal, the American Academy of Pediatrics Committee on Infectious Diseases identifies doxycycline as

the drug of choice for treating presumed or confirmed human ehrlichioses in children of any age [127]. The recommended dosage of doxycycline is 100 mg for adults and 2.2 mg/kg for children 8 years of age or older given orally every 12 h. Adults can receive the dosage intravenously, if required. Tetracycline should be administered orally at 6-h intervals at a dosage of 500 mg for adults and 25–50 mg/kg/day for children 8 years of age or older. The clinical response to doxycycline or tetracycline treatment is pronounced, with marked improvement in overall well-being and fever within 24–48 h. The absence of such a response should prompt consideration of alternate diagnoses, such as a non-ehrlichial infection, a complicating infection by another tick-borne pathogen, or a secondary infection that is not susceptible to tetracycline antibiotics [11,27]. Tetracyclines are generally not prescribed for pregnant women because of risks of malformation of teeth and bones in the fetus and hepatotoxicity and pancreatitis in the mother [11]. However, tetracycline and doxycycline have been used to successfully treat HME and HGA in pregnant women, respectively [103,128]. Thus, the use of tetracycline antibiotics might be warranted in the case of a life-threatening situation [11].

Although no trials for efficacy have been conducted, a small number of pediatric-age and pregnant HGA patients have been successfully treated with rifampin [107,129–131]. It is only recommended when the patient has a history of allergy to tetracycline antibiotics, is under 8 years of age, or is pregnant [107]. The dosage for rifampin is 300 mg orally twice daily for adults or 10 mg/kg for children (maximum of 300 mg/dose) [90]. Treatment should continue until the patient is afebrile for 3 days. The fluoroquinolone, levofloxacin, has *in vitro* MICs that would predict efficacy [132,133]. However, treatment of HGA with this antibiotic in at least one patient suppressed, but did not resolve, infection. Moreover, *E. chaffeensis* exhibits resistance *in vitro* to fluoroquinolone antibiotics. Therefore, fluoroquinolones should not be used to treat human ehrlichioses [134]. *In vitro* evidence suggests that chloramphenicol might not be effective for treating HME or HGA [132,135]. There is a paucity of evidence for alternative antibiotic treatments as *in vitro* β -lactams, cephalosporins, macrolides and aminoglycosides are inactive against both *E. chaffeensis* and *A. phagocytophilum* and evidence of *in vivo* efficacy of these drugs is lacking.

Prophylactic antibiotic therapy for human ehrlichial infections is not recommended for patients that recall having been bitten by a tick and are not ill. Because the percentages of ticks infected with the causative agents of human ehrlichioses are relatively low [12,28,29,96], the risk for infection with these agents following a tick bite is low.

Expert commentary

The best method for the prevention of human ehrlichioses is to limit one's exposure to ticks. Since the infectious agents can be transmitted within a few hours after tick attachment, rapid removal of the tick does not necessarily exclude the chance for infection. Wearing protective clothing and using repellent sprays can reduce the risk of tick attachment. DEET (*N*, *N*-diethylmeta-toluamide) and permethrin-based products are deemed safe for human topical application or application to clothing, respectively [136,137]. Alternative repellents containing natural compounds such as citrodiol or *p*-menthane-3,8-diol, which are derived from lemon-scented eucalyptus oil [138], are also available and have proven effective at deterring tick attachment [139].

Obtaining a thorough patient history is critical for presumptively diagnosing human ehrlichioses early on in infection in order to promptly initiate antibiotic therapy prior to confirmatory diagnosis. This is especially important in areas where *Ixodes* spp. or *A. americanum* ticks are endemic. Doxycycline is the antibiotic of choice for all patients unless they are pregnant or have a documented history of allergy to doxycycline or tetracycline. For

those unable to take doxycycline or tetracycline, rifampin is an alternative with good *in vitro* MICs and anecdotal clinical success. While serologic testing is the most sensitive diagnostic confirmatory method, PCR is rapidly becoming the choice diagnostic method at or soon after presentation. Also, PCR is presently the only method available for diagnosing *E. ewingii* infection. In the USA, suspected and confirmed cases of HGA, HME and *E. ewingii* ehrlichiosis are required to be reported to the appropriate public-health authority to assist in control measures and public-health education efforts. Sustained surveillance of these tick-transmitted diseases will continue to identify endemic regions around the world and delineate their seroepidemiologic trends.

Five-year view

We expect that the numbers of reported cases of HGA, HME and *E. ewingii* ehrlichiosis will continue to rise as clinicians continue to become more aware of these infections, their clinical presentations and their areas of geographic distribution, and as research scientists continue to refine sensitive and rapid methods for diagnosing each disease. The latter will be facilitated by identification of *A. phagocytophilum*-, *E. chaffeensis*- and *E. ewingii*- specific antigens that are expressed during infection and elicit the production of non-cross-reactive antibodies. Improved clinical recognition of human ehrlichioses will in turn aid the efforts of epidemiologists and ecologists to better understand these emerging pathogens and further define their areas of endemicity. A critical area of cellular microbiology research is to identify the bacterial adhesins that facilitate adherence to and invasion of host leukocytes. Accomplishing this task will delineate novel targets for nonantibiotic-based therapeutic and/or protective measures against the etiologic agents of human ehrlichioses.

Key issues

- *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis* and *Ehrlichia ewingii* are obligate intracellular bacterial pathogens and are the etiologic agents of human granulocytic anaplasmosis (HGA), human monocytic ehrlichiosis (HME) and *E. ewingii* ehrlichiosis, respectively. *A. phagocytophilum* is transmitted by ticks of the *Ixodes persulcatus* complex, while *E. chaffeensis* and *E. ewingii* are transmitted by *Amblyomma americanum*.
- *A. phagocytophilum*, *E. chaffeensis* and *E. ewingii* infect peripherally circulating leukocytes to cause infections that range in clinical spectra from asymptomatic seroconversion to mild or severe disease. These infections are fatal in less than 3% of cases.
- Nonspecificity can occur in indirect immunofluorescence assay due to cross-reactivity between *A. phagocytophilum* and *E. chaffeensis* antibodies. Likewise, this technique cannot distinguish between *E. chaffeensis* and *E. ewingii* antibodies. Seroconversion or fourfold increase in antibody titers is strongly preferred over single titers for diagnostic confirmation.
- Early clinical signs associated with HGA, HME and *E. ewingii* ehrlichiosis can resemble nonspecific symptoms of other infections or noninfectious diseases, which could obfuscate correct diagnosis. Therefore, it is imperative to obtain a thorough patient history regarding recent tick bites; exposure (including travel) to their habitats particularly around times of known high tick activity and clustering of similar illness in family members, coworkers, other defined groups, or pets.
- Clinicians who practice in areas that are nonendemic for human ehrlichioses should still be familiar with the epidemiology of these diseases as such knowledge

will be helpful in thoroughly querying patients that have recently travelled to endemic regions, and to detect their emergence in new regions.

- The onset of human ehrlichiosis can be fast and potentially fatal. Therefore, antibiotic therapy should be administered as soon as infection is suspected even before laboratory diagnosis has been confirmed.
- For accurate diagnosis upon clinical presentation, it is imperative that blood samples be taken before initiation of treatment since antibiotic therapy rapidly reduces the detected quantities of infected cells and bacterial DNA in peripheral blood.
- Leukopenia, thrombocytopenia and mildly elevated hepatic transaminase levels are commonly observed for human ehrlichiosis and are useful clinical features for making presumptive diagnoses of such infections. Absence of such features does not exclude diagnosis of a human ehrlichiosis.
- Not all patients with HGA, HME or *E. ewingii* ehrlichiosis will require hospitalization.
- Doxycycline is the antibiotic of choice for the treatment of HME, HGA and *E. ewingii* unless the patient is pregnant or has a documented history of allergy to doxycycline or tetracycline. For those unable to take doxycycline or tetracycline, rifampin is a suitable alternative.

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Figure 1. Number of human monocytic ehrlichiosis, *Ehrlichia ewingii* ehrlichiosis, human granulocytic anaplasmosis and undetermined human ehrlichiosis cases per year (2003–2008)
Undetermined cases occur when all clinical criteria support *Ehrlichia* spp./*Anaplasma phagocytophilum* infection, but there is a lack of sufficient clarity to definitively classify the causative agent (e.g., morulae may have been identified in white cells in the absence of other supportive laboratory results).

EEE: *Ehrlichia ewingii* ehrlichiosis; HGA: Human granulocytic anaplasmosis; HME: Human monocytic ehrlichiosis; UD: Undetermined.

Data taken from [20].

Figure 2. Examples of *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* morulae in peripheral blood smears and tissue culture cells

(A & B) *Anaplasma phagocytophilum* and (C & D) *Ehrlichia chaffeensis* morulae within peripheral blood leukocytes of (A & C) infected human patients and (B & D) cultivated *in vitro* in (B) HL-60 human promyelocytic cells or (D) canine DH82 histiocytic lymphoma cells. Representative morulae are indicated by arrows. (A & C) Wright stain, original magnification $\times 1000$, (B & D) LeukoStat stain, original magnification $\times 1000$. (E & F) Electron micrographs of *A. phagocytophilum*-infected HL-60 cells. (E) *A. phagocytophilum* exhibits a biphasic developmental cycle and transitions between two distinct morphological forms, the dense cored cell (DC) and reticulate cell (RC); original magnification $\times 8200$. Arrows and arrowheads delineate representative morulae containing DC or RC, respectively. (F) An arrow points to an *A. phagocytophilum* DC bound to the surface of and inducing its own phagocytic uptake by a HL-60 cell; original magnification $\times 26,500$. Electron micrographs are provided by Matthew J Troese of Virginia Commonwealth University (VA, USA).

Table 1
Overview of the three most common causes of human ehrlichiosis.

Agent	Disease	Target leukocyte	Geographical distribution	Tick vector	Primary mammalian reservoir(s)
<i>Anaplasma phagocytophilum</i>	Human granulocytic anaplasmosis	Neutrophil	Northeast and upper midwest USA, Europe, Asia	<i>Ixodes scapularis</i> (USA) <i>Ixodes pacificus</i> (USA) <i>Ixodes ricinus</i> (Europe) <i>Ixodes persulcatus</i> (Asia)	White-footed mouse, dusky-footed wood rat
<i>Ehrlichia chaffeensis</i>	Human monocytic ehrlichiosis	Monocyte	Southeastern, southcentral, and mid-Atlantic USA	<i>Amblyoma americanum</i>	White-tailed deer
<i>Ehrlichia ewingii</i>	Human <i>ewingii</i> ehrlichiosis	Neutrophil	Southeastern and southcentral USA	<i>A. americanum</i>	Dog

Table 2

Preferred laboratory diagnostic methods by interval after onset of illness with human granulocytic anaplasmosis and human monocytic ehrlichiosis.

Interval after fever onset	Preferred method	Alternate method	Lower sensitivity
<7 days	PCR, blood smear	Blood culture in HL-60 cells (<i>Anaplasma phagocytophilum</i>) or DH82 cells (<i>Ehrlichia chaffeensis</i>)	Serology
7–14 days	Serology (acute and convalescent pair)*		PCR, blood smear
>14 days	Serology (acute and convalescent pair)*		

* Serology: indirect immunofluorescence assay using both *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* antigens; a fourfold increase in antibody titer or seroconversion between acute and convalescent sera is strongly preferred. IgM assays are less sensitive than IgG even early after infection.