Human Babesiosis and Ehrlichiosis – Current Status

Jyotsna S Shah,[1] Richard Horowitz[2] and Nick S Harris[3]

1. Vice President, IGeneX Inc., California; 2. Medical Director, Hudson Valley Healing Arts Center, New York; 3. CEO and President, IGeneX Inc., California, US

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*

Disclosure: Jyotsna Shah and Nick Harris are employees of IGeneX. Richard Horowitz is an employee of Hudson Valley Healing Arts Center.
Acknowledgements: The authors would like to thank Eddie Caoili, and Sohini Stone, for providing technical assistance.
Received: 13 September 2011 **Accepted:** 11 April 2012 **Citation:** *European Infectious Disease*, 2012;6(1):49–56
Correspondence: Jyotsna Shah, Vice-President and Laboratory Director, IGeneX, Inc., 795–797 San Antonio Road, Palo Alto, CA 94303, US. E: jyotsna@aol.com

Lyme disease (LD), caused by the *Borrelia burgdorferi* complex, is the most frequently reported arthropod-borne infection in North America and Europe.[1] The bacteria are transmitted to humans by the bites of infected *Ixodes* ticks, *Ixodes scapularis* and *Ixodes pacificus* in the US, *Ixodes ricinus* in Europe and *Ixodes persulcatus* in Russia and Asia. The *Ixodes* tick can also carry and transmit several other pathogens, such as *Babesia*, *Ehrlichia, Bartonella* and *Rickettsia* species and various viruses leading to human babesiosis, ehrlichiosis, bartonellosis, Rocky Mountain spotted fever, tick-borne encephalitis (TBE) and a number of other diseases.[2,3] Babesiosis and ehrlichiosis are the two most common co-infections among patients with LD.[4–7] In the US, among early LD patients, depending on their location, 2–12 % also have human granulocytic anaplasmosis (HGA)-type ehrlichiosis and 2–40 % have babesiosis.[8,9] Less than 10 % have triple infections (LD, ehrlichiosis and babesiosis).[8] Serological confirmation of concurrent babesiosis and LD was first reported in 1983 in a 36-year-old asplenic male from Shelter Island, NY, who experienced recurrent fevers, erythema chronicum migrans and monoarticular arthritis.[10] Serological evidence of concurrent infection with HGA and LD was reported in 1995 and molecular evidence was reported in 1997.[2,11] The Centers for Disease Control and Prevention (CDC) studied 100 ticks in rural New Jersey and found 55 % of the ticks were infected with at least one of the pathogens.[12] Co-infections complicate LD symptoms and treatment. It is possible for a tick to carry and transmit one of the co-infections and not *Borrelia*, making diagnosis difficult and often elusive.[12]

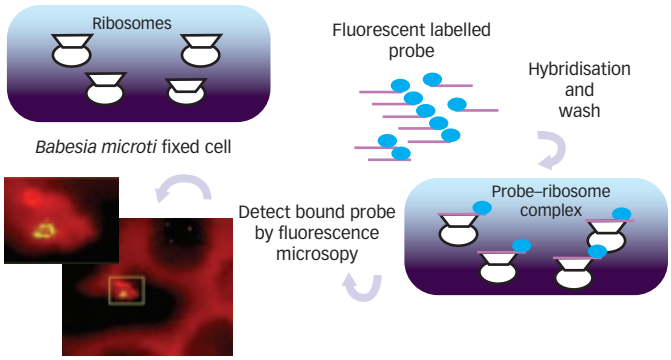
Human babesiosis is caused by protozoan parasites of the genus *Babesia*, including *Babesia microti*, *Babesia duncani*, *Babesia divergens*, *Babesia divergens*-like (also known as *Babesia* MOI), *Babesia* EU1 and *Babesia* KO1.[13] Ehrlichiosis includes human sennetsu ehrlichiosis (HSE), HGA, human monocytic ehrlichiosis (HME), human ewingii ehrlichiosis (HEE) and the recently discovered human ehrlichiosis Wisconsin–Minnesota (HWME).[14–19] The causative agents of HSE, HGA, HME, HEE and HWME are *Neorickettsia sennetsu*, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii* and *Ehrlichia* Wisconsin–Minnesota, respectively.[14–19] Given that the two most common co-infections with LD are babesiosis and ehrlichiosis, the focus of the present article is human babesiosis and human ehrlichiosis. The geographical distribution, clinical findings, diagnosis and treatment will be discussed.

Human Babesiosis

Babesiosis is a tick-borne infectious disease caused by haemoprotozoan parasites of the genus *Babesia*, family *Babesiidae*, order *Piroplasmida*.[13] Babesiosis is acquired through a tick bite or by blood transfusion.[13,20] The tick vectors are the hard-bodied *I. scapularis* in the US and *I. ricinus* in Europe.[13] In addition, approximately 150 cases of transfusion-transmitted *Babesia* (TTB) between 2000 and 2009 have been confirmed in the US by Herwaldt et al.[21]

While more than 100 species have been reported, only a few have been identified as causing human infections. The book of Exodus contained the first reference to babesiosis, alluding to a plague of 'murrain' that affected cattle, camels, sheep and other domestic animals.[13] Centuries later in 1888, Romanian Biologist Victor Babeş identified a protozoan parasite from cattle with febrile haemoglobinuria

Figure 1: Fluorescent *In Situ* Hybridisation (FISH)



as Babesia.[22] In 1893, Smith and Kilbourne found that Texas cattle fever was caused by the Babesia parasite, thus linking Babesia with its vector, the tick.[23] The first case of human babesiosis was reported in 1957 near Zagreb, Croatia (then in Yugoslavia) in an asplenic farmer.[24] Since then, several species of *Babesia* that cause human babesiosis have been described in the US, Europe and Asia. Earlier reports involved splenectomised patients with fulminant babesiosis. However, in 1969, infection with *B. microti* was described in a patient with an intact spleen from Nantucket Island off the coast of Massachusetts.[25] Over the past 50 years, the epidemiology of the disease has changed from a few isolated cases to the establishment of endemic areas in the northern Midwest and the eastern and western coasts of the US.[13,25–30]

Geographical Distribution

Currently, in the US, three species of *Babesia* have been identified as the causative agents of human babesiosis: *B. microti*, *B. duncani* (formerly known as *Babesia* WA1-type and related parasites in the states of Washington and California) and *B. divergens*-like (also known as *B.* MOI).[13] *B. microti* is the agent most frequently identified in the North-east and Midwest and can occur in non-splenectomised individuals.[5,25] 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.[31] *B. microti* is rapidly spreading in areas of the North-east not previously known to be endemic for babesiosis.[26,27,32–40] 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.[32] This is due to ticks now containing polymicrobial infections in these emerging endemic areas.[33] *B. duncani* is most frequently identified on the West Coast.[26,34–37,41] *B. divergens*-like organisms have been identified in three cases, two from the Midwest (Missouri and Kentucky) and one from Washington state.[27,38,39] 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.[40]

Worldwide, little is known about the prevalence of *Babesia* in malaria-endemic countries, where misidentification as *Plasmodium* probably occurs. *B. divergens* is responsible for the majority of cases of babesiosis in Europe.[42–45] To date, approximately 40 cases of *B. divergens* infection have been documented in Europe, mostly from countries with extensive cattle industries such as France, Ireland and Great Britain.[42–44] Cases have sporadically been reported from Sweden, Switzerland, Spain, Portugal and Croatia (index case).[45] Nearly all patients had been splenectomised prior to the onset of *Babesia* infection.[42–45] However, two cases of babesiosis in immunocompetent patients were reported in France in 2011; one of the two cases was confirmed to be due to *B. divergens*.[46] In July 2011, the first two cases of human babesiosis caused by *B. divergens* were reported in China.[47]

There are fewer worldwide reports of babesiosis due to the other *Babesia* species. Isolated cases due to *B. microti*-like parasites have been reported over a wide geographical range in Europe, Asia, Africa, South America and Australia.[48–57] In East Asia, cases of *B. microti* have been reported in Japan and Taiwan.[52,57] In Australia, 13 patients who had never travelled outside Australia tested positive for *Babesia*.[48] Three cases of human infection with *B.* EU1 have been described in Europe.[58,59] All three were men over 50 years of age who had been splenectomised.[58,59] The first case of babesiosis reported from Korea was nearly fatal and was caused by *B.* KO1 that was isolated from sheep in China.[54]

New emerging species of babesiosis are also being found in areas not previously known to be endemic.[60] The first zoonotic case of babesiosis was recently found in Tennessee in a patient who had not travelled outside the state, but had had multiple tick exposures during hunting trips.[60] *Babesia* parasites were seen on the blood smear and molecular analysis revealed a novel species, but attempts to isolate it were unsuccessful.[60] Patients can therefore have babesiosis without travelling to known endemic areas and without testing positive for previously known species.

Clinical Findings

Infection with *Babesia* is usually asymptomatic.[61–64] Elderly and immunosuppressed people, especially those without a spleen or with impaired cellular immunity, are more likely to become symptomatic.[61–64] Symptoms, including fever, malaise, headache, nausea and generalised aching, may last weeks to months.[61–64] Hepatomegaly, splenomegaly, jaundice, and dark urine are also common findings in symptomatic patients.[61–64] These may be accompanied by elevation in hepatic transaminases, proteinuria and haemoglobinuria.[61–64] Severe haemolysis, often accompanied by thrombocytopenia, leukopenia and atypical lymphocytosis, is more common in high-risk patients.[61–64]

Babesia microti Infection

The disease spectrum of *B. microti* infection in humans depends on their immune status.[1] The symptoms vary from asymptomatic infection through flu-like symptoms to severe disease, sometimes leading to death in immunocompromised and splenectomised patients.[29] 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.[65,66] In fact, patients co-infected with LD and *B. microti* often have subclinical presentations.[67]

Asymptomatic Infection: The incubation period is between one and nine weeks following infection by *Babesia*.[13,28] According to one report, approximately 30 % of patients have asymptomatic infection.[29] Asymptomatic infection also occurs following resolution of symptomatic babesiosis, with parasites persisting in the blood for months or years.[68] These asymptomatic patients therefore pose a risk

for transmission of babesiosis through blood donation, as evidenced by the first confirmed case of *B. microti* transmission via blood donation in Germany in 2007.[53,68–70]

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 that contracts *Babesia* infection gets flu-like symptoms with fever and chills.[65,68,71–76] 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.[65,71–73] Other, less common, symptoms include gastrointestinal symptoms such as vomiting, diarrhoea and weight loss, conjunctival injection and emotional instability.[72–76] The illness can last anywhere from a week to over a year.[65,68,72,75,76] Although a patient may feel well, parasites can be detected in the blood up to two years after the initial episode.[68]

Severe Disease: Severe disease usually occurs in immunocompromised or splenectomised patients and patients on immunosuppressive medication.[59,75,77–79] 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.[74,77,80] Complications of severe disease are acute respiratory failure, congestive heart failure, liver and renal failure and rupture of the spleen.[60] Mortality rates between 5 and 21 % have been reported.[30,71,81] 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 109/l.[71,73,82] These patients require comprehensive and aggressive medical care.

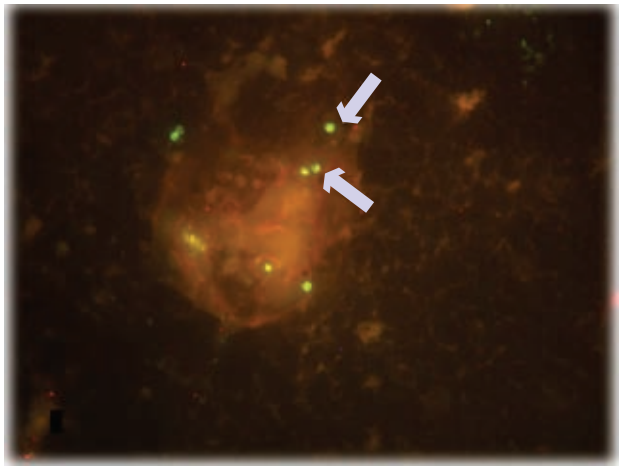
Babesia duncani Infection

The severity of *B. duncani* infection is variable, depending primarily on the immune status of the host.[1] 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.[26] Although studies performed in hamsters have demonstrated that infection due to *B. duncani* is more pathogenic than *B. microti* infection,[83] 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.[26,51,52,54,84] The other eight had mild clinical symptoms or were asymptomatic.[26,51,52,54,84]

Babesia divergens Infection

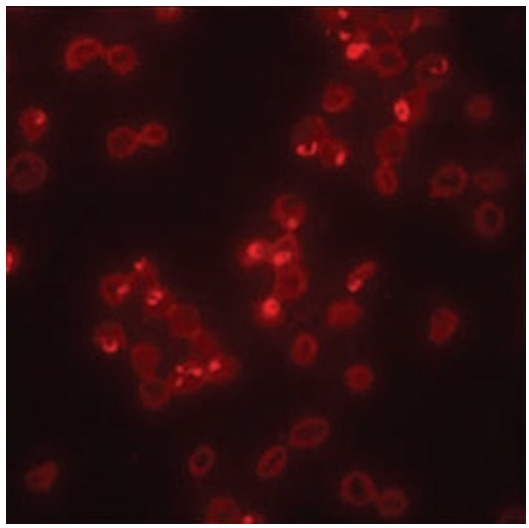
In Europe, approximately 40 human cases of *B. divergens* infection have been reported, all in splenectomised patients.[42–44] These patients suffered a severe form of babesiosis.[42–44] Signs and symptoms begin one to three weeks after tick bite and consist of high fever, headache, chills, intense sweating, myalgia, abdominal pains with severe intravascular haemolysis that results in haemoglobinaemia, haemoglobinuria and jaundice.[42] In more than 50 % of the patients, there was a rapid onset of renal failure and pulmonary oedema.[42] Ecchymoses, petechiae, congestive heart failure and coma have also been reported.[42–44] The illness is generally fulminant, lasting about a week and, for more than one-third of patients, ending in prolonged convalescence or death.[42–44] Recently, *B. divergens* infection was reported from two anaemic patients in Shandong province, China.[47] These two patients also had hepatic injury, haemoglobinuria and renal failure.[47]

Figure 2a: Patient Blood Smear Positive for *Babesia microti* by Fluorescent *In Situ* Hybridisation Assay Using Fluorescent-labelled *B. microti*-specific Probe



Babesia appear green.

Figure 2b: Hamster Blood Smear Positive for *Babesia duncani* by Fluorescent In Situ Hybridisation Assay Using Texas Red-labelled *B. duncani*-specific Probe



Babesia appear red.

Babesia EU1 Infection

B. EU 1, together with *B. odocoilei* that infects white-tailed deer in the US, forms a sister group to that of *B. divergens*.[58,59] Unlike *B. divergens* infections, which are fulminant and often fatal, *B.* EU1 infections have varied from mild to severe, but have not been fatal.[58,59] All three reported cases of *B.* EU1 infection had a history of Hodgkin's disease and had been splenectomised.[58,59] One patient experienced mild babesiosis, whereas two had moderate to severe illness.[58,59] Symptoms included fever, dark urine, fatigue, chills, headache, confusion, jaundice, sweats and shortness of breath.[58,59] Peak parasitaemia ranged from 1 to 30 %. In the two patients with moderate to severe illness, babesiosis was concurrent with a relapse of nodular lymphocyte-predominant Hodgkin's lymphoma or with stage IIIA diffuse large B-cell lymphoma.[58,59] All three patients were admitted to the hospital and recovered after antibabesial therapy.[58,59] One had a prolonged relapsing illness that eventually cleared.[58,59]

Table 1: Human Babesiosis Pharmacological Treatment Regimens

Regimen	Patient	Dosing
Atovaquone (oral) + azithromycin (oral)	Adults	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
Atovaquone (oral) + azithromycin (oral)	Children	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)
Clindamycin (oral) + quinine (oral)	Adults	Clindamycin 600 mg every eight hours and quinine 650 mg every six hours
Clindamycin (intravenous) + quinine (oral)	Adults	Clindamycin intravenously 300–600 mg every six hours and quinine orally 650 mg every six hours
Clindamycin (intravenous or oral) + quinine (oral)	Children	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)

Diagnosis

When patients living or travelling in areas endemic for babesiosis are bitten by *Ixodes* ticks or have received a recent blood transfusion, and have flu-like or malaria-like symptoms in late spring, summer or autumn, a diagnosis of babesiosis should be considered. As the signs and symptoms are relatively non-specific, often imitating malaria, laboratory testing is required for definitive diagnosis.[61–64] In most patients with babesiosis, there is some degree of haemolytic anaemia with an elevated reticulocyte count[61–64] and, in about 50 % of patients, serum liver enzymes are elevated. In severe cases, urine protein level, blood urea nitrogen and serum creatinine are elevated.[61–64] Thus it is common for physicians to order screening tests, such as complete blood count (CBC) and liver function tests, prior to ordering *Babesia*-specific tests. Specific laboratory tests currently available for diagnosis of babesiosis are described below.

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.[85] 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.[26] The tetrad form, referred to as a Maltese cross, is pathognomonic of small *Babesia* species such as *B. microti* and *B. duncani*.[26] *B. duncani* displays more tetrad forms than *B. microti* (see *Figures 1–3*).[26] Parasitaemia is usually low (5 %) but can go as high as 85 %.[74]

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* parasitaemia.[86] However, there are drawbacks to PCR: the time to result can be several hours, PCR cannot be performed directly on blood, and DNA has to 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.

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.

Serology by the Indirect Immunofluorescence Assay

The indirect immunofluorescence assay (IFA) is the most commonly used serological test for diagnosis of babesiosis.[87,88] 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*).[88] Sensitivity is high two to four weeks after disease onset (versus a few days for PCR or blood smear microscopy).[88] 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.[88] 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.[36,37] 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).[89] *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.[89] Thus, IgG titres of 1:1,024 or greater usually signify active or recent infection.[89] The detection of IgM is indicative of recent infection.[87] Although seroconversion occurs in virtually all immunocompetent individuals infected with *B. microti*, the diagnosis of active *babesia*l 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*.[36,37,84]

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.[83] The infected patient's blood is injected by 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.[86]

Treatment

According to Vannier et al., the following treatment protocols are recommended (see *Table 1*).[13] Note that dosing regimens are for 7–10 days except for persistent relapsing infection.[13]

For immunocompromised patients, successful outcomes have been reported using atovaquone combined with higher doses of azithromycin (600–1,000 mg orally per day).[13] For severe cases of babesiosis, partial or complete exchange transfusion should also be considered, particularly in

patients co-infected with LD[13,68,90] since failures of classical treatment regimens using clindamycin and quinine as well as atovaquone and azithromycin have been reported in this patient group.[91] Atovaquone and azithromycin do not cross the placenta, whereas clindamycin and quinine do cross the placenta. Therefore, during pregnancy, clindamyacin and quinine are recommended to treat fetal infections.[32]

Human Ehrlichiosis

Human ehrlichiosis is caused by tick-transmitted bacteria of the genera *Neorickettsia*, *Anaplasma* and *Ehrlichia*, all belonging to the *Anaplasmataceae* family.[14] Ehrlichiosis includes HSE, HGA, HME, HEE and the recently discovered HWME.[14–19] The causative agents of HSE, HGA, HME, HEE and HWME are *N. sennetsu*, *A. phagocytophilum*, *E. chaffeensis*, *E. ewingii* and *E.* Wisconsin–Minnesota, respectively.[14–19] 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*.[15,92] In 1953, the first human case of sennetsu ehrlichiosis caused by *N. sennetsu* (formally known as *Ehrlichia sennetsu*) was reported in Japan.[93,94] Infections with *Ehrlichia* and *Anaplasma* were recognised in 1986 and 1990, respectively.[14,15] These two genera comprise the majority of the human infections.[14,15] *E. ewingii*, the canine pathogen discovered in 1992, was first recognised as a causative agent of human ehrlichiosis in 1998.[17,18] *E. ewingii* is serologically very similar to *E. chaffeensis* but, like *A. phagocytophilum*, propagates within neutrophils.[17,18] *E.* Wisconsin–Minnesota was reported to be the cause of four cases of human ehrlichiosis for the first time in 2011.[19]

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.[15,93,94] *N. sennetsu* is thought to be transmitted by a fluke, a trematode considered to be the reservoir and vector.[95]

E. chaffeensis is the causative agent of HME.[16] 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*.[16] 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.[16] In 1990, the agent of HME was isolated from the blood of a US Army reservist at Fort Chaffee, AR.[96] The newly recognised organism was named *E. chaffeensis*.[97]

A. phagocytophilum (formerly called *Ehrlichia phagocytophila* or *Ehrlichia equi*), transmitted by black-legged ticks of the *Ixodes* group, is the causative agent of HGA.[98] 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.[98] 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.[48,99] 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.[99]

In Europe, HGA infection was first reported in a Slovenian woman aged 70 years, with evidence of potential co-infection with *B. burgdorferi sensu lato* determined through a rise in the IgG antibody titre.[100] 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 %.[101] 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.[101]

E. ewingii, recognised as a human pathogen in 1999, is the causative agent of HEE.[17,18] It is primarily transmitted by the lone star tick, *A. americanum*.[18] Disease caused by *E. ewingii* has been limited to a few patients in Missouri, Oklahoma and Tennessee, most of whom have been immunosuppressed.[102] 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.[19] It is transmitted by *I. scapularis*.[19] Four human cases have been reported to date, two from Wisconsin and two from Minnesota.[19]

Clinical Findings

The multiple types of ehrlichiosis share many non-specific clinical and laboratory manifestations, including fever, headache, myalgia, malaise, nausea, vomiting, diarrhoea, cough, arthralgias, rash, stiff neck, confusion, thrombocytopenia, leukopenia and elevated serum alanine aminotransferase and aspartate aminotransferas.[99,103,104] The median age of patients is approximately 50 years and slightly more males than females are infected (57 to 61 %).[105]

Although immunosuppressed patients are at a higher risk of HEE, there are far fewer complications and no fatalities have been reported.[18,106] Similarly, HSE is very rare and is usually benign, with no fatalities ever having been reported.[94] Of the four patients with HWME, all presented with fever, malaise, headache and lymphopenia.[19] In addition, three had thrombocytopenia and two had elevated liver enzyme levels. All recovered after receiving antimicrobial treatment.[19]

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.[99,103,107,108] HME patients can develop a fulminant 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).[106] 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.[105,107,109] HGA patients can develop peripheral neuropathies such as brachial plexopathy, demyelinating polyneuropathy and even isolated facial palsy.[110]

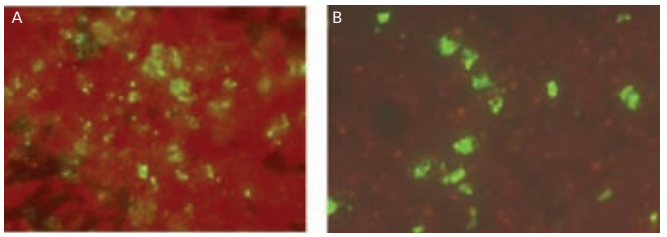
Diagnosis

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

Table 2: Human Ehrlichiosis Pharmacological Treatment Regimens

Patient Group	Medication	Dosing
Adults	Doxycycline	100 mg orally or intravenously twice daily
	Tetracyclines	500 mg orally every six hours
Children 8 years +	Doxycycline	2.2 mg/kg orally or intravenously twice daily (maximum 100 mg/dose)
	Tetracyclines	25–50 mg/kg/day orally divided every six hours (maximum 500 mg/dose)
Children <8 years	Rifampin	10 mg/kg orally twice daily (maximum 300 mg/dose)
Pregnancy	Rifampin	300 mg orally twice daily

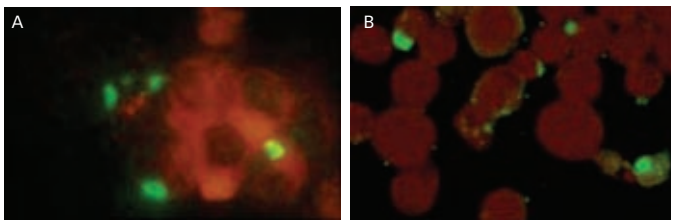
Figure 3: Patient Serum Positive by *Babesia microti* Indirect Immunofluorescence Assay Test (A) and Patient Serum Positive by *Babesia duncani* Indirect Immunofluorescence Assay Test (B)



Patient's serum positive by B. microti IFA | Patient's serum positive by Babesia duncani IFA

In Figure 3A, Babesia microti *are stained green; in Figure 3B,* B. 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)



Positive serum by HGA IFA | Positive serum by HME IFA

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

diagnosis. Specific laboratory tests that are used for the diagnosis of ehrlichiosis are described below. Specific tests for concurrent LD and babesiosis should be performed in patients presenting with clinical manifestations that suggest co-infection, such as an erythema migrans-type rash or haemolytic anaemia.[111]

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).[111] This test should be carried out within a week of disease onset, as sensitivity is highest at this timE.[112,113] Blood samples must be taken prior to administering antibiotic therapy, since morulae disappear from the blood within 24–72 hours after treatment begins.[114,115] Unfortunately, microscopic examination is very insensitive for HME, detecting less than 10 % of infected patients.[115] For HGA, the sensitivity varies between 25 and 75 %, depending on the expertise of the examiner and the time of testing.[114] 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.[112] After the first week, the bacteraemic phase of infection rapidly wanes, thereby limiting the effectiveness of PCR as a diagnostic technique.[112] Sensitivity is 60–85 % for detecting *E. chaffeensis* DNA and 67–90 % for detecting *A. phagocytophilum* DNA.[116,117] 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.[96,97,116,118] Several PCR targets have been employed towards conserved genes among different *Ehrlichia* isolates, including the *rrs* (*16S* rRNA) and *groESL* heat shock operon.[119] Other genes have been used, such as genus-specific disulphide bond formation protein gene (dsb), the *E. chaffeensis*-specific 120 kDa and *TRP32* protein (*VLPT*) genes, and the 28 kDa outer-membrane proteins (*P28*).[120]

Serology by the Indirect Immunofluorescence Assay

Ehrlichia infection can be confirmed by serological testing using IFA.[121] IFA is the most frequently used assay for clinical diagnosis.[121] 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*).[121] Sensitivity is high two to four 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.[122,123] 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.[123] Moreover, seropositivity against *E. chaffeensis* or *A. phagocytophilum* can sometimes last from months to years after initial exposure.[121,122] 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 %.[121] For *E. chaffeensis*, IgG antibody sensitivity ranges from 88 to 90 % and IgM sensitivity is approximately 44 %.[104,111,124] Currently, there are no specific IFA tests available for *E. ewingii*. Notably, the *E. chaffeensis* IFA test cannot distinguish between HME and HEE antibodies.[17,125] 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.[124]

Culture Confirmation

Culture confirmation is the gold standard for diagnosis of ehrlichiosis. Although culture confirmation of *E. chaffeensis* and *A. phagocytophilum* from a patient's blood is possible in antibiotic-free

mammalian cell culture, it can take anywhere from two to 36 days.[126,127] *A. phagocytophilum* and *E. chaffeensis* are usually cultivated in the human promyelocytic leukaemia 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.[116,126,127]

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.[126] Intracellular organisms can be visualised as early as five days post-inoculation or can remain undetectable for more than two weeks.[126] *E. chaffeensis* morulae can be visible in susceptible cells from two to 36 days post-inoculation.[128] 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.[129] 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.[129] 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.[32,33] ■

1. Nadelman RB, Wormser GP, Reinfection in patients with Lyme disease, *Clin Infect Dis*, 2007;45(8):1032–8.
2. Tokarz R, Jain K, Bennett A, et al., Assessment of Polymicrobial Infections in Ticks in New York State, *Vector Borne Zoonotic Dis*, 2010;10(3):217–21.
3. Lindgren E, Gustafson R, Tick-borne encephalitis in Sweden and climate change, *Lancet*, 2001;358(9275):16–8.
4. Schwartz I, Fish D, Daniels T, Prevalence of the rickettsial agent of human granulocytic ehrlichiosis in ticks from a hyperendemic focus of Lyme disease [letter], *N Engl J Med*, 1997;337:49–50.
5. Nadelman RB, Horowitz HW, Hsieh TC, et al., Simultaneous human granulocytic ehrlichiosis and Lyme borreliosis, *N Engl J Med*, 1997;337:27–30.
6. Krause P, Telford S, Spielman A, et al., Concurrent Lyme disease and babesiosis: evidence for increased severity and duration of illness, *JAMA*, 1996;275:1657–60.
7. Sweeney C, Ghassemi M, Agger W, Persing D, Coinfection with Babesia microti and Borrelia burgdorferi in a western Wisconsin resident, *Mayo Clin Proc*, 1998;73(4):338–41.
8. Krause PJ, McKay K, Thompson CA, et al., Disease-specific diagnosis of coinfecting tickborne zoonoses: babesiosis, human granulocytic ehrlichiosis, and Lyme disease, *Clin Infect Dis*, 2002;34:1184–91.
9. Swanson SJ, Neitzel D, Reed KD, Belongia EA, Coinfections acquired from ixodes ticks, *Clin Microbiol Rev*, 2006;19(4):708–27.
10. Grunwaldt E, Barbour AG, Benach JL, Simultaneous occurrence of babesiosis and Lyme disease, *N Engl J Med*, 1983;308:1166.
11. Kolbert CP, Pancholi P, Mitchell PD, et al., Evidence for human granulocytic ehrlichiosis in patients with suspected Lyme disease, Abstract C-204, p. 36, Presented at: 95th General Meeting of the American Society for Microbiology, Washington, DC, 21–25 May 1995.
12. Varde S, Beckley J, Schwartz I, Prevalence of tick-borne pathogens in Ixodes scapularis in a rural New Jersey County, *Emerg Infect Dis*, 1998;4(1):97–9.
13. Vannier E, Gewurz BE, Krause PJ, Human babesiosis, *Infect Dis Clin North Am*, 2008;22(3):469–88.
14. Dumler JS, Barbet AF, Bekker CP, et al., Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: Unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophila, *Int J Syst Evol Microbiol*, 2001;51(Pt 6):2145–65.
15. Bakken JS, Dumler JS, Chen SM, et al., Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging?, *JAMA*, 1994;272:212–8.
16. Maeda K, Markowitz N, Hawley RC, et al., Human infection with Ehrlichia canis, a leukocytic Rickettsia, *N Engl J Med*, 1987;316:853–6.
17. Buller RS, Arens M, Hmiel SP, et al., Ehrlichia ewingii, a newly recognized agent of human ehrlichiosis, *N Engl J Med*, 1999;341:148–55.
18. Paddock CD, Liddell AM, Storch GA, Other causes of tick-borne ehrlichioses, including Ehrlichia ewingii. In: Goodman JL, Dennis DT, Sonenshine DE (eds), *Tick-borne diseases of humans*, Washington, DC: ASM Press, 2005;258–67.
19. Pritt BS, Sloan LM, Johnson DK, et al., Emergence of a new pathogenic Ehrlichia species, Wisconsin and Minnesota, 2009, *N Engl J Med*, 2011;365(5):422–9.
20. Leiby DA, Transfusion-transmitted Babesia spp.: bull's-eye on Babesia microti, *Clin Microbiol Rev*, 2011;24(1):14–28.
21. Herwaldt BL, Linden JV, Bosserman E, et al., Transfusion-associated babesiosis in the United States: a description of cases, *Ann Intern Med*, 2011;155(8):509–19.
22. Babes V, [On bacterial haemoglobinuria in cattle], *Comptes Rendus de l'Academie des Sciences*, 1888;107:692–4.
23. Smith T, Kilborne FL, *Investigations into the nature, causation, and prevention of Texas or southern cattle fever*, Washington, DC: US Department of Agriculture Bureau of Animal Industry, 1893;177–304.
24. Skrabalo Z, Deanovic Z, Piroplasmosis in man: report of a case, *Doc Med Geogr Trop*, 1957;9:1, 11–6.
25. Western KA, Benson GD, Gleason NN, et al., Babesiosis in a Massachusetts resident, *N Engl J Med*, 1970;283(16):854–6.
26. Conrad PA, Kjemtrup AM, Carreno RA, et al., Description of Babesia duncani n.sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms, *Int J Parasitol*, 2006;36(7):779–89.
27. Herwaldt BL, Persing DH, Précigout EA, et al., A fatal case of babesiosis in Missouri: Identification of another piroplasm that infects humans, *Ann Intern Med*, 1996;124(7):643–50.
28. Hunfeld KP, Hildebrandt A, Gray JS, Babesiosis: recent insights into an ancient disease, *Int J Parasitol*, 2008;38(11):1219–37.
29. Krause PJ, McKay K, Gadbaw J, et al., Increasing health burden of human babesiosis in endemic sites, *Am J Trop Med Hyg*, 2003;68(4):431–6.
30. Meldrum SC, Birkhead GS, White DJ, et al., Human babesiosis in New York state: an epidemiological description of 136 cases, *Clin Infect Dis*, 1992;15(6):1019–23.
31. Menis M, Anderson SA, Izurieta HS, et al., Babesiosis among elderly Medicare beneficiaries, United States, 2006-2008, *Emerg Infect Dis*, 2012;18(1):128–31.
32. Joseph JT, Roy SS, Shams N, et al., Babesiosis in Lower Hudson Valley, New York, USA, *Emerg Infect Dis*, 2011;17(5):843–7.
33. Tokarz R, Jain K, Bennett A, et al., Assessment of Polymicrobial Infections in Ticks in New York State, *Vector Borne Zoonotic Dis*, 2010;10(3):217–21.
34. Herwaldt BL, Kjemtrup AM, Conrad PA, et al., Transfusion-transmitted babesiosis in Washington state: first reported case caused by a WA1-type parasite, *J Infect Dis*, 1997;175(5):1259–62.
35. Kjemtrup AM, Lee B, Fritz CL, et al., Investigation of transfusion transmission of a WA1-type babesial parasite to a premature infant in California, *Transfusion*, 2002;42(11):1482–7.
36. Persing DH, Conrad PA, Babesiosis: new insights from phylogenetic analysis, *Infect Agents Dis*, 1995;4(4):182–95.
37. Quick RE, Herwaldt BL, Thomford JW, et al., Babesiosis in Washington State: a new species of Babesia?, *Ann Intern Med*, 1993;119(4):284–90.
38. Beattie JF, Michelson ML, Holman PJ, Acute babesiosis caused by Babesia divergens in a resident of Kentucky, *N Engl J Med*, 2002;347(9):697–8.
39. Herwaldt BL, de Bruyn G, Pieniazek NJ, et al., Babesia divergens-like Infection, Washington State, *Emerg Infect Dis*, 2004;10(4):622–9.
40. Holman PJ, Phylogenetic and biologic evidence that Babesia divergens is not endemic in the United States, *Ann N Y Acad Sci*, 2006;1081:518–25.
41. Prince HE, Lape-Nixon M, Patel H, Yeh C, Comparison of the Babesia duncani (WA1) IgG detection rates among clinical sera submitted to a reference laboratory for WA1 IgG testing and blood donor specimens from diverse geographic areas of the United States, *Clin Vaccine Immunol*, 2010;17(11):1729–33.
42. Gorenflot A, Moubri K, Precigout E, et al., Human babesiosis, *Ann Trop Med Parasitol*, 1998;92(4):489–501.
43. Zintl A, Gray JS, Skerrett HE, Mulcahy G, Possible mechanisms underlying age-related resistance to bovine babesiosis, *Parasite Immunol*, 2005;27(4):115–20.
44. Zintl A, Mulcahy G, Skerrett HE, et al., Babesia divergens, a bovine blood parasite of veterinary and zoonotic importance, *Clin Microbiol Rev*, 2003;16(4):622–36.
45. Meer-Scherrer L, Adelson M, Mordechai E, et al., Babesia microti in Europe, *Curr Microbiol*, 2004;48(6):435–7.
46. Martinot M, Zadeh MM, Hansmann Y, et al., Babesiosis in Immunocompetent Patients, Europe, *Emerg Infect Dis*, 2011;17(1):114–6.
47. Qi C, Zhou D, Liu J, et al., Detection of Babesia divergens using molecular methods in anemic patients in Shandong Province, China, *Parasitol Res*, 2011;109(1):241–5.
48. Mayne PJ, Emerging incidence of Lyme borreliosis, babesiosis, bartonellosis, and granulocytic ehrlichiosis in Australia, *Int J Gen Med*, 2011;4:845–52.
49. Bush JB, Isaacson M, Mohamed AS, et al., Human babesiosis—a preliminary report of 2 suspected cases in South Africa, *S Afr Med J*, 1990;78(11):699.
50. Foppa IM, Krause PJ, Spielman A, et al., Entomologic and serologic evidence of zoonotic transmission of Babesia microti, eastern Switzerland, *Emerg Infect Dis*, 2008;8(7):722–6.
51. Gorenflot A, Bazin C, Ambroise-Thomas P, [Human babesiosis, Treatment of severe forms], *Presse Med*, 1987;16(22):1099.
52. Saito-Ito A, Tsuji M, Wei Q, et al., Transfusion-acquired, autochthonous human babesiosis in Japan: isolation of Babesia microti-like parasites with hu-RBC-SCID mice, *J Clin Microbiol*, 2000;38(12):4511–6.
53. Hildebrandt A, Hunfeld KP, Baier M, et al., First confirmed autochthonous case of human Babesia microti infection in Europe, *Eur J Clin Microbiol Infect Dis*, 2007;26(8):595–601.
54. Kim JY, Cho SH, Joo HN, et al., First case of human babesiosis in Korea: detection and characterization of a novel type of Babesia sp. (KO1) similar to ovine Babesia, *J Clin Microbiol*, 2007;45(6):2084–7.
55. Marathe A, Tripathi J, Handa V, Date V, Human babesiosis—a case report, *Indian J Med Microbiol*, 2005;23(4):267–9.
56. Rios L, Alvarez G, Blair S, Serological and parasitological study and report of the first case of human babesiosis in Colombia, *Rev Soc Bras Med Trop*, 2003;36(4):493–8.
57. Shih CM, Liu LP, Chung WC, et al., Human babesiosis in Taiwan: asymptomatic infection with a Babesia microti-like organism in a Taiwanese woman, *J Clin Microbiol*, 1997;35(2):450–4.
58. Herwaldt BL, Cacciò S, Gherlinzoni F, et al., Molecular characterization of a non-Babesia divergens organism causing zoonotic babesiosis in Europe, *Emerg Infect Dis*, 2003;9(8):942–8.
59. Haselbarth K, Tenter AM, Brade V, et al., First case of human babesiosis in Germany—clinical presentation and molecular characterisation of the pathogen, *Int J Med Microbiol*, 2007;297(3):197–204.
60. Mosites EM, Wheeler PW, Pieniazek NJ, et al., Human Babesiosis Caused by a Novel Babesia Parasite – Tennessee, 2009. Abstract number 310, Presented at: International Conference on Emerging Infectious Diseases (ICEID), Atlanta, GA, 10–14 July 2010.
61. Gorenflot A, Moubri K, Precigout E, et al., Human babesiosis, *Ann Trop Med Parasitol*, 1998;92:489–501.
62. Villar BF, White DJ, Benach JL, Human babesiosis, *Prog Clin Parasitol*, 1991;2:129–43.
63. Sun T, Tenenbaum MJ, Greenspan J, et al., Morphologic and clinical observations in human infection with Babesia microti, *J Infect Dis*, 1983;148(2):239–48.
64. Benach JL, Habicht GS, Clinical characteristics of human babesiosis, *J Infect Dis*, 1981;144:481.
65. Krause PJ, Telford SR 3rd, Spielman A, et al., Concurrent Lyme disease and babesiosis: evidence for increased severity and duration of illness, *JAMA*, 1996;275(21):1657–60.
66. Krause PJ, McKay K, Thompson CA, et al., Disease-specific diagnosis of coinfecting tickborne zoonoses: babesiosis, human granulocytic ehrlichiosis, and Lyme disease, *Clin Infect Dis*, 2002;34(9):1184–91.
67. Horowitz RI, Lyme Disease and Babesiosis: New Therapeutic Options for Chronic Persistent Disease, Presented at: 13th International Scientific Conference on Lyme Disease and Other Tick-Borne Disorders, Farmington, CT, 25–26 March 2000.
68. Krause PJ, Spielman A, Telford SR 3rd, et al., Persistent parasitemia after acute babesiosis, *N Engl J Med*, 1998;339(3):160–5.
69. Leiby DA, Babesiosis and blood transfusion: flying under the radar, *Vox Sang*, 2006;90(3):157–65.
70. Leiby DA, Chung AP, Gill JE, et al., Demonstrable parasitemia among Connecticut blood donors with antibodies to Babesia microti, *Transfusion*, 2005;45(11):1804–10.
71. Hatcher JC, Greenberg PD, Antique J, Jimenez-Lucho VE, Severe babesiosis in Long Island: review of 34 cases and their complications, *Clin Infect Dis*, 2001;32(8):1117–25.
72. Ruebush TK 2nd, Juranek DD, Chisholm ES, Human babesiosis on Nantucket Island. Evidence for self-limited and

subclinical infection, *N Engl J Med*, 1997;297(15):825–7.
73. White DJ, Talarico J, Chang HG, et al., Human babesiosis in New York State: review of 139 hospitalized cases and analysis of prognostic factors, *Arch Intern Med*, 1998;158(19):2149–54.
74. Sun T, Tenenbaum MJ, Greenspan J, et al., Morphologic and clinical observations in human infection with Babesia microti, *J Infect Dis*, 1983;148(2):239–48.
75. Mathewson HO, Anderson AE, Hazard GW, Self-limited babesiosis in a splenectomized child, *Pediatr Infect Dis*, 1984;3(2):148–9.
76. Ortiz JM, Eagle RC Jr, Ocular findings in human babesiosis (Nantucket fever), *Am J Ophthalmol*, 1982;93(3):307–11.
77. Rosner F, Zarrabi MH, Benach JL, Habicht GS, Babesiosis in splenectomized adults: review of 22 reported cases, *Am J Med*, 1984;76(4):696–701.
78. Krause PJ, Gewurz BE, Hill D, et al., Persistent and relapsing babesiosis in immunocompromised patients, *Clin Infect Dis*, 2008;46(3):370–6.
79. Reubush TK 2nd, Cassaday PB, Marsh HJ, et al., Human babesiosis on Nantucket Island: clinical features, *Ann Intern Med*, 1977;86(1):6–9.
80. Stowell CP, Gelfand JA, Shepard JAO, Kratz A, Case 17-2007: a 25-year-old woman with relapsing fevers and recent onset of dyspnea, *N Engl J Med*, 2007;356(22):2313–9.
81. Spielman A, Wilson ML, Levine JF, Piesman J, Ecology of Ixodes dammini-borne human babesiosis and Lyme disease, *Annu Rev Entomol*, 1985;30:439–60.
82. Benach JL, Habicht GS, Clinical characteristics of human babesiosis, *J Infect Dis*, 1981;144(5):481.
83. Kjemtrup AM, Conrad PA, Human babesiosis: an emerging tick-borne disease, *Int J Parasitol*, 2000;30(12–13):1323–37.
84. Persing DH, Herwaldt BL, Glaser C, et al., Infection with a Babesia-like organism in Northern California, *N Engl J Med*, 1995;332(5):298–303.
85. Healy GR, Ruebush TK 2nd, Morphology of Babesia microti in human blood smears, *Am J Clin Pathol*, 1980;73(1):107–9.
86. Krause PJ, Telford SR 3rd, Spielman A, et al., Comparison of PCR with blood smear and inoculation of small animals for diagnosis of Babesia microti parasitemia, *J Clin Microbiol*, 1996;34(11):2791–4.
87. Krause PJ, Ryan R, Telford SR 3rd, et al., Efficacy of immunoglobulin M serodiagnostic test for rapid diagnosis of acute babesiosis, *J Clin Microbiol*, 1996;34(8):2014–6.
88. Krause PJ, Telford SR 3rd, Ryan R, et al., Diagnosis of babesiosis: evaluation of a serologic test for the detection of Babesia microti antibody, *J Infect Dis*, 1994;169(4):923–6.
89. Ruebush TK 2nd, Chisholm ES, Sulzer AJ, Healy GR, Development and persistence of antibody in persons infected with Babesia microti, *Am J Trop Med Hyg*, 1981;30(1):291–2.
90. Wormser GP, Prasad A, Neuhaus E, et al., Emergence of Resistance to Azithromycin-Atovaquone in Immunocompromised Patients with Babesia microti Infection, *Clin Infect Dis*, 2010;50(3):381–6.
91. Horowitz RI, Chronic Persistent Babesiosis After Acute Treatment with Cleocin and Quinine, and Atovaquone and Azithromycin, Abstract, Presented at: 12th International Scientific Conference on Lyme Disease and Other Spirochetal Disorders, NYC, April 1999.
92. Keefe TJ, Holland CJ, Salyer PE, Ristic M, Distribution of Ehrlichia canis among military working dogs in the world and selected civilian dogs in the United States, *J Am Vet Med Assoc*, 1982;181(3):236–8.
93. Misao T, Kobayashi Y, [Studies on mononucleosis. 1 report. Isolation of the infectious agent], *Fukuoka Med J*, 1954;45:519.
94. Tachibana N, Sennetsu fever: the disease, diagnosis, and treatment. In: Leive L (ed.), *Microbiology*, Washington, DC: American Society for Microbiology, 1986;205–8.
95. Newton PN, Rolain JM, Rasachak B, et al., Sennetsu Neorickettsiosis: A Probable Fish-Borne Cause of Fever Rediscovered in Laos, *Am J Trop Med Hyg*, 2009;81(2):190–4.
96. Dawson JE, Anderson BE, Fishbein DB, et al., Isolation and characterization of an Ehrlichia sp. from a patient diagnosed with human ehrlichiosis, *J Clin Microbiol*, 1991;29:2741–5.
97. Anderson BE, Dawson JE, Jones DC, et al., Ehrlichia chaffeensis, a new species associated with human ehrlichiosis, *J Clin Microbiol*, 1991;29:2838–42.
98. Chapman AS, Bakken JS, Folk SM, et al., Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis-United States: a practical guide for physicians and other health-care and public health professionals, *MMWR Recomm Rep*, 2006;55(RR-4):1–27.
99. Dumler JS, Choi KS, Garcia-Garcia JC, et al., Human granulocytic anaplasmosis and Anaplasma phagocytophilum, *Emerg Infect Dis*, 2005;11:1828–34.
100. Krause PJ, Telford SR 3rd, Ryan R, et al., Geographical and temporal distribution of babesial infection in Connecticut, *J Clin Microbiol*, 1991;29:1–4.
101. Strle F, Human granulocytic ehrlichiosis in Europe, *Int J Med Microbiol*, 2004;293(Suppl. 37):27–35.
102. Thomas LD, Hongo I, Bloch KC, et al., Human ehrlichiosis in transplant recipients, *Am J Transplant*, 2007;7(6):1641–7.
103. Stone JH, Dierberg K, Aram G, Dumler JS, Human monocytic ehrlichiosis, *JAMA*, 2004;292:2263–70.
104. Olano JP, Masters E, Hogrefe W, Walker DH, Human monocytotropic ehrlichiosis, Missouri, *Emerg Infect Dis*, 2003;9:1579–86.
105. Demma LJ, Holman RC, McQuiston JH, et al., Epidemiology of human ehrlichiosis and anaplasmosis in the United States, 2001–2002, *Am J Trop Med Hyg*, 2005;73:400–9.
106. Paddock CD, Folk SM, Shore GM, et al., Infections with Ehrlichia chaffeensis and Ehrlichia ewingii in persons coinfected with human immunodeficiency virus, *Clin Infect Dis*, 2001;33:1586–94.
107. Dumler JS, Anaplasma and Ehrlichia infection, *Ann N Y Acad Sci*, 2005;1063:361–73.
108. Olano JP, Walker DH, Human ehrlichioses, *Med Clin North Am*, 2002;86:375–92.
109. Paddock CD, Childs JE, Ehrlichia chaffeensis: a prototypical emerging pathogen, *Clin Microbiol Rev*, 2003;16:37–64.
110. Bakken JS, Dumler JS, Human granulocytic ehrlichiosis, *Clin Infect Dis*, 2000;31:554–60.
111. Dumbler JS, Madigan JE, Pusterla N, Bakken JS, Ehrlichioses in humans: epidemiology clinical presentation, diagnosis and treatment, *Clin Infect Dis*, 2007;45(Suppl. 1):S45–S51.
112. Bakken JS, Dumler JS, Clinical diagnosis and treatment of human granulocytotropic anaplasmosis, *Ann N Y Acad Sci*, 2006;1078:236–47.
113. Horowitz HW, Aguero-Rosenfeld ME, McKenna DF, et al., Clinical and laboratory spectrum of culture-proven human granulocytic ehrlichiosis: comparison with culture-negative cases, *Clin Infect Dis*, 1998;27:1314–7.
114. Goodman JL, Human granulocytic anaplasmosis. In: Goodman JL, Dennis DT, Sonenshine DE (eds), *Tick-Borne Diseases of Humans*, Washington, DC: ASM Press, 2005;218–38.
115. Dawson JE, Ewing SA, Davidson WR, Human monocytotrophic Ehrlichiosis. In: Goodman JL, Dennis DT, Sonenshine DE (eds), *Tick-Borne Diseases of Humans*, Washington, DC: ASM Press, 2005;239–57.
116. Standaert SM, Yu T, Scott MA, et al., Primary isolation of Ehrlichia chaffeensis from patients with febrile illnesses: clinical and molecular characteristics, *J Infect Dis*, 2000;181(3):1082–8.
117. Horowitz HW, Kilchevsky E, Haber S, et al., Perinatal transmission of the agent of human granulocytic ehrlichiosis, *N Engl J Med*, 1998;339(6):375–8.
118. Tan HP, Dumler JS, Maley WR, et al., Human monocytic ehrlichiosis: an emerging pathogen in transplantation, *Transplantation*, 2001;71:1678–80.
119. Sumner JW, Nicholson WL, Massung RF, PCR amplification and comparison of a nucleotide sequences from the groESL heat shock operon of Ehrlichia species, *J Clin Microbiol*, 1997;35:2087–92.
120. Sumner JW, Childs JE, Paddock CD, Molecular cloning and characterization of the Ehrlichia chaffeensis variable-length PCR target: an antigen-expressing gene that exhibits interstrain variation, *J Clin Microbiol*, 1999;37:1447–53.
121. Walls JJ, Aguero-Rosenfeld M, Bakken JS, et al., Inter- and intralaboratory comparison of Ehrlichia equi and human granulocytic ehrlichiosis (HGE) agent strains for serodiagnosis of HGE by the immunofluorescent-antibody test, *J Clin Microbiol*, 1999;37(9):2968–73.
122. Bakken JS, Haller I, Riddell D, et al., The serological response of patients infected with the agent of human granulocytic ehrlichiosis, *Clin Infect Dis*, 2002;34(1):22–7.
123. Walker DH, Diagnosing human ehrlichioses: current status and recommendations, *ASM News*, 2000;66:287–90.
124. Dawson JE, Fishbein DB, Eng TR, et al., Diagnosis of human ehrlichiosis with the indirect fluorescent antibody test: kinetics and specificity, *J Infect Dis*, 1990;162(1):91–5.
125. Rikihisa Y, Ewing SA, Fox JC, Western immunoblot analysis of Ehrlichia chaffeensis, E. canis, or E. ewingii infections in dogs and humans, *J Clin Microbiol*, 1994;32(9):2107–12.
126. Goodman JL, Nelson C, Vitale B, et al., Direct cultivation of the causative agent of human granulocytic ehrlichiosis, *N Engl J Med*, 1996;334(4):209–15.
127. Carlyon JA, Laboratory Maintenance of Anaplasma phagocytophilum, *Curr Protoc Microbiol*, 2005;3:3A.2.
128. Dawson JE, Ewing SA, Davidson WR, Human monocytotrophic Ehrlichiosis. In: Goodman JL, Dennis DT, Sonenshine DE (eds), *Tick-Borne Diseases of Humans*, Washington, DC: ASM Press: 2005;239–57.
129. Thomas RJ, Dumbler JS, Carlyon JA, Nulocytic anaplasmosis, human monocytic ehrlichiosis and Ehrlichia ewingii ehrlichiosis. Current management of human granulocytic ehrlichiosis, *Expert Rev Anti Infect Ther*, 2009;7(6):709–22.