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Tick-borne ehrlichiosis infection in human beings

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Abstract

Human monocytic ehrlichiosis is a tick-borne infectious disease transmitted by several tick species, especially *Amblyomma* spp caused by *Ehrlichia chaffeensis. E. chaffeensis* is an obligatory intracellular, tick-transmitted bacterium that is maintained in nature in a cycle involving at least one and perhaps several vertebrate reservoir hosts. Two additional *Ehrlichia* spp, *Anaplasma* (formerly *Ehrlichia*) *phagocytophila* (the agent of human granulocytic ehrlichiosis [HGE]) and *E. ewingii* (a cause of granulocytic ehrlichiosis in dogs) act as human pathogens. Human *E. chaffeensis* infections have generally been reported in North America, Asia and Europe, but recently human cases have been reported in Brazil only. Human monocytic ehrlichiosis is diagnosed by demonstration of a four-fold or greater change in antibody titer to *E. chaffeensis* antigen by IFA in paired serum samples, or a positive PCR assay and confirmation of *E. chaffeensis* DNA, or identification of morulae in leukocytes and a positive IFA titer to *E. chaffeensis* antigen, or immunostaining of *E. chaffeensis* antigen in a biopsy or autopsy sample, or culture of *E. chaffeensis* from a clinical specimen.

Key words *Amblyomma* spp – *Ehrlichia chaffeensis* – ehrlichiosis – human monocytic

Ehrlichia chaffeensis is an obligatory intracellular, tick-transmitted bacterium that is maintained in nature in a cycle involving at least one and perhaps several vertebrate reservoir hosts. This animal serves as a keystone host for all life stages of the principal tick vector (*Amblyomma americanum*) and is perhaps the most important vertebrate reservoir host for *E. chaffeensis*. Disease caused by *E. chaffeensis* in human has been termed human monocytic ehrlichiosis or human monocytotropic ehrlichiosis (HME).

Epidemiology and geographic distribution

Human ehrlichiosis is a zoonotic disease, caused by a rickettsia that infects leukocytes. It was described for the first time in the United States of America in 1986. More than 300 cases have been reported in that country. *E. chaffeensis* belong to the family *Anaplasmataceae* and is a member of the subdivision of the *Proteobacteria.* Martínez *et al*[1], conducted serologic studies which indicated *E. chaffeensis* infection in Latin American countries—Venezuela, Mexico, Argentina, Chile and Brazil. However, no molecular evidence for *E. chaffeensis* has been reported.

During the 1990s, Bakken *et al*[2] and Buller *et al*[3], identified two additional *Ehrlichia* spp, *Anaplasma* (formerly *Ehrlichia*) *phagocytophila* (the agent of human granulocytic ehrlichiosis [HGE]) and *E. ewingii* (a causative agent of granulocytic ehrlichiosis in dogs) as human pathogens, and these reports greatly expanded the geographic region and the size of the human population at risk for acquiring one of these potentially lethal infections. While most of the cases of ehrlichiosis caused by *E. chaffeensis* were

being identified by McQuiston *et al*[4] in the south-eastern and south-central United States of America, within a relatively few years of the initial recognition of human ehrlichiosis (HE), the number of cases of HE identified in the north-eastern and north-central states surpassed other regional totals. A retrospective review of all medical and laboratory records from six sites located in the 'tick belt' of the south-eastern U.S.A. was carried out by Schutze and Jacobs[5]. Demographic, history and laboratory data were abstracted from the identified medical records of patients.

One case has been reported in Portugal, two in France and one more in a tourist coming from Mali (Africa)[6]. In Venezuela, a tropical country, where ehrlichiosis is endemic in dogs and horses, the first case of human ehrlichiosis was reported in a 17-month old girl[6].

At least 21 isolates of *E. chaffeensis* have been obtained from patients with HME, infected in Arkansas[7,8], Oklahoma[9], Florida and Georgia[10,11], Tennessee[12,13] and Maryland[14]. Isolates of *E. chaffeensis* from sources other than human tissues are few and include five from white-tailed deer[15] and one from a domestic goat[16], each obtained in Georgia.

Tick vectors

Amblyomma americanum: As a geographic portrait of HME emerged in the late 1980s, investigators identified a predominance of patients from the south-central, south-eastern, and mid-Atlantic states. Because this region closely approximates the recognised distribution of *A. americanum* (commonly known as the lone star tick for the silvery white spot on its dorsal surface), this tick was soon implicated as a potential vector for *E. chaffeensis*[17]. This hypothesis was supported further by amplification of *E. chaffeensis* DNA from pools of *A. americanum* adults collected from various locations in the eastern United States[18], experimental transmission of *E. chaffeensis* among white-tailed deer by adult and nymphal lone star ticks[19], and retrospective studies demonstrating temporal and spatial associations between lone star tick infestations and the presence of antibodies reactive with *E. chaffeensis* in white-tailed deer[20,21].

Other tick species: PCR has been used to detect DNA of *E. chaffeensis* in other tick species, including the dog tick, *Dermacentor variabilis*[18,22,23], the western black-legged tick, *Ixodes pacificus*[22], *Ixodes ricinus* in Russia[24], and the ticks *A. testudinarium* and *Haemaphysalis yeni* collected from domesticated and wild animals in southern China[25]. Detection of DNA of ehrlichiae within a particular tick species does not conclusively incriminate that tick as an efficient vector[26], and the role of these or other tick species as natural vectors of HME has not been established definitively. Similarly, the Gulf Coast tick (*A. maculatum*) has been implicated as a potential vector because of feeding proclivities and distribution range similar to those of the lone star tick, although insufficient data exist to support or refute the role of this tick in the transmission of *E. chaffeensis*[27].

Vertebrate reservoirs

E. chaffeensis is maintained in nature as a complex zoonosis, potentially involving many vertebrate species that serve as competent reservoirs for the bacterium, as sources of blood for tick vectors, or as both. The catholic feeding proclivity of *A. americanum* for the blood of a wide range of mammalian and avian species is well-described[28,29]. Considerably less is known about vertebrates which can serve as competent reservoirs for *E. chaffeensis.*

White-tailed deer: The white-tailed deer (*Odocoileus virginianus*) currently stands as the sole vertebrate species recognised as a complete and sufficient host for maintaining the transmission cycle of *E. chaffeensis*. White-tailed deer is an important source of blood for adult and immature stages of *A. americanum*[28,29]. These deer are also naturally infected with *E. chaffeensis* in the southeastern United States based

on PCR results and isolation of the bacterium[30].

Goats: Domestic goats (*Ovis* species) may serve as hosts for all stages of the life cycle of *A. americanum*[28,29,31].

Domestic dogs: Can serve as hosts for all stages of the life cycle of *A. americanum* and provide a convenient vehicle for transport of ticks from various habitats into the peridomestic environment[28, 29].

Red foxes (Vulpes vulpes): Can serve as hosts for all stages of *A. americanum*[32]. Raccoons (*Procyon lotor*) are frequently parasitised by all life stages of *A. americanum*[28,32]. Raccoons occur throughout much of North America and reach some of their highest population densities in areas coinhabited by humans[33], making them a potentially important reservoir for *E. chaffeensis*. The Virginia opossum (*Didelphis virginianus*) can serve as a host for nymphal *A. americanum*[28].

Birds: The role of birds as a natural reservoir for *E. chaffeensis* is yet to be investigated, although many ground-feeding species can serve as important sources of blood for immature stages of *A. americanum*[29].

Transmission cycle

Non-infected larvae obtaining blood from a bacteremic vertebrate reservoir host (e.g. white-tailed deer [shaded]) become infected and maintain ehrlichiae to the nymphal stage. Infected nymphs may transmit *E. chaffeensis* to susceptible reservoir hosts (unshaded) or to humans during acquisition of blood (Fig. 1)[34]. Infected adult ticks, having acquired ehrlichiae either by trans-stadial transmission from infected nymphal stage or during blood meal acquisition as non-infected nymphs on infected deer, may also pass *E. chaffeensis* to humans or other susceptible reservoirs. Transovarial transmission has not been demonstrated, and eggs and unfed larvae are presumably not infected.

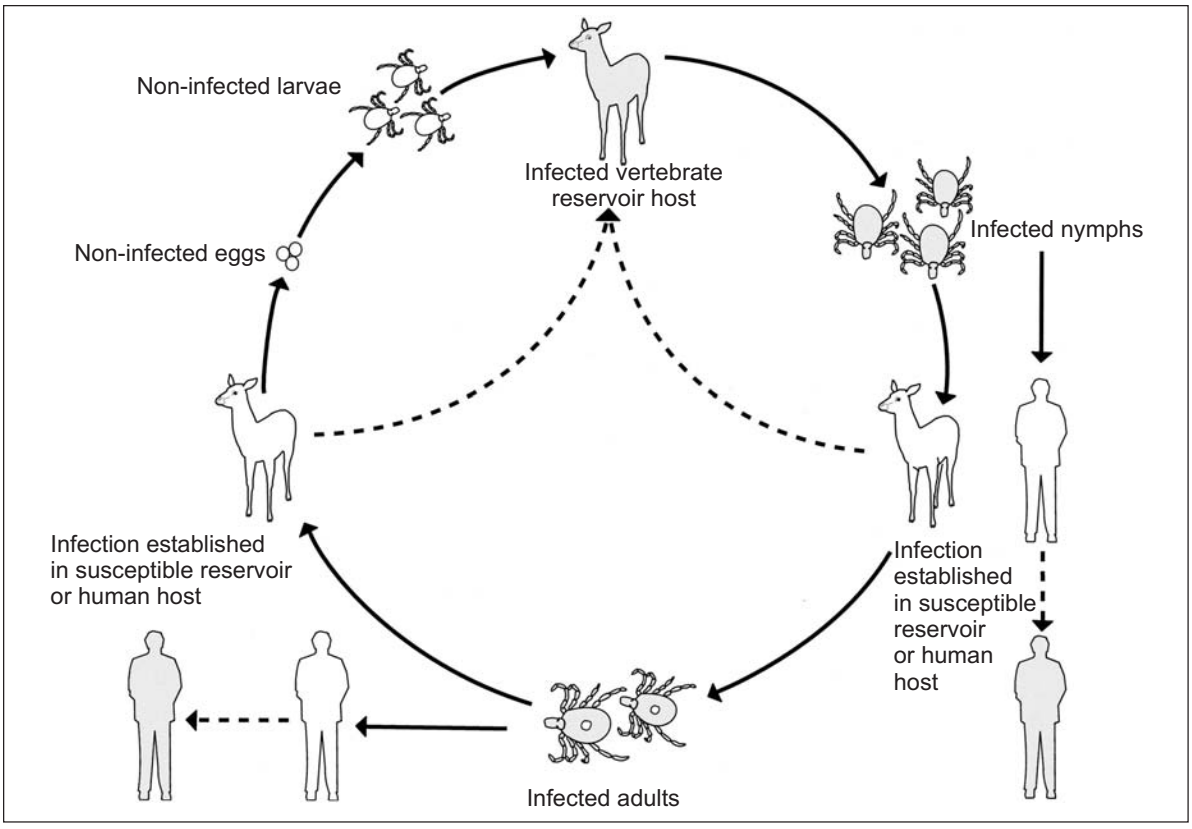

Fig. 1: A life cycle of *E. chaffeensis* (adapted from Ref. 34)

Pathogenesis

In vertebrate hosts, *E. chaffeensis* infects predominantly mononuclear phagocytic cells. The most frequently infected blood cells are monocytes, however, infections in other cell types have been described, including lymphocytes, atypical lymphocytes, promyelocytes, metamyelocytes, and band and segmented neutrophils[10,35–37]. Although *E. chaffeensis* appears capable of inhabiting other phagocytic cells (e.g. granulocytes), it is likely that mononuclear phagocytes maintain the productive infection[36]. Infected cells typically contain only 1 or 2 morulae, although as many as 15 have been observed in leukocytes of immunosuppressed patients[38–40].

General clinical features

Within 1 to 2 weeks (median, 9 days) following exposure to an infecting tick, patients experience a prodrome characterised by malaise, low-back pain, or gastrointestinal symptoms or may develop sudden onset of fever (often >39°C). Patients with HME are most likely to seek medical attention within 3 to 4 days after the onset of symptoms, and the presenting clinical features frequently include fever (>95%), headache (60 to 75%), myalgias (40 to 60%), nausea (40 to 50%), arthralgias (30 to 35%), and malaise (30 to 80%)[41,42]. During the course of the illness, other manifestations of multisystem disease develop in approximately 10 to 40% of patients, including cough, pharyngitis, lymphadenopathy, diarrhoea, vomiting, abdominal pain, and changes in mental status[41–44]. Less frequently reported manifestations include conjunctivitis[45,46], dysuria[39,47], and peripheral edema[17].

Laboratory diagnosis

Serologic testing: The most widely available laboratory diagnostic tests detect and measure antibody reactive with *E. chaffeensis*[48]. Currently available serologic tests may provide negative results for the majority of patients during the first week of illness.

Indirect immunofluorescence assay: Most patients with HME have been diagnosed by the indirect immunofluorescence assay (IFA). The original IFA format for detecting antibodies reactive with *E. chaffeensis* used a surrogate antigen *E. canis,* as substrate[49]. Paired sera collected during a 3 to 6-week interval represent the preferred specimens for serologic evaluation of HME. Both immunoglobulin IgM and IgG antibodies can be measured using the IFA[50], however, the IgG IFA test is negative in as many as 80% of patients during the first week of illness and the IgM titers may also be uninformative at this time. It is important to obtain a convalescent-phase serum specimen since most (>80%) patients develop diagnostic IFA titers by six weeks post-infection[13,50].

Western blotting: The use of Western blotting has permitted the identification of antigenic variability among isolates of *E. chaffeensis* and identified variability in the reactivity of patient sera to a number of *E. chaffeensis* antigens[51,52].

Visualization of morulae and staining methods: Morulae have been identified in smears of peripheral blood, buffy coat preparations, and bone marrow aspirates by using various eosin-azure (Romanovsky)-type stains, including Wright's, Diff-Quik, Giemsa's, and Leishman's. Although this technique offers the most rapid method of diagnosis, it is considered relatively insensitive and is seldom confirmatory in clinical practice. In this context, morula-positive smears are characteristically seen in a minority of patients, even in patients from whom the organism has been isolated[7,10,13,14,50].

PCR amplification: PCR assays to identify DNA from *Ehrlichia* spp in whole blood, CSF, and serum are becoming standard complements to serologic assays. Frequently, positive results can be obtained by PCR using an acute-phase whole-blood sample from *E. chaffeensis* patients at a time when serologic

testing is still negative[50]. Historically, the 16S rRNA gene has been the primary molecular target for diagnosing *E. chaffeensis* infections in humans[41,53]. This gene has also been the most widely used to identify *E. chaffeensis* DNA in ticks[18,23,54] and vertebrate reservoirs[27,30].

Isolation: The isolation of *Ehrlichia* species from blood, CSF, and other tissues requires a laboratory capable of processing clinical specimens using cell culture techniques. In this context, a clinical laboratory equipped for virus isolations could potentially culture *E. chaffeensis.* In some cases, primary isolation has required several weeks[7,10,55], although morulae have been identified in some primary cultures as early as two days following inoculation[12,13].

Treatment and control: E. chaffeensis is susceptible to tetracyclines and their derivatives broad spectrum antimicrobials which act by inhibiting protein synthesis of various bacterial species by reversibly binding to the 30S ribosomal subunit to prevent the addition of new amino acids during the formation of peptide chains. Particularly doxycycline, represents the treatment of choice for all persons with HME. Most patients become afebrile within 1 to 3 days following the treatment with a tetracycline[41,42,56].

Reducing contact with infected ticks lowers the risk of acquiring HME. Wearing light-coloured clothing that facilitates the detection of crawling or attached ticks and the use of repellents containing DEET 9*n*,*n*-diethyl-*m*-toluamide) can minimize the risk of tick bites. However, the best protective measures consist of a thorough body examination for ticks after returning from potentially tick-infested areas. It is not known how long *A. americanum* must remain attached before it can transmit *E. chaffeensis* to a host; however, because other tick species generally require several hours of attachment before bacteria are transmitted[57,58], frequent inspections for and prompt removal of attached ticks by using forceps or tweezers is an important method to minimize the risk of HME.

Conclusion

More than 20 years have elapsed since the first case of HME was reported in 1986, presented to medical attention. During this interval, much has been learnt about the pathogen, the disease, and the multiple ecological elements involved in the maintenance of this zoonosis. However, as is true for all emerging pathogens, many questions remain unanswered. Among the numerous areas for future research include studies that provide a better understanding of the interactions between the pathogen and the vector, that define pathogenic mechanisms involved in the maintenance of *E. chaffeensis* in vertebrate reservoirs and factors influencing disease and immunity in human hosts, and that estimate the incidence of disease in areas where *E. chaffeensis* is endemic.

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