Human Babesiosis and Ehrlichiosis – Current Status

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Abstract

Lyme disease (LD), caused by the *Borrelia burgdorferi* complex, is the most frequently reported arthropod-borne infection in North America and Europe. The ticks that transmit LD also carry other pathogens. The two most common co-infections in patients with LD are babesiosis and ehrlichiosis. Human babesiosis is caused by protozoan parasites of the genus *Babesia* including *Babesia microti*, *Babesia duncani*, *Babesia divergens*, *Babesia divergens*-like (also known as *Babesia MOI*), *Babesia EU1* and *Babesia KO1*. Ehrlichiosis includes human sennetsu ehrlichiosis (HSE), human granulocytic anaplasmosis (HGA), human monocytic ehrlichiosis (HME), human ewingii ehrlichiosis (HEE) and the recently discovered human ehrlichiosis Wisconsin–Minnesota (HWME). The resulting illnesses vary from asymptomatic to severe, leading to significant morbidity and mortality, particularly in immunocompromised patients. Clinical signs and symptoms are often non-specific and require the medical provider to have a high degree of suspicion of these infections in order to be recognised. In this article, the causative agents, geographical distribution, clinical findings, diagnosis and treatment protocols are discussed for both babesiosis and ehrlichiosis.

Keywords

*Babesia*, *Ehrlichia*, babesiosis, ehrlichiosis, human, *Borrelia*

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Human Babesiosis

Babesiosis is a tick-borne infectious disease caused by haemoproteozoon parasites of the genus *Babesia*, family *Babesiidae*, order *Piroplasmida*. Babesiosis is acquired through a tick bite or by blood transfusion. The tick vectors are the hard-bodied *I. scapularis* in the US and *I. ricinus* in Europe. In addition, approximately 150 cases of transfusion-transmitted *Babesia* (TBI) between 2000 and 2009 have been confirmed in the US by Herwaldt et al. While more than 100 species have been reported, only a few have been identified as causing human infections. The book of Exodus contains the first reference to babesiosis, alluding to a plague of ‘murrain’ that affected cattle, camels, sheep and other domestic animals. Centuries later in 1888, Romanian Biologist Victor Babes identified a protozoan parasite from cattle with febrile haemoglobinuria...
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Figure 1: Fluorescent In Situ Hybridisation (FISH)

Geographical Distribution

Currently, in the US, three species of Babesia have been identified as the causative agents of human babesiosis: *B. microti*, *B. duncanii* (formerly known as *Babesia WA1-type* and related parasites in the states of Washington and California) and *B. divergens*-like (also known as *B. MO1*). *B. microti* is the agent most frequently identified in the North-east and Midwest and can occur in non-splenectomised individuals. A recent study of babesiosis among elderly Medicare patients indicates that the highest rates of babesiosis in this group are in Connecticut, Rhode Island, New York and Massachusetts, with men affected slightly more than women. *B. microti* is rapidly spreading in areas of the North-east not previously known to be endemic for babesiosis. For example, although LD has been endemic to parts of the lower Hudson valley, NY, for more than two decades, babesiosis has emerged there only since 2001. This is due to ticks now containing polymicrobial infections in these emerging endemic areas. *B. duncanii* is most frequently identified on the West Coast. *B. divergens*-like organisms have been identified in three cases, two from the Midwest (Missouri and Kentucky) and one from Washington state. All three patients had risk factors for severe disease, namely age greater than 50 years and splenectomy. Sequence analysis of the entire 18S ribosomal RNA (rRNA) gene indicated that the *Missouri isolate* (MO1) and the *Kentucky isolate* (KY) were identical to each other and to *piroplasms* found in eastern cottontail rabbits on Nantucket Island.

Clinical Findings

Infection with *Babesia* is usually asymptomatic. Elderly and immunosuppressed people, especially those without a spleen or with impaired cellular immunity, are more likely to become symptomatic. Symptoms, including fever, malaise, headache, nausea and generalised aching, may last weeks to months. Hepatomegaly, splenomegaly, jaundice, and dark urine are also common findings in symptomatic patients. These may be accompanied by elevation in hepatic transaminases, proteinuria and haemoglobinuria. Severe haemolysis, often accompanied by thrombocytopenia, leucopenia and atypical lymphocytosis, is more common in high-risk patients.

*Babesia microti* Infection

The disease spectrum of *B. microti* infection in humans depends on their immune status. The symptoms vary from asymptomatic infection through flu-like symptoms to severe disease, sometimes leading to death in immunocompromised and splenectomised patients. Although several physicians believe that the severity of LD increases in patients with concurrent infections with Babesia (personal communication), Krause et al. have reported that in patients with babesiosis concurrent infection with LD does not increase the number or duration of symptoms of babesiosis. In fact, patients co-infected with LD and *B. microti* often have subclinical presentations.

Asymptomatic Infection: The incubation period is between one and nine weeks following infection by Babesia. According to one report, approximately 30 % of patients have asymptomatic infection. Asymptomatic infection also occurs following resolution of symptomatic babesiosis, with parasites persisting in the blood for months or years. These asymptomatic patients therefore pose a risk...
Mild to Moderate Illness: An incubation period of one to eight weeks elapses between the tick bite and the onset of Babesia symptoms. Almost everyone who contracts Babesia infection gets flu-like symptoms with fever and chills.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\) In addition, patients get one or more of these non-specific symptoms: generalised weakness, fatigue, night sweats, headaches, muscle pain, joint pains, loss of appetite, nausea and cough.\(^7\)\(^8\)\(^9\)\(^10\) Other, less common, symptoms include gastrointestinal symptoms such as vomiting, diarrhoea and weight loss, conjunctival injection and emotional instability.\(^11\)\(^12\)\(^13\) The illness can last anywhere from a week to over a year.\(^14\)\(^15\)\(^16\) Although a patient may feel well, parasites can be detected in the blood up to two years after the initial episode.\(^17\)

Severe Disease: Severe disease usually occurs in immunocompromised or splenectomised patients and patients on immunosuppressive medication.\(^18\)\(^19\)\(^20\) Specific symptoms include: jaundice, shortness of breath, night sweats and hot flashes, muscle pain, swollen spleen and dark urine, retinal infarcts or ecchymoses and petechiae.\(^21\)\(^22\)\(^23\) Complications of severe disease are acute respiratory failure, congestive heart failure, liver and renal failure and rupture of the spleen.\(^24\) Mortality rates between 5 and 21% have been reported.\(^25\)\(^26\)\(^27\) In case studies, strong predictors of severe outcome have included male gender, infection at 50 years and older, alkaline phosphatase values greater than 125 U/l and white blood cell (WBC) counts greater than 5 x 10^9/l.\(^28\)\(^29\)\(^30\)\(^31\)\(^32\) These patients require comprehensive and aggressive medical care.

Babesia duncani Infection

The severity of B. duncani infection is variable, depending primarily on the immune status of the host.\(^33\) Symptoms are very similar to B. microti infection and include flu-like symptoms with fever and chills, headache, sweats, nausea, vomiting, diarrhoea, fatigue and dark urine.\(^34\) Although studies performed in hamsters have demonstrated that infection due to B. duncani is more pathogenic than B. microti infection,\(^35\) there is not enough evidence in the literature to support this in humans. In fact, of the nine cases reported to date, only one patient experienced pulmonary oedema and renal insufficiency and died.\(^36\)\(^37\)\(^38\)\(^39\)\(^40\) The other eight had mild clinical symptoms or were asymptomatic.\(^41\)\(^42\)\(^43\)\(^44\)

Babesia divergens Infection

In Europe, approximately 40 human cases of B. divergens infection have been reported, all in splenectomised patients.\(^45\)\(^46\) These patients suffered a severe form of babesiosis.\(^47\)\(^48\) Signs and symptoms begin one to three weeks after tick bite and consist of high fever, headache, chills, intense sweating, myalgia, abdominal pains with severe intravascular haemolysis that results in haemoglobinuria, haemoglobinuria and jaundice.\(^49\) In more than 50% of the patients, there was a rapid onset of renal failure and pulmonary oedema.\(^50\) Ecchymoses, petechiae, congestive heart failure and coma have also been reported.\(^51\)\(^52\)\(^53\) The illness is generally fulminant, lasting about a week and, for more than one-third of patients, ending in prolonged convalescence or death.\(^54\)\(^55\) Recently, B. divergens infection was reported from two anaemic patients in Shandong province, China.\(^56\) These two patients also had hepatic injury, haemoglobinuria and renal failure.\(^57\)
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### Microscopic Examination of Giemsa-stained Smear

A specific diagnosis of babesiosis can be made by microscopic identification of the organism using Giemsa-stained thin blood smears. The ring form is most common and can be mistaken for early-stage ring forms of *Plasmodium falciparum*, though subtle morphological differences are detectable by trained practitioners. The tetrad form, referred to as a Maltese cross, is pathognomonic of *Babesia* species such as *B. microti* and *B. duncani*. *B. duncani* displays more tetrad forms than *B. microti* (see Figures 1–3). Parasitaemia is usually low (5 %) but can go as high as 85 %.

### Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a sensitive and specific method for detecting *Babesia* DNA in blood and identifies *Babesia* species within a few days of infection. The most common target is the 18S ribosomal DNA (rDNA) gene of Babesia. The PCR test may be a useful adjunct to the Giemsa stain since it is highly specific for detecting *B. microti* parasites. However, there are drawbacks to PCR: the time to result can be several hours, PCR cannot be performed directly on blood, and DNA must be purified from blood samples to avoid inhibition by haemoglobin. Even after extensive purification, PCR inhibition remains in about 5 % of samples. Therefore only a few laboratories offer this PCR test, because special precautions are necessary to avoid contamination.

### Serology by the Indirect Immunofluorescence Assay

The indirect immunofluorescence assay (IFA) is the most commonly used serological test for diagnosis of babesiosis. The assay employs the detection of the patient’s immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies that are reactive against *B. microti* or *B. duncani* parasites grown in hamster blood and fixed onto glass slides (see Figures 3a and 3b). Sensitivity is high two to four weeks after disease onset (versus a few days for PCR or blood smear microscopy). A diagnosis of babesia infection is confirmed by a fourfold increase in antibody titre between acute and convalescent sera or a seroconversion to a titre of 128 or higher. During the acute phase of *B. microti* illness, IgG titres from previous infections with *B. duncani* or related organisms do not cross-react with *B. microti* antigen. Sera from patients infected with one of several *Babesia* species may cross-react with antigen from *Plasmodium* species, but titres are almost always low (1:16 or lower). *Babesia* titres exceed 1:1,024 during the active phase of the disease and then decline to 1:64 or less within 8–12 months. Thus, IgG titres of 1:1,024 or greater usually signify active or recent infection. The detection of IgM is indicative of recent infection. Although seroconversion occurs in virtually all immunocompetent individuals infected with *B. microti*, the diagnosis of active babesial infection based on serological findings alone is suspect. Serology is usually not considered in cases attributed to *B. divergens* because the illness becomes fulminant before the antibody can be detected. Sera from patients infected with *B. divergens*-like organisms or *B. EU1* cross-react with antigen from *B. divergens*.

### Amplification of Babesia in Laboratory Animals

*Babesia* parasites can multiply in hamsters and gerbils. *B. microti* is easily detected in hamsters, whereas *B. duncani* is often lethal in these animals. The infected patient’s blood is injected in intravenous or intraperitoneal route into hamsters or gerbils and the animal blood is tested for the presence of *Babesia* on a regular basis. However, this approach is not suited to rapid diagnosis, as *Babesia* usually do not appear in the blood of the laboratory animal until two to four weeks after inoculation.

### Treatment

According to Vannier et al., the following treatment protocols are recommended (see Table 1). Note that dosing regimens are for 7–10 days except for persistent relapsing infection.

For immunocompromised patients, successful outcomes have been reported using atovaquone combined with higher doses of azithromycin (600–1,000 mg orally per day). For severe cases of babesiosis, partial or complete exchange transfusion should also be considered, particularly in

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Patient</th>
<th>Dosing</th>
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<tbody>
<tr>
<td>Atovaquone (oral) + azithromycin (oral)</td>
<td>Adults</td>
<td>Atovaquone 750 mg every 12 hours and azithromycin 500–1,000 mg once daily on day 1 and 250–1,000 mg daily on subsequent days</td>
</tr>
<tr>
<td>Atovaquone (oral) + azithromycin (oral)</td>
<td>Children</td>
<td>Atovaquone 20 mg/kg/dose every 12 hours (maximum 750 mg/dose) and azithromycin 10 mg/kg/dose once on day 1 (maximum 500 mg/dose) and 5 mg/kg/dose daily on subsequent days (maximum 250 mg/dose)</td>
</tr>
<tr>
<td>Clindamycin (oral) + quine (oral)</td>
<td>Adults</td>
<td>Clindamycin 600 mg every eight hours and quinine 650 mg every six hours</td>
</tr>
<tr>
<td>Clindamycin (intravenous) + quine (oral)</td>
<td>Adults</td>
<td>Clindamycin intravenously 300–600 mg every six hours and quinine orally 650 mg every six hours</td>
</tr>
<tr>
<td>Clindamycin (intravenous or oral) + quine (oral)</td>
<td>Children</td>
<td>Clindamycin orally or intravenously 7–10 mg/kg/dose every 6–8 hours (maximum 600 mg/dose) and quinine orally 8 mg/kg/dose every eight hours (maximum 650 mg/dose)</td>
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</tbody>
</table>

**Fluorescent In Situ Hybridisation**

The *Babesia* fluorescent in situ hybridisation (FISH) assay detects *Babesia*-specific rRNA directly on a blood smear (see Figures 2a and 2b). This test has a greater than 98 % specificity and is more sensitive than Giemsa. The specificity of the *Babesia* FISH test is greater than PCR (unpublished results). This is because, unlike PCR assays, FISH assays do not have inhibition issues. Despite this, the FISH test is currently offered by only one reference laboratory in the US.

**Table 1: Human Babesiosis Pharmacological Treatment Regimens**

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patients co-infected with LD, since failures of classical treatment regimens using clindamycin and quinine as well as atovaquone and azithromycin have been reported in this patient group. Atovaquone and azithromycin do not cross the placenta, whereas clindamycin and quinine do cross the placenta. Therefore, during pregnancy, clindamycin and quinine are recommended to treat fetal infections.

Human Ehrlichiosis

Human ehrlichiosis is caused by tick-transmitted bacteria of the genera Neorickettsia, Anaplasa, and Ehrlichia, all belonging to the Anaplasmataceae family. Ehrlichiosis includes HSE, HGA, HME, and HEE. The causative agents of HSE, HGA, HME, and HEE are Neorickettsia sennetsu, A. phagocytophilum, E. chaffeensis, E. ewingii, and E. Wisconsin–Minnesota, respectively.

Ehrlichiosis was first described in Algerian dogs in 1935 and, in the 1960s, a number of military guard dogs stationed in Vietnam died from complications of a haemorrhagic illness caused by Ehrlichia canis. In 1993, the first human case of sennetsu ehrlichiosis caused by Neorickettsia sennetsu (formally known as Ehrlichia sennetsu) was reported in Japan. Infections with Ehrlichia and Anaplasma were recognised in 1986 and 1990, respectively. These two genera comprise the majority of the human infections.

E. chaffeensis is the canine pathogen discovered in 1992, was first recognised as a causative agent of human ehrlichiosis in 1998. E. ewingii is serologically very similar to E. chaffeensis but, like A. phagocytophilum, propagates within neutrophils. E. Wisconsin–Minnesota was reported to be the cause of four cases of human ehrlichiosis for the first time in 2011.

Geographical Distribution

N. sennetsu is the causative agent of HSE, commonly known as sennetsu fever, a mononucleosis-type illness that primarily occurs in Japan and Malaysia. N. sennetsu is thought to be transmitted by a fluke, a trematode considered to be the reservoir and vector.

E. chaffeensis is the causative agent of HME. The disease occurs mostly in the south-eastern and south-central regions of the US. It is primarily transmitted by the lone star tick, Amblyomma americanum. The first diagnosed case of HME occurred in 1986 in a 51-year-old man from Detroit who had been exposed to ticks in a rural area of Arkansas. In 1990, the agent of HME was isolated from the blood of a US Army reservist at Fort Chaffee, AR. The newly recognised organism was named E. chaffeensis.

A. phagocytophilum (formerly called Ehrlichia phagocytophila or Ehrlichia equi), transmitted by black-legged ticks of the Ixodes group, is the causative agent of HGA. Small mammals, such as white-footed mice (Peromyscus leucopus), dusky-footed wood rats (Neotoma fuscipes), wood mice (Apodemus), and voles (Microtus or Clethrionomys species), are likely reservoirs. The disease occurs internationally, including the US (North-eastern, mid-Atlantic, upper Midwest and Pacific North-west states), Europe, Asia (China, Siberian Russia and Korea) and Australia. These regions correspond to areas where Ixodes ticks bite humans: I. scapularis in the eastern US, I. pacificus in the western US, I. ricinus in Europe and I. persulcatus in Asia.

In Europe, HGA infection was first reported in a Slovenian woman aged 70 years, with evidence of potential co-infection with A. phagocytophilum sensu lato determined through a rise in the IgG antibody titre. Serological evidence of HGA infection has since been reported in more than 17 European countries. Seroprevalence rates among examined populations range from zero to 28%. The highest number of incident cases of HGA has been reported in Central Europe and Sweden, and seroepidemiological evidence of HGA infection has been reported to be higher among persons frequently exposed to ticks (e.g. forestry workers) and among patients with LD or TBE.

E. ewingii, recognised as a human pathogen in 1999, is the causative agent of HEE. It is primarily transmitted by the lone star tick, A. americanum. Disease caused by E. ewingii has been limited to a few patients in Missouri, Oklahoma and Tennessee, most of whom have been immunosuppressed. The full extent of the geographical range of this species, its vectors and its role in human disease is currently under investigation. The associated disease may be clinically indistinguishable from infection caused by E. chaffeensis or A. phagocytophilum.

E. Wisconsin–Minnesota is the most newly recognised pathogen of human ehrlichiosis, causing HWME. It is transmitted by I. scapularis. Four human cases have been reported to date, two from Wisconsin and two from Minnesota.

Clinical Findings

The multiple types of ehrlichiosis share many non-specific clinical and laboratory manifestations, including fever, headache, myalgia, malaise, nausea, vomiting, diarrhea, cough, arthralgias, rash, stiff neck, confusion, thrombocytopenia, leukopenia and elevated serum alanine aminotransferase and aspartate aminotransferase. The median age of patients is approximately 50 years and slightly more males than females are infected (57 to 61%). Although immunosuppressed patients are at a higher risk of HEE, there are far fewer complications and no fatalities have been reported. Similar, HSE is very rare and is usually benign, with no fatalities ever having been reported. Of the four patients with HWME, all presented with fever, malaise, headache and lymphopenia. In addition, three had thrombocytopenia and two had elevated liver enzyme levels. All recovered after receiving antimicrobial treatment.

Complications of HME and HGA are infrequent but may occur at any time – at the time of presentation, within several days after the onset of symptoms or, rarely, later – and persist for long intervals in the absence of active disease. HME patients can develop a fulminating toxic or septic shock-like syndrome, particularly individuals with underlying compromised immune systems (e.g. patients infected with HIV, organ transplant recipients, patients undergoing immunosuppression therapy for cancer or patients with immune disorders). About 20% of patients with HME have central nervous system (CNS) involvement (meningitis or meningoencephalitis). In addition, fatalities occur in approximately 3% of patients, most commonly in immunosuppressed persons with respiratory distress syndrome, hepatitis or opportunistic infections. HGA patients can develop peripheral neuropathies such as brachial plexopathy, demyelinating polyneuropathy and even isolated facial palsy.

Diagnosis

The diagnosis of ehrlichiosis should be considered in patients who live or travel in areas that are endemic for ehrlichiosis, experience a viral-like illness in the late spring, summer, or autumn and have been bitten by Ixodes ticks or the lone star tick, A. americanum. As the signs and symptoms are relatively non-specific, laboratory testing is required for
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**Table 2: Human Ehrlichiosis Pharmacological Treatment Regimens**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Medication</th>
<th>Dosing</th>
</tr>
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<tbody>
<tr>
<td>Adults</td>
<td>Doxycycline</td>
<td>100 mg orally or intravenously twice daily</td>
</tr>
<tr>
<td></td>
<td>Tetracyclines</td>
<td>500 mg orally every six hours</td>
</tr>
<tr>
<td>Children 8 years +</td>
<td>Doxycycline</td>
<td>2.2 mg/kg orally or intravenously twice daily (maximum 100 mg/dose)</td>
</tr>
<tr>
<td></td>
<td>Tetracyclines</td>
<td>25–50 mg/kg/day orally divided every six hours (maximum 500 mg/dose)</td>
</tr>
<tr>
<td>Children &lt;8 years</td>
<td>Rifampin</td>
<td>10 mg/kg orally twice daily (maximum 300 mg/dose)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Rifampin</td>
<td>300 mg orally twice daily</td>
</tr>
</tbody>
</table>

**Figure 3: Patient Serum Positive by Babesia microti Indirect Immunofluorescence Assay Test (A) and Patient Serum Positive by Babesia duncani Indirect Immunofluorescence Assay Test (B)**

In Figure 3A, Babesia microti is stained green; in Figure 3B, Babesia duncani parasites are stained green.

**Figure 4: Patient Serum Positive by Human Granulocytic Anaplasmosis Indirect Immunofluorescence Assay Test (A) and Patient Serum Positive by Human Monocytic Ehrlichiosis Indirect Immunofluorescence Assay Test (B)**

In Figure 4A, Anaplasma phagocytophilum bacteria are stained green; in Figure 4B, Ehrlichia chaffeensis are stained green.

**Microscopic Examination of Giemsa-stained Smear**

Microscopic examination of a Giemsa-stained smear is the most rapid and inexpensive method for detection of intracytoplasmic inclusions (morulae) that may be seen as stippled blue inclusions of bacteria in monocytes (HME) or neutrophils and bands (HGA and HEE). This test should be carried out within a week of disease onset, as sensitivity is highest at this time. Blood samples must be taken prior to administering antibiotic therapy, since morulae disappear from the blood within 24–72 hours after treatment begins. Unfortunately, microscopic examination is very insensitive for HME, detecting less than 10% of infected patients. For HGA, the sensitivity varies between 25 and 75%, depending on the expertise of the examiner and the time of testing. Information on the sensitivity for HEE is not available.

**Polymerase Chain Reaction**

During the first week of infection, when antibody levels are low or undetectable and the disease is in the acute phase, PCR on ethylenediaminetetraacetic acid (EDTA) or citrated whole blood is the most sensitive method for detecting *Ehrlichia* infections. After the first week, the bacteraemic phase of infection rapidly wanes, thereby limiting the effectiveness of PCR as a diagnostic technique. Sensitivity is 60–85% for detecting *E. chaffeensis* DNA and 67–90% for detecting *A. phagocytophilum* DNA. At present, PCR is the only specific diagnostic test available for *E. ewingii* infection because *E. ewingii* and *A. phagocytophilum* morulae are indistinguishable and culture isolation of *E. ewingii* has yet to be achieved. While PCR of cerebrospinal fluid (CSF) may be positive, the sensitivity is lower than for whole blood, probably due to the significantly lower volume of infected cells.

Several PCR targets have been employed towards conserved genes among different *Ehrlichia* isolates, including the *rps16* (*rRNA*) and *groEL* heat shock operon. Other genes have been used, such as genus-specific disulphide bond formation protein gene (*dsb*), the *E. chaffeensis*-specific 120 kDa and 32 kDa protein (*VLP*) genes, and the 28 kDa outer-membrane proteins (*P28*).

**Serology by the Indirect Immunofluorescence Assay**

_Ehrlichia_ infection can be confirmed by serological testing using IFA. IFA is the most frequently used assay for clinical diagnosis. The assay employs the detection of IgM and IgG antibodies that are reactive against *A. phagocytophilum* or *E. chaffeensis*-infected tissue culture cells or purified bacteria fixed to glass slides (Figure 4). Sensitivity is high to two weeks following disease onset compared with the first few days of infection for PCR, blood smear microscopy and cell culture. A diagnosis of *A. phagocytophilum* or *E. chaffeensis* infection is confirmed by a fourfold increase in antibody titre between acute and convalescent sera or a seroconversion to a titre of 128 or higher. However, the Consensus Approach for Ehrlichiosis Task Force has suggested that patients with single titres of 64 and 128 be considered probable cases of HME and those with single titres greater than 256 be considered confirmed HME cases. Moreover, seropositivity against *E. chaffeensis* or *A. phagocytophilum* can sometimes last from months to years after initial exposure. Thus an antibody titre must be considered in the context of other clinical evidence of infection and should not be the sole criterion for a diagnosis. For *A. phagocytophilum*, IgG antibody sensitivity ranges from 82 to 100% and IgM sensitivity from 27 to 37%. For *E. chaffeensis*, IgG antibody sensitivity ranges from 88 to 90% and IgM sensitivity is approximately 44%. Currently, there are no specific IFA tests available for *E. ewingii*. Notably, the *E. chaffeensis* IFA test cannot distinguish between HME and HEE antibodies. Thus it is likely that some patients diagnosed as having HME based on HME IFA test results may in fact have HEE. In addition, false-positive results can be obtained on patients with Rocky Mountain spotted fever, typhus, Q fever, brucellosis, LD, Epstein-Barr virus or various autoimmune disorders.
mammalian cell culture, it can take anywhere from two to 36 days.

A. phagocytophilum and E. chaffeensis are usually cultivated in the human promyelocytic leukemia cell line HL-60 and canine histiocytic cell line DH82, respectively, by direct inoculation of cell cultures with peripheral blood from a potentially infected patient.

The A. phagocytophilum bacteria develop within vacuoles to form morulae in the cytoplasm of infected cells that can be detected using Giemsa staining or PCR. Intracellular organisms can be visualised as early as five days post-inoculation or can remain undetectable for more than two weeks.

E. chaffeensis morulae can be visible in susceptible cells from two to 36 days post-inoculation.

However, currently very few laboratories offer these culture confirmation tests. Also, at present it is not possible to culture E. ewingii.

Treatment

The recommended treatment protocols for human ehrlichiosis are as follows (see Table 2). Treatment should be continued until the patient is afebrile for three days, typically resulting in 5–14 days of treatment.

The clinical response to doxycycline or tetracycline treatment is fast, with patients demonstrating an improvement in fever curve and an overall decrease in symptoms within 24–48 hours of initiating treatment.

The absence of such a response should cause clinicians to consider alternative diagnoses, particularly non-ehrlichial infections that are not susceptible to the tetracycline class of antibiotics.
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