Babesiosis: Recent insights into an ancient disease

K.-P. Hunfeld a,*, A. Hildebrandt b, J.S. Gray c

Abstract

Ever since the discovery of parasitic inclusions in erythrocytes of cattle in Romania by Victor Babes at the end of the 19th century, newly recognised babesial pathogens continue to emerge around the world and the substantial public health impact of babesiosis on livestock and man is ongoing. Babesia are transmitted by ixodid ticks and infection of the host causes a host-mediated pathology and erythrocyte lysis, resulting in anemia, hyperbilirubinuria, hemoglobinuria, and possibly organ failure. Recently obtained molecular data, particularly for the 18S rRNA gene, has contributed significantly to a better understanding of the sometimes puzzling phylogenetic situation of the genus Babesia and new information has been added to help determine the taxonomic position of many species. Moreover, it seems that owing to higher medical awareness the number of reported cases in humans is rising steadily. Hitherto unknown zoonotic babesias are now being reported from geographical areas where babesiosis was not known to occur and the growing numbers of immunocompromised individuals suggest that the frequency of cases will continue to rise. This review covers recent insights into human babesiosis with regard to phylogeny, diagnostics and treatment in order to provide new information on well known as well as recently discovered parasites with zoonotic potential.

Keywords: Babesia; Ticks; Zoonosis; Phylogeny; Human disease; Blood transfusion; IFAT; PCR; Diagnostics; Treatment; Prevention

1. Introduction

Tick-transmitted hemoparasites of the protozoan genus Babesia (phylum Apicomplexa) are the second most common blood-borne parasites of mammals after the trypanosomes (Telford et al., 1993). It was Victor Babes who at the end of the 19th century first discovered microorganisms in erythrocytes of cattle in Romania and associated them with bovine hemoglobinuria or red water fever (Babes, 1888). Five years later, Smith and Kilbourne established Pyrosoma – later renamed Babesia bigemina – as the causative agent of Texas Cattle Fever (Smith and Kilbourne, 1893), a finding of historic significance because this piroplasm was the first recognised arthropod-borne pathogen of vertebrates (Kjemtrup and Conrad, 2000). Since then, newly recognised babesia with zoonotic potential continue to emerge around the world and the substantial economic impact of babesiosis on livestock and companion animals especially in the tropics and subtropics is ongoing (Collett, 2000; Kivaria et al., 2007). A fatal Babesia divergens infection in 1956 was the first confirmed case of human babesiosis (Skrabalo and Deanovic, 1957) and, ever since, babesiosis came into view as a potentially life threatening zoonotic infection in humans (Homer et al., 2000; Herwaldt et al., 2003). Although, several babesia species have been involved in human infections worldwide (Gorenflo, et al., 1998), the major public health burden on man lies in North America and is due to Babesia microti, especially in the eastern parts of the US (Homer et al., 2000). In these classic areas of endemicity, babesiosis is on the rise and the number of cases appears to be increasing in some parts of the US relative to the number of Lyme...
disease cases (Meldrum et al., 1992; Krause et al., 2003). Moreover, during the last decade, newly recognised babesia parasites (Table 1) have been implicated in human disease and it seems that owing to higher medical awareness the number of reported cases is rising steadily (Hildebrandt et al., 2007). In addition, the occurrence of hitherto unknown zoonotic parasites is now reported from geographical areas where babesiosis was not known to occur and obviously the growing population of immunocompromised individuals is ever more involved (Hunfeld and Brade, 2004; Häselbarth et al., 2007; Hildebrandt et al., 2007; Karp and Auwaerter, 2007). Most significantly, molecular analysis of the implicated pathogens suggests that the host-range of many babesia is less restricted than believed previously and also that hitherto unrecognised species can cause infections in a variety of animal hosts and in humans (Zahler et al., 2000; Cho et al., 2002; Herwaldt et al., 2003, 2004; Conrad et al., 2006; Kjemtrup et al., 2006; Häselbarth et al., 2007; Kim et al., 2007). Therefore, many past cases of human babesiosis on both sides of the Atlantic that were attributed, based on traditional methods, to classic species such as B. divergens or B. microti, may indeed be due to species not yet known to cause such infections in humans (Herwaldt et al., 2003; Gray, 2006; Hildebrandt et al., in press). This notion is further substantiated by the recent recognition of Babesia duncani and B. divergens-like organisms as pathogens of medical significance for humans in the US (Herwaldt et al., 1996; Beattie et al., 2002; Conrad et al., 2006). Moreover, confirmed autochthonous B. microti infections have been reported in Taiwan, Japan and Europe (Shih et al., 1997; Saito-Itô et al., 2000; Hildebrandt et al., 2007), and a new European B. divergens-like organism (EU1), provisionally named Babesia venatorum, has been discovered, which is probably a parasite of deer (Telford and Goethert, 2004; Bonnet et al., 2007). This parasite was involved in the first documented cases of human babesiosis in Italy, Austria and Germany (Herwaldt et al., 2003; Häselbarth et al., 2007). Such new findings now clearly challenge the dogma that human babesiosis in North America is almost exclusively caused by B. microti and that human babesiosis in Europe is solely due to B. divergens infection in splenectomized individuals. This review covers recent developments and important new information on well known and recently discovered babesias with zoonotic potential.

2. Classification and life cycle characteristics of Babesia spp.

Babesia are classified as apicomplexan parasites of the suborder Piroplasmidea and family Babesiidae on the basis of their exclusive invasion of erythrocytes, multiplication by budding rather than schizogony, and lack of hemozoin. The life cycles of the parasites are very similar (Fig. 1). All species of babesia are naturally transmitted by the bite of infected ticks (almost all ixodids rather than argasids) and the main lifecycle difference amounts to the presence of transovarial transmission in some species (Babesia spp. sensu stricto) and not in others (B. microti-like). During the tick bite, sporozoites are injected into the host and directly infect red blood cells (Fig. 1). This phenomenon separates Babesia spp. from Theileria spp., where sporozoites do not readily infect red blood cells but initially penetrate a lymphocyte or macrophage in which development into schizonts takes place (Ultenberg, 2006). In the host, babesia sporozoites develop into piroplasms inside the infected erythrocyte resulting in two or sometimes four daughter cells that leave the host cell to infect other erythrocytes until the host dies or the immunity of the host clears the parasites. The spleen with its lympho-reticular filter function is essential in resisting primary infections of Babesia spp. by specifically removing infected cells from circulation, probably through a combination of spleen microcirculation and stimulated phagocytic cell activity (de Vos et al., 1987; Gray and Weiss, 2008).

To date, more than 100 species have been identified, infecting many mammalian and some avian species (Gray and Weiss, 2008). Traditionally, babesias were mainly grouped on the basis of their morphology, host/vector specificity, and susceptibility to drugs. Pragmatically, they are divided into the small babesias (trophozoites of 1.0–2.5 µm; including Babesia gibsoni, B. microti, and Babesia rodhaini and large babesias (2.5–5.0 µm; including Babesia bovis, Babesia caballi, and Babesia canis). These morphological classifications are generally consistent with the phylogenetic characterization based on nuclear small subunit-ribosomal RNA gene (18S rDNA) sequences, which shows that the large and small babesias fall into two phylogenetic clusters, with the small babesias being more related to Theileria spp. than the large (with the exception of B. divergens, which appears small on blood smears [0.4–1.5 µm] but is genetically related to large babesias (Homer et al., 2000). Recently, molecular genetic analyses clarified the somewhat confused phylogenetic situation, sometimes resulting in the emergence of new groups and 18S rDNA analysis added new information to the taxonomic position of many piroplasm species (Kjemtrup and Conrad, 2006). A careful study by Criado-Fornelio et al. (2003) recently suggested division of the piroplasms into five distinct clades: (i) B. microti group containing B. rodhaini, Babesia felis, Babesia leo, B. microti, and a B. microti-like canine isolate, (ii) western US Theileria-like group, containing Babesia conradae, (iii) Theileria-group, containing all Theileria species from bovines, (iv) a first group of ‘true’ Babesia spp. (sensu stricto) including B. canis and B. gibsoni from canines together with B. divergens and Babesia odocoilei, and (v) a second Babesia spp. sensu stricto group composed mainly of Babesia spp. from ungulates: B. caballi, B. bigemina, B. ovis, B. bovis, and other Babesia spp. from cattle.

2.1. New developments in the phylogeny of B. microti and B. microti-like organisms

Latest research suggests that B. microti is only distantly related to Babesia species sensu stricto (B. bigemina, B. bovis, and B. divergens), that are best known as parasites
Table 1

Important Babesia spp. with zoonotic potential including recently recognised defined parasites

<table>
<thead>
<tr>
<th>Species</th>
<th>Vector</th>
<th>Vertebrate host</th>
<th>Geographical occurrence</th>
<th>Reported human cases (N)</th>
<th>Reported mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Large babesia</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>B. divergens s.s.</td>
<td>Ixodes ricinus, Ixodes ventralloi (?)</td>
<td>Larvae, nymphs, adults</td>
<td>Cattle, wild ruminants</td>
<td>Europe</td>
<td>&gt;30</td>
</tr>
<tr>
<td>B. venatorum (EU1)</td>
<td>Ixodes ricinus</td>
<td>Larvae, nymphs, adults</td>
<td>Deer</td>
<td>Europe</td>
<td>3</td>
</tr>
<tr>
<td>MO1 and related parasites</td>
<td>Ixodes dentatus (?)</td>
<td>?</td>
<td>Cottontail rabbits</td>
<td>USA</td>
<td>3</td>
</tr>
<tr>
<td><strong>B. ovis-like</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KO1</td>
<td>Hemaphysalis longicornis (?)</td>
<td>?</td>
<td>Sheep (?)</td>
<td>Korea, Asia (?)</td>
<td>1</td>
</tr>
<tr>
<td>B. bovis(^b)</td>
<td>Boophilus spp., Ixodes spp.(^a,)</td>
<td>Larvae</td>
<td>Cattle, water buffalo,</td>
<td>Southern Europe, Africa,</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Rhipicephalus bursa</td>
<td></td>
<td>wild ruminants</td>
<td>America, Asia, Australia</td>
<td></td>
</tr>
<tr>
<td>B. canis(^b)</td>
<td>Rhipicephalus sanguineus(^a,)</td>
<td>Nymphs, adults</td>
<td>dogs, <em>Vulpes vulpes</em>,</td>
<td>Europe, Asia, Africa,</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Hemaphysalis leachi,</td>
<td></td>
<td>wild canines</td>
<td>America, Australia</td>
<td></td>
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<tr>
<td></td>
<td>Dermacentor reticularis(^a)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Small babesia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. microti &amp; B. microti-like</td>
<td>Ixodes trianguliceps, Ixodes ricinus(^a,)</td>
<td>Nymphs, adults</td>
<td>Rodents</td>
<td>Europe, Asia, America</td>
<td>&gt;200</td>
</tr>
<tr>
<td>B. microti complex</td>
<td>Ixodes ovatus(^a,), Ixodes scapularis(^a,), Ixodes spinipalpis, Ixodes angustus, Ixodes maurus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. duncani &amp; B. duncani-like</td>
<td>?</td>
<td>?</td>
<td>USA</td>
<td>9 (?)</td>
<td>11%</td>
</tr>
</tbody>
</table>

\(^a\) Known to regularly parasitize humans; ?: unknown; (?) : questionable.

\(^b\) Unverified infections with *B. canis* and *B. bovis* have been reported (Gorenflot et al., 1998), though it is likely that *B. bovis* infections are in fact *B. divergens*.
of domestic livestock (Fig. 2). In a seminal study on *B. microti* by Goethert and Telford (2003a), the molecular analysis of 18S rRNA and β-tubulin gene fragments of parasites collected in several countries (USA, Switzerland, Spain, Russia), from a variety of vertebrate hosts (humans, voles, woodmice, shrews, foxes, skunks, raccoons, dogs) and from ticks resulted in the identification of three separate clades (Fig. 3). As a consequence *B. microti*, long regarded as a single species, is now regarded as a genetically diverse species complex. Clade 1 contained mostly rodent parasites and also the majority of strains thought to be zoonotic. Clade 2 contained carnivore parasites, and Clade 3 contained rodent parasites that are probably not zoonotic (Fig. 3). In a separate comparison of babesial 18S rDNA sequences Gray (2006) demonstrated that a European rodent isolate, ‘Munich’ (GenBank AB071177) is clearly distinct from any of the Goethert and Telford (2003b) rodent isolates and more recent studies have shown that the Munich strain is identical to isolates from ticks and *Microtus* spp. from Poland (Pieniazek et al., 2006; Sinski et al., 2006). In Japan, the first *B. microti* strain to be characterized (Kobe) was isolated from a human case in the central region of the country (Saito-Ito et al., 2000). Another strain (Hobetsu) was found to be much more widespread within Japan than the Kobe strain. Neither was closely related to the American zoonotic strains and both seemed to be especially associated with the large Japanese field mouse, *Apodemus speciosus* (Tsuji et al., 2001). A third Japanese strain, referred to as a ‘US-type’, is closely related to the American zoonotic strains, according to β-tubulin gene homology, and has been found in rodents in a limited region in Hokkaido, northern Japan (Zamoto...
This strain also occurs in South Korea, Vladivostok in Russia, and Xinjiang in China, and appears to utilise a wider range of hosts, including insectivores, than the Hobetsu and Kobe strains (Zamoto et al., 2004b).

The rather loosely constituted Clade 2 group of Goethert and Telford’s study (2003a) are all parasites of carnivores, and the recently described \textit{B. microti}-like parasite of dogs in Spain (GenBank AY534602, \textit{Theileria annae}? ) clearly belongs to this group since it shows 100% 18S rDNA homology to a Clade 2 parasite (GenBank AY144702) from a fox in Cape Cod, MA, USA (Fig. 3). The assigning of this Spanish dog parasite to the genus \textit{Theileria} by
Camacho et al. (2001) emphasises the lack of certainty in the classification of *B. microti*-like parasites (Fig. 2).

At least 11 cases of human babesiosis have occurred in the western USA caused by *B. microti*-like pathogens (Gray and Weiss, 2008). Recently, isolates from Washington State (WA1) and from California (CA5) were characterised in detail and the parasite named as *B. duncani* (Conrad et al., 2006). *B. duncani* shows only subtle morphological differences from *B. microti*, but analysis of the 18S rDNA clearly demonstrated that it belongs to a separate phylogenetic group, together with isolates from dogs and wildlife, and was furthermore indistinguishable from two other human isolates, CA6 and WA2-clone 1, though distinguishable from the dog isolate *B. conradae* (Fig. 1). The manifestations of the *B. duncani* disease are very similar to those caused by *B. microti* but unlike *B. microti*, the reservoir hosts and vectors of *B. duncani* are unknown at present. In addition, uncharacterized *B. microti*-like babesias in human patients have been recorded in Brazil, China, Egypt, Mexico, South Africa and Taiwan (Gorenflo et al., 1998), and most recently in India (Marathe et al., 2005).

### 2.2. New developments in the phylogeny of Babesia sensu stricto human pathogens

New phylogenetic information has also emerged for other recently recognised zoonotic *Babesia* spp. such as *B. venatorum* (EU1-3) in Europe (Herwaldt et al., 2003; Häselbarth et al., 2007) and *B. divergens*-like organisms (Gray, 2006) identified in the US based on 18S rDNA and ITS2 sequence analysis. Phylogenetic analysis of *B. venatorum* clearly demonstrates that it clusters together with *B. odocoieli*, a parasite of deer from the US, and these two organisms form a sister group with *B. divergens* (Fig. 4). The clustering of these organisms was identical, regardless of which phylogenetic method was used (Herwaldt et al., 2003). *B. divergens*-like parasites isolated from humans in the US: MO1 from Missouri (Herwaldt et al., 1996); Washington State (Herwaldt et al., 2004); Kentucky (Beattie et al., 2002) are so close to *B. divergens* in terms of 18S rDNA homology (Gray, 2006) they were sometimes referred to as *B. divergens*. The Kentucky parasite appears to be identical for the 18S rRNA gene to the Nantucket cottontail rabbit ‘*B. divergens*’ described by Goethert and Telford (2003b) and also to the Missouri parasite (MO1) (Holman et al., 2005; Gray, 2006). However, all bovine isolates of *B. divergens*, including reassigned isolates of the original GenBank depositions, were found to be identical for this gene (Herwaldt et al., 2003; Slemenda et al., unpublished) so that parasites showing less than 100% homology should perhaps not be considered *B. divergens*. Indeed, a recent study by Holman (2006) on the cottontail rabbit isolate, involving differential infectivity for cattle, parasite size and morphology in vitro, host erythrocyte specificity in vitro and ribosomal RNA gene sequences suggest that *B. divergens* sensu stricto is not endemic to the US. At least two European isolates from human cases that were identified at the time as *B. divergens*, also show less than 100% homology with the *B. divergens* 18S rDNA (Olmeda et al., 1997; Centeno-Lima et al., 2003) so that their identity as *B. divergens* is questionable. This could mean that there are at least three *B. divergens*-like parasites with zoonotic potential in Europe. Another human case occurred recently in Korea (Kim et al., 2007) and this parasite, designated KO1, was found to be most closely related to *Babesia* spp of sheep that were described by Liu et al. (2007). This large babesia is clearly separate from all the other agents of human babesiosis so far analysed, as shown in the phylogenetic tree constructed by Kim et al. (2007).

### 3. Reservoir hosts and tick vectors

*Babesia microti* is now recognised as a diverse species complex, parasitising a variety of hosts including rodents, insectivores and carnivores but the majority of the zoonotic strains utilise microtine rodents as reservoir hosts (Goethert and Telford, 2003a). In the US the white-footed mouse *Peromyscus leucopus*, is the main reservoir host and the vector is the human-biting *Ixodes scapularis*, the deer or black-legged tick (Spielman, 1976). Other vectors of various strains of *B. microti* include *Ixodes spinipalpis*, *Ixodes angustus* and *Ixodes muris* which transmit the parasites to various species of voles. However, these ticks do not bite humans and the zoonotic potential of the strains of *B. microti* that they transmit is unknown. The vectors and reservoirs of the main west coast US zoonotic babesia, *B. duncani*, are not known.

European strains of *B. microti* also parasitise a variety of microtine rodents and two vectors are involved, *Ixodes trianguliceps* is a specialised rodent tick that rarely if ever bites man and is probably responsible for the transmission of *B. microti* throughout Europe. *Ixodes ricinus*, which is closely related to *I. scapularis*, and is well known to infest humans, was identified as a vector of *B. microti* in Germany by Walter (1984), with the field vole, *Microtus agrestis* as the natural reservoir. *B. microti* transmission of a European strain by *I. ricinus* using Mongolian gerbils in the laboratory was confirmed by Gray et al. (2002) and the parasite was detected in *I. ricinus* specimens collected from vegetation in several countries (Gray and Weiss, 2008). It seems likely, therefore, that this tick species is responsible, for transmission to humans as evidenced by reports of positive serology (Hunfeld et al., 2002) and a single confirmed case (Hildebrandt et al., 2007). The only vector implicated in transmission of *B. microti* in Japan, where an autochthonous human case transmitted by blood transfusion was reported (Saito-Ito et al., 2000), is *Ixodes ovatus*, specimens of which were found in tick salivary glands (Saito-Ito et al., 2004; Yano et al., 2005), although neither of the two types of *B. microti* that were detected matched the zoonotic (Kobe) strain. Microtine rodents are the likely reservoir hosts.

The reservoir host for *B. divergens* sensu stricto, which is implicated in most cases of human babesiosis in Europe, is
cattle and the vector for this parasite is *I. ricinus*. However, *B. divergens*-like parasites have also been described from deer and from *I. ricinus* (Duh et al., 2001, 2005; Bonnet et al., 2007) and these parasites may have caused some infections attributed to *B. divergens* sensu stricto. The studies by Duh et al. (2001, 2005) and Bonnet et al. (2007) suggest strongly that the newly described *B. venatorum*, which has caused infections in Austria, Italy, and Germany (Herwaldt et al., 2003; Häselbarth et al., 2007) also have deer as a reservoir host and *I. ricinus* as a vector. The vector and reservoir host for the *B. divergens*-like parasite in a human case from the Canary Isles are unknown (Olmeda et al., 1997). *I. ricinus* is not thought to be involved but a close relative, *Ixodes ventralloi*, occurs there and may be the vector.

In the US, the reservoir hosts for the *B. divergens*-like isolates from Missouri (MO1) and from Kentucky are probably species of cottontail rabbits in view of the findings of Goethert and Telford (2003b) in Nantucket. The vectors are unknown but it is likely that *Ixodes dentatus* maintains the infection in the Nantucket rabbit population. Neither vector nor reservoir host are known for the *B. divergens*-like parasite isolated from a human case in Washington State (Herwaldt et al., 2004), though interestingly, parasites from Texan black-tailed jackrabbits showed 99.9% homology with this isolate (Yabsley et al., 2006).

### 4. Human disease due to *Babesia* spp

To date, seven distinct babesia parasites have been found to cause human babesiosis (Table 1): *B. microti* and related organisms, *B. divergens*, *B. bovis*, *B. canis*, *B. duncanii*, *B. venatorum*, and a novel type of *Babesia* sp. similar to ovine babesias provisionally named KO1 (Calvo De Mora et al., 1985, Marsaudon et al., 1995; Gorenflo et al., 1998; Homer et al., 2000; Gray and Weiss, 2008; Hildebrandt et al., in press). Most patients infected with *Babesia* spp. sensu stricto share splenectomy as a risk factor for acquiring the disease. In addition, for all babesia infections advanced age and depressed cellular immunity are associated with a higher risk of symptomatic infection and more severe illness (Telford and Maguire, 2006). This is why, the rising number of HIV positive individuals and the increasing population of immunocompromised individuals may serve to boost the number of human babesiosis cases (Kjemtrup and Conrad, 2000; Häselbarth et al., 2007; Hildebrandt et al., 2007; Karp and Auwaerter, 2007).

Humans acquire the disease through tick bites or transfusion of contaminated blood products (Homer et al., 2000). An exceptional way of infection which is rarely observed in humans is transplacental transmission (Kjemtrup and Conrad, 2000). So far, only two confirmed human cases have been documented (Esernio-Jenssen et al., 1987; New et al., 1997). In case of maternal infection, treatment can successfully prevent infection of the foetus (Raucher et al., 1984).

The clinical features in patients with babesiosis vary substantially from asymptomatic to life threatening, depending on the conditions of the patient and the parasite involved. In general, patients of all ages including children are affected, but most present clinically in their 40s to 60s (Mylonakis, 2001; Hunfeld and Brade, 2004). For *B. microti* children and adults are infected at similar frequencies, but the proportion of symptomatic infections in adults is higher (Krause et al., 2003). Peak transmission occurs from May to September and incubation periods vary from five to 33 days after a tick bite. However, most individuals do not remember a tick infestation (Homer et al., 2000; Hunfeld and Brade, 2004; Häselbarth et al., 2007; Hildebrandt et al., in press). In transfusion-transmitted cases or in immunocompromised individuals, cases may arise at any time of the year and incubation periods can be much longer depending on the exact time point and means of transmission (Hildebrandt et al., in press). In immunocompetent individuals parasitemia can hardly be detected. Patients may show up with, in part, non-specific symptoms like fever, flu-like disease, headache, chills, sweats, and myalgia (Table 2). Clinical diagnosis of human babesiosis can be further complicated by long persistence of subclinical infections (Krause et al., 1998) and in Europe.
and the US may underlie other tick-borne diseases (Hunfeld and Brade, 2004; Swanson et al., 2006), particularly Lyme borreliosis (Table 2). Symptoms, however, usually abate within a few weeks (Leiby, 2006). In HIV patients fever may occur for more than 4 weeks and show high parasitemias (>30%), and the parasite may persist despite active treatment (Kjemtrup and Conrad, 2000). Clinical symptoms in immunocompromised patients include high fever (up to 40 °C), high parasitemia (20–80%) diaphoresis, severe anemia, shortness of breath, weakness, and fatigue. Later, patients may develop more specific symptoms like jaundice, dark urine, CNS involvement, or complications like congestive heart failure, and respiratory distress syndrome (Homer et al., 2000; Hatcher et al., 2001; Mylonakis, 2001; Herwaldt et al., 2003; Hunfeld and Brade, 2004; Hildebrandt et al., 2007). Babesia microti infections may persist despite multiple courses of treatment and may be associated with relapsing symptoms for more than a year in immunocompromised individuals as described in a recent case control study (Krause et al., 2008). A recent study on 139 patients with B. microti infection in New York State revealed that 25% required hospitalisation and 6.5% died. On average, a 12- to 14-day delay after the onset of symptoms was noted before initiation of appropriate antibiotic treatment (White et al., 1998).

Infections with B. divergens generally present as fulminating, life-threatening infections. To date, about 30 cases of B. divergens infections have been reported in Europe predominantly in asplenic patients (Homer et al., 2000; Zintl et al., 2003; Hildebrandt et al., in press). Symptoms appear rapidly within 1–3 weeks post infection with septic fever, hemoglobinuria, or jaundice due to severe hemolysis, and up to 42% of patients die (Homer et al., 2000; Leiby, 2006). In contrast to the findings in human B. divergens infections, the first cases of B. venatorum babesiosis in Italy and Austria, which occurred in two asplenic men with Hodgkin’s disease and large B-cell lymphoma, respectively, were different in that the disease manifestations ranged from mild to moderately severe and both patients were cured after successful chemotherapy with clindamycin and/or quinine (Herwaldt et al., 2003). Likewise, infections with the newly recognised B. divergens-like parasite that occurred in Washington State and a case of babesiosis in Korea due to a babesia parasite closely related to ovine babesias and provisionally named KO1, took a more benign clinical course and the patients recovered after treatment.

Similar to the Italian and Austrian cases, clinical symptoms in the first German B. venatorum-infected patient included elevated body temperature, chills, anemia, weakness, fatigue, anorexia, and headache followed by jaundice and dark urine from hemoglobinuria (Häselbarth et al., 2007; Hildebrandt et al., in press). There was significant delay between the onset of symptoms and the initiation of specific chemotherapy. Such a delay can be fatal because patients can deteriorate due to congestive heart failure, intravascular coagulation, renal failure, and respiratory distress syndrome if left untreated (Homer et al., 2000; Mylonakis, 2001). Therefore, such patients, most of whom are immunocompromised or asplenic must be regarded as medical emergencies requiring immediate treatment to arrest hemolysis and to prevent renal failure (Gelfand, 2000; Homer et al., 2000). The recently described German case due to B. venatorum, however, was unique in that the patient remained seronegative for specific antibodies for several months and suffered from relapse after initial treatment. Moreover, re-treatment with atovaquone and azithromycin did not clear the parasite and low level parasitemia persisted for several months despite maintenance therapy with atovaquone, possibly due to the previous combined application of rituximab and prednisolone which have highly immunosuppressive effects (Häselbarth et al., 2007). Corticosteroids and depressed cellular immunity are associated with severe human babesiosis (Rosner et al., 1984; Meldrum et al., 1992; Telford and Maguire, 2006). In such cases, monitoring of parasitemia by blood smear examination and PCR analysis, and clinical long-term follow-up is important (Häselbarth et al., 2007; Hildebrandt et al., in press). Clinicians should be aware that in these patients relapse and persistence of the parasite may occur despite treatment (Hildebrandt et al., in press; Krause et al., 2008).

### Table 2

<table>
<thead>
<tr>
<th>Symptom</th>
<th>% of patients exhibiting the indicated symptoms</th>
<th>Babesiosis (n = 10)</th>
<th>LD (n = 214)</th>
<th>Both infections (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>60</td>
<td>49</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>60</td>
<td>42</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Erythema migrans (EM)</td>
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<td>85</td>
<td>62</td>
<td></td>
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<tr>
<td>Fever</td>
<td>80</td>
<td>42</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Sweats</td>
<td>20</td>
<td>11</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Chills</td>
<td>50</td>
<td>23</td>
<td>42</td>
<td></td>
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<tr>
<td>Myalgia</td>
<td>20</td>
<td>31</td>
<td>38</td>
<td></td>
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<tr>
<td>Anorexia</td>
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<td>14</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>50</td>
<td>36</td>
<td>27</td>
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<tr>
<td>Nausea</td>
<td>10</td>
<td>5</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Neck stiffness</td>
<td>30</td>
<td>21</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Multiple EM</td>
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<td>14</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>20</td>
<td>10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Sore throat</td>
<td>20</td>
<td>9</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>0</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>10</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Joint swelling</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**4.1. Pathogenesis and immunobiology**

Erythrocytic parasitism by babesia leads to anemia, hyperbilirubinemia, hemoglobinuria possibly followed by kidney failure, adult respiratory distress syndrome (ARDS), and central nervous system impairment (Hunfeld and Brade, 2004; Telford and Maguire, 2006). Upon
of immunity to *B. microti* lowered the fatality risk whereas way as well as CD4 and CD8 gene knockout in animals (Maguire, 2006). Antibody-derived immunity is less important pathogenetic cofactor for severe babesiosis, as demonstrated for example in a mouse model of *B. microti* infection because B-cell deficient mice remain less susceptible to infection, whereas T-cell receptor-deficient mice are readily infected (Clawson et al., 2002; Telford and Maguire, 2006). Immunological studies on mice also indicate an important role of CD4+ T cells in controlling parasitemia (Hemmer et al., 2000). This corresponds to the known difficulties of individuals with depressed cellular immunity to control persistent parasitemia (Kjemtrup and Conrad, 2000; Kjemtrup and Maguire, 2006; Häselbarth et al., 2007; Hildebrandt et al., in press). Similarly, depletion of host macrophages and natural killer cells increases susceptibility to infection (Aguilar-Delfín et al., 2003). Animal studies with *B. duncanii* show overwhelming production of pro-inflammatory cytokines such as TNF-α and interferon-γ suggesting that the pathobiology mainly results from the host response and not from the parasite itself. In humans, symptoms occur at parasitemias of less than 1% and on several *Babesia* spp. suggest that an excessive host immune response is an important pathogenetic cofactor for severe babesiosis, thereby resembling fulminate malaria due to *Plasmodium falciparum* except for cerebral symptoms (Krause et al., 2007; Gray and Weiss, 2008).

Correspondingly, genetic disruption of the TNF-α pathway as well as CD4 and CD8 gene knockout in animals lowered the fatality risk whereas γδ T-cell knockout mice and controls readily died after *B. duncanii* infection (Hemmer et al., 2000; Telford and Maguire, 2006). This suggests contribution of CD8+ T cells to the pathobiology of *B. duncanii* infections (Hemmer et al., 2000; Telford and Maguire, 2006). Antibody-derived immunity is less important as passive transfer of immune sera to SCID and nude mice fails to protect such animals against challenge with *B. microti* (Matsubara et al., 1993).

Other authors, however, demonstrated that some degree of immunity to *B. microti* and *B. rodhaini* can be transferred to cattle and mice with serum containing anti-babesia antibodies although it could not prevent infection or ultimate death (Mahoney, 1967; Abdalla et al., 1978). Whether such data hold true for human infection with newly recognised babesias requires further research. It is interesting to note though, that in a recently described *B. venatorum*-infection it took the patient 7 months to finally develop babesia-specific antibodies following prednisolone treatment and rituximab induced depletion of CD20 positive cells (Häselbarth et al., 2007). With the appearance of the antibodies the patient started clearing the parasites and smears turned negative. Six weeks later, PCR turned negative also. Although not for certain, the production of anti-babesia antibodies obviously contributed to clearing the parasite in this patient (Häselbarth et al., 2007). The hypothesis of a significant role of the humoral immune response for the successful control of babesia infections is further supported by the data presented in a recent case control study on immunocompromised patients that also identified rituximab as an important risk factor for persistent and relapsing babesiosis (Krause et al., 2008).

5. Laboratory diagnostics in human babesiosis

Besides a general lack of awareness, the major problem with diagnosing babesia infections in humans is that convenient and well evaluated diagnostic tools designed for rapid and reliable detection of such pathogens are not yet readily available to most routine diagnostic laboratories. Giemsa-stained slides are important in endemic areas where the diagnostic services are experienced. Outside endemic areas, however, Giemsa-stained blood smears are not regularly performed in patients with fever of unknown origin (Hildebrandt et al., in press). Moreover, automated blood analyzers cannot always reliably detect infected erythrocytes so that diagnosis of babesiosis can be missed (Bruckner et al., 1985; Hildebrandt et al., in press). Laboratory testing in apparent cases of human babesiosis may show non-specific findings such as elevated transaminases, alkaline phosphatases, unconjugated bilirubin, and lactic dehydrogenase. Normochromia, normocytic anemia, thrombocytopenia, and, occasionally, leucopenia may also be observed, probably owing to a TNF-mediated immune response similar to that seen in severe cases of malaria (Krause and Telford, 1999; Gelfand, 2000; Homer et al., 2000; Sharan and Krause, 2000; Mylonakis, 2001). A positive Coombs test in combination with hemolytic anemia and elevated procalcitonine levels is highly suspicious of babesiosis (Häselbarth et al., 2007; Hildebrandt et al., in press) and should prompt further diagnostic tests.

5.1. Direct detection of parasites

Thus far, microbiological diagnosis of human babesiosis in general has been based on the detection of the parasites in Giemsa-stained thin blood smears (Fig. 5). However, early in the infection the level of parasitemia in the non-immunocompromised host can be lower than 1% (Krause
et al., 1998; Gelfand, 2000; Homer et al., 2000; Sharan and Krause, 2000; Mylonakis, 2001). In these patients, serial blood smears must be investigated. On the other hand, high level parasitemia ranging from 50–80% may be seen in spleenectomized individuals (Kjemtrup and Conrad, 2000). In Giemsa-stained blood smears Babesia spp. appear as intra-erythrocyte ring forms or pyriform inclusions with light blue cytoplasm (Fig. 5). Malaria is the most important differential diagnosis as Plasmodium spp. may also show intraerythrocytic rings (Fig. 5). In malaria parasitic pigment (hemozoin) is usually detectable, but early parasitic stages may lack pigment and sometimes co-infections with both agents cannot be ruled out (Bush et al., 1990). In such cases, Babesia spp. may be easily misdiagnosed as Plasmodium spp. and vice versa (Fig. 5). This remains especially true in malaria endemic regions or in travelers returning from areas where malaria is known to occur but where babesiosis has never been reported before.

In B. microti infections erythrocytes often show several parasites that appear as rings, sometimes with incomplete closures. Basket-shaped merozoites (Fig. 5) can be approximately 1.5–2.5 μm in size. Although B. microti is considered a diverse species complex, parasites appear monomorphic in human blood smears (Telford and Goehert, 2004). In B. divergens and also in B. capreoli, and B. duncani infections, parasites are pleomorphic with accé forms, and paired pyriform ring forms (Fig. 5). Merozoites can be 1–3 μm in size. The morphology of intraerythrocytic B. divergens parasites (Fig. 5) is very similar to B. duncani, B. venatorum, and B. microti, except for the fact that in B. divergens and B. venatorum paired forms are typically found at an obtuse or diverging angle usually in the centre of human erythrocytes (Telford et al., 1993; Gorenflot et al., 1998) (Fig. 5). Tetrad forms are more common in B. microti infections but are still relatively rare (Gray and Weiss, 2008; Hildebrandt et al., in press). Consequently, although helpful for diagnostic purposes, microscopy of parasites in blood smears without additional molecular analysis of the pathogen is not reliable for species identification.

5.2. Detection of Babesia spp. by animal inoculation and in vitro culture

Babesiosis can also be confirmed by directly inoculating 1.0 ml ETDA whole blood into the peritoneum of golden hamsters, jirds, or mice (Krause and Telford, 1999; Homer et al., 2000; Sharan and Krause, 2000). Blood smears of the inoculated animal usually become positive within 2–4 weeks. This is much too long to wait in acute human cases. Moreover, parasitemia in infected animals can be transient. Therefore, blood smears need to be monitored at least daily which is time-consuming. As such, animal inoculation is not useful in emergency situations but still has its place in the diagnosis of cryptic chronic babesia infections and especially for genomic studies (Hildebrandt et al., in press). Similarly, bioassays are not suitable for parasites that do not provoke parasitemia in laboratory animals (Telford and Maguire, 2006). For example, jirds are highly susceptible to infection with B. divergens, but could not be infected by inoculation of human blood containing B. venatorum in all three cases reported so far (Herwaldt et al., 2003; Häselbarth et al., 2007). In cases involving parasites where suitable animal hosts are not yet known, such Babesia spp. can be cultivated directly from blood by in vitro culture using autologous erythrocyte preparations (Malandrin et al., 2004; Bonnet et al., 2007). Such cultures then need to be monitored for parasitemia by examination of blood smears. If positive, parasites can be adapted to culture in blood from appropriate animal species after several passages (Chauvin et al., 2002). These laboratory methods, however, are not readily available in most routine diagnostic laboratories facing a first case of babesiosis.

5.3. Indirect detection of babesia infection by antibody testing

IFAT for B. microti is the only serological test for human babesiosis that has so far been standardised to any extent (Homer et al., 2000; Kjemtrup and Conrad, 2000; Hunfeld and Brade, 2004). Studies suggest B. microti-specific IFAT to be specific, sensitive, and reproducible with reported test specificities ranging from 90% to 100% (Krause et al., 1994; Gelfand, 2000; Homer et al., 2000; Hunfeld et al., 2002). Moreover, titres from 1:32 to 1:160 are diagnostic and specific, with positive predictive values of 69–100% and negative predictive values of 96–99% (Krause et al., 1994). For IFAT based on B. divergens antigen and the more recently encountered parasites such data are lacking. A B. duncani IFAT was used to screen potentially infected individuals (Kjemtrup and Conrad, 2000). However, a positive cutoff titer has not been established.

Specific IgG antibodies are found in patients with acute and chronic babesia infections. The detection of IgM antibodies (>1:64) may indicate acute infection even in the absence of demonstrable parasitemia but is less specific than IgG antibody testing (Homer et al., 2000; Hunfeld et al., 2002). In immunocompromised patients with prolonged prepatent periods a significant delay of seroconversion may occur (Telford and Maguire, 2006). After infection titers persist from 13 months up to 6 years (Persing et al., 1995).

Our knowledge of the serodiagnosis of human babesiosis refers to B. microti-infected individuals mainly because observations involving other Babesia spp. are based on case reports rather than on clinical studies (Hunfeld and Brade, 2004). In acute cases, whether caused by B. divergens-like parasites or B. microti in immunocompromised patients, IFAT are not reliable because seronegative results at disease onset are common and may delay treatment (Persing et al., 1995; Häselbarth et al., 2007; Hildebrandt et al., in press). Nevertheless, IFAT are important in demonstrating seroconversion to retrospectively confirm the diagnosis by
examining paired acute and convalescent sera (Telford and Maguire, 2006). Cross-reactivity between *B. venatorum* and *B. divergens* exists, and *B. divergens* IFAT proved useful to detect seroreactivity in patients infected with *B. venatorum* (Herwaldt et al., 2003; Häselbarth et al., 2007). Low-titer cross-reactivity (predominantly for IgM) can be observed with *B. microti* antigen (Häselbarth et al., 2007). Similarly, in several of the reported *B. duncanii* infections from California and Washington State testing of patients’ sera demonstrated cross-reactivity with *B. conraudi* and *B. microti* although the height of titers depended on disease duration and type of antigen used (Kjemtrup and Conrad, 2000). In a patient from Washington State infected with a *B. divergens*-like organism, however, the patient’s serum did not react in *B. microti* or *B. duncanii* IFAT but showed marked reactivity to *B. divergens* (Herwaldt et al., 2004). Until more information becomes available on the appropriate isolates and antigens, concurrent *B. microti* and *B. divergens* IFAT testing of sera from patients with suspected babesiosis is suggested for endemic areas, because such tests are readily available and may cover the antibody response in patients infected with newly recognised organisms.

Test sensitivity and specificity can change significantly as a function of the prevalence of the pathogen in the population (Rothman and Greenland, 1998). Therefore, test results for *Babesia* spp. must be interpreted with caution outside endemic areas (WHO, 1995; Hildebrandt et al., in press). Sera should be tested for IgG antibodies first and only positive sera should then be tested for anti-babesia IgM-antibodies indicating a more recent infection. Such a stepwise approach can minimise false-positive IgM-IFAT results in areas with low prevalence of *Babesia* spp. infections (Hunfeld et al., 2002). IFAT readings depend on the investigator, the conjugate used, and the antigen preparation. Photomicrographs (Fig. 6) can aid in the correct reading of such tests, but the fluorescence pattern may vary slightly with the type (anti-*B. divergens*, anti-*B. microti*, or anti-*B. venatorum*) and the class (IgG or IgM) of the antibodies. Diagnostic reading of samples and controls (Fig. 6)
should start with a magnification of 400× and, in case of any doubt, be confirmed by checking the fluorescence pattern with a magnification of 1000× with oil (Fig. 6). This is done to exclude rare non-specific fluorescence of babesial antigen which mainly involves the apex of the parasite (Hildebrandt et al., in press). False positive results have been described in Plasmodium spp. and Toxoplasma gondii infections, and in lupus erythematosus or rheumatoid arthritis (Homer et al., 2000; Hildebrandt et al., in press).

ELISA and immunoblot can be used to confirm IFAT results and to identify babesia-positive carriers. ELISA detection of anti-Babesia spp. antibodies is less subjective than that of IFAT and can be automated. A peptide-based ELISA has recently been described using secreted antigens of babesia parasites (Homer et al., 2003) but, compared with IFAT, greater amounts of antigen are required and test specificity depends on optimised blocking conditions and purification of the antigens (Zintl et al., 2003). Moreover, tests such as ELISAs and immunoblot are not yet sufficiently standardised for routine application in diagnostic laboratories (Ryan et al., 2001; Hunfeld and Brade, 2004; Gray and Weiss, 2008).

5.4. New tools for a more rapid molecular biological diagnosis of Babesia spp. infection

The development of sensitive and specific multiplex PCR assays may be an important future improvement in the laboratory diagnosis of human disease following single or multiple infection with tick-borne pathogens (Hildebrandt et al., in press). Detection may then be attempted by use of a single diagnostic test covering agents such as Babesia spp., Borrelia spp., Anaplasma spp., and Rickettsia spp. (Swanson et al., 2006). This seems attractive because patients suffering from Lyme disease, human babesiosis, human anaplasmosis, or most other tick-borne diseases can all present with relatively non-specific influenza-like illnesses complicating any clinical differential diagnostic considerations (Swanson et al., 2006). With the development of more sensitive molecular techniques for diagnosing fastidious organisms like Babesia spp. and Anaplasma spp., PCR is increasingly relied on to detect such rare pathogens in patients with suspected infection but low parasite loads and negative blood smears (Hunfeld and Brade, 2004; Swanson et al., 2006; Hildebrandt et al., in press).

In patients with suspected babesiosis, PCR results for Babesia spp. can be available within one day and as few as three parasites in 100 μl blood can yield a positive result (Persing et al., 1992; Armstrong et al., 1998). Studies have shown that PCR targeting the 18S rDNA (Fig. 6) is more sensitive than and equally specific as blood smear evaluation or bioassay in the detection of acute Babesia spp. infections (Homer et al., 2000). For purposes of quality control, the 12S rDNA of ticks or the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene can be used as housekeeping genes to control for high quality of DNA extraction from ticks or human blood, respectively (Barber et al., 2005; Casati et al., 2006). As mentioned above, such new rapid and sensitive PCR methods are also attractive in cases of human babesiosis where the effectiveness of antimicrobial therapy has to be monitored. Detection of babesia DNA indicates a parasitemia and persistent DNA detection clearly points to ongoing infection (Krause et al., 1998; Homer et al., 2000; Häselbarth et al., 2007). Nevertheless, PCR suffers from limitations such as the difficulties to distinguish between nucleic acids from viable and non-viable organisms and, therefore, reversion to PCR negativity may significantly lag behind a clinical response to antimicrobial therapy (Persing, 1993). In this context, the major advantage of detecting RNA instead of DNA targets is a more direct correlation of rRNA and specific gene transcript quantities with growth of the organism. This is why a more sensitive indicator of therapeutic response in the near future may be to quantitatively monitor for RNA target sequences such as rRNA rather than specific DNA because of their higher turnover (Hildebrandt et al., in press). Whether PCR indeed can be useful in monitoring the success of specific treatment in patients with Babesia spp. infection and prolonged persistent parasitemia, however, awaits further evidence from clinical studies (Häselbarth et al., 2007). For purposes of epidemiology and phylogeny, PCR technology and sequence analyses of the amplicons proved powerful in more exact species identification. Because a high degree of 18S rDNA sequence identity exists between many Babesia spp., the complete 18S rRNA gene (about 1700 bp) should always be analysed especially in newly recognised organisms (Gray, 2006; Hildebrandt et al., in press).

6. Treatment and outcome of babesiosis

Although babesiosis is a common disease in domestic animals and many drugs have been developed for treatment, none have been adopted for routine use in humans, primarily because of licensing problems. Current knowledge on clinical course and treatment of human babesiosis is mainly derived from clinical data on B. microti and B. divergens-infected patients. Several drugs have been tested in vitro against a variety of babesia species, but results for pyrimethamine, tetracyclines, primaquine, and pentamidines have varied. Moreover, their use in humans at appropriate dosage is associated with significant side effects and treatment failure. These drugs, therefore, have not been applied consistently in the clinical context (Pudney and Gray, 1997; Homer et al., 2000; Gray and Weiss, 2008). The anti-malaria drug chloroquine is regarded as ineffective against Babesia spp. (Homer et al., 2000; Zintl et al., 2003). This remains true also for antimalarials such as mefloquine and artemisinin at least in the animal model (Wittner et al., 1996; Marley et al., 1997). Imidocarb is highly effective in vitro and has been used successfully to treat two Irish patients infected with B. divergens (Zintl et al., 2003) but has not been approved for general use in humans (Mylonakis, 2001; Vial and Gorenflot, 2006). Since
treatment of human babesiosis with a combination of quinine and clindamycin (Table 3) for seven to 10 days is applied, disease outcome has dramatically improved (Rowin et al., 1982; Wittner et al., 1982). Quinine can be exchanged for quinidine and administered intravenously along with clindamycin, if necessary (Vial and Gorenflot, 2006). The mortality rate for clinically apparent B. microti infections is ~5% and most infections resolve on their own (Meldrum et al., 1992). Chemotherapy, thus, is indicated only in moderately to severely ill cases with such infections. Individuals with B. divergens infection, however, are usually regarded as medical emergencies and require immediate treatment to arrest hemolysis and prevent renal failure. Many severe B. divergens infections in the past ended fatally with general organ failure 4–7 days after the manifestation of hemoglobinuria, and outcome data in severely ill individuals with asplenia suggest a mortality rate of 42% (Gorenflot et al., 1998; Homer et al., 2000; Kjemtrup and Conrad, 2000; Zintl et al., 2003; Leiby, 2006). The in vivo effectiveness of quinine, however, has always been questioned and the drug related toxicity of this drug is significant (Brasseur et al., 1996). It is noteworthy that in recent years, a more favourable outcome was increasingly reported in B. divergens-infected patients with severe complications even though they were not treated with a full course of quinine and clindamycin, mainly because of quinine side effects (Denes et al., 1999; Corpelet et al., 2005). These findings underscore the impact of improved adjunctive measures provided by modern intensive care medicine, including exchange transfusion which is usually reserved for extremely ill individuals, i.e., those with massive hemolysis, asplenia, immunosuppression, and parasitemia of more than 10% (Mylonakis, 2001; Zintl et al., 2003), but has been recommended for all emergency cases involving B. divergens (Gorenflot et al., 1987).

This measure is particularly helpful because in humans babesia have no exo-erythrocytic stages. Therefore, the removal of parasitised erythrocytes is curative. In addition,
anemia is corrected and toxic and harmful metabolites are removed. Clindamycin monotherapy has been proposed when accompanied by such adjunctive measures (Brasseur et al., 1996; Denes et al., 1999; Corpelet et al., 2005). It is notable, however, that in an experimental B. divergens/gerbil model, atovaquone alone proved more effective than the best animal antibabesial, imidocarb dipropionate, (Pudney and Gray, 1997) and perhaps this drug should also be considered for emergency treatment of B. divergens-like infections (Table 3).

For treatment of B. microti infections, animal studies showed that azithromycin in combination with quinine (Weiss et al., 1993), azithromycin with atovaquone (Witten et al., 1996), and atovaquone with clindamycin (Gray and Pudney, 1999), were all effective. More recently, randomized trials in humans infected with B. microti showed that atovaquone plus azithromycin therapy was as effective as the standard quinine/clindamycin combination and there were fewer side effects (15% versus 72%) (Krause et al., 2000). In view of the low risk of side effects associated with atovaquone/azithromycin and the possibility of parasite persistence and occasional recrudescence, it has been argued that all patients diagnosed with B. microti infection be treated with this drug combination. In severe cases similar adjunctive measures to those used for B. divergens infections may be necessary. In HIV patients and in otherwise immunocompromised individuals substantially higher dosage regimens and longer treatment schedules may be required for clearing the infection (Kjemtrup and Conrad, 2000; Krause et al., 2008). Little is known so far about the exact in vitro susceptibilities to potential antibabesia drugs of the newly recognised Babesia spp. such as B. duncani, B. venatorum or the B. divergens-like organisms from the US, though the cottontail rabbit babesia thought to be responsible for cases in Kentucky and Missouri has now been adapted to an in vitro system (Spencer et al., 2006). Having reviewed the currently available information on the antimicrobial susceptibility data and in vivo treatment outcome available from in vitro susceptibility studies, case reports, and clinical investigations (Homer et al., 2000; Herwaldt et al., 2003; Vial and Gorenflo, 2006; Häselbarth et al., 2007) published so far, it appears that there is no convincing scientific evidence for any clinically relevant differences in the susceptibilities of pathogenic Babesia spp. with regard to the therapeutic agents commonly used to treat human disease, though it is known that in animal models B. microti-like infections are more difficult to treat than those caused by Babesia species s.s., such as B. divergens (Gray and Pudney, 1999). The clindamycin and quinine combination appears to be the regimen of choice for human cases, despite problems with side effects and the requirement for aggressive adjunctive procedures in rapidly fulminating infections. Most of the recent cases of human babesiosis caused by previously unknown babesias have responded well to this drug combination (Quick et al., 1993; Persing et al., 1995; Saito-Ito et al., 2000; Kjemtrup et al., 2002; Herwaldt et al., 2003, 2004; Conrad et al., 2006; Wormser et al., 2006; Häselbarth et al., 2007; Hildebrandt et al., 2007; Kim et al., 2007). However, problems with speed of response to therapy and also parasite persistence remain (Krause et al., 1998; Häselbarth et al., 2007), emphasising the importance of closely monitoring the course of parasitemia, the necessity for long-term follow-up in such patients, and the need for further antibabesial drug research.

7. Babesia spp. and blood products

In recent years, transfusion medicine massively focused on HIV, hepatitis B, and hepatitis C virus, whereas patho-

Table 3
Commonly used effective drugs for the treatment of human babesiosis (according to Telford and Maguire, 2006; Vial and Gorenflo, 2006; Wormser et al., 2006)

<table>
<thead>
<tr>
<th>Drug (generic name)</th>
<th>Regular single dose (Adults, 70 kg)</th>
<th>Application</th>
<th>Dosage regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinine</td>
<td>650 mg</td>
<td>p.o.</td>
<td>3 times daily</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>600 mg</td>
<td>p.o., i.v.</td>
<td>3 times daily</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>500 mg/1st day, 250 mg thereafter a</td>
<td>p.o.</td>
<td>Once daily</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>750 mg</td>
<td>p.o.</td>
<td>Twice daily</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>200 mg</td>
<td>p.o.</td>
<td>Once daily</td>
</tr>
<tr>
<td>Drug (generic name)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinine</td>
<td>8 mg      c</td>
<td>p.o.</td>
<td>3 times daily</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>7–10 mg      a</td>
<td>p.o., i.v.</td>
<td>3 times daily</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>10 mg/1st day, 5 mg/day thereafter a</td>
<td>p.o.</td>
<td>Once daily</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>20 mg/day        f</td>
<td>p.o.</td>
<td>Twice daily</td>
</tr>
</tbody>
</table>

a In immunocompromised patients higher doses (600–1000 mg/day) may be required.
b In immunocompromised patients higher dose may be required.
c Maximum: 650 mg per dose.
d Maximum: 600 mg per dose.
e Maximum: 250 mg per dose.
f Maximum: 750 mg per dose.
gens such as babesia that represent a more scattered but ongoing blood safety risk continue to “fly under the radar” (Leiby, 2006). Characteristics of Babesia spp. clearly favor successful transmission via blood products: First, once they are introduced into a mammalian host they infect and replicate in red blood cells. Infected blood or residual erythrocytes in platelet units, therefore, represent suitable vehicles for transmission to transfusion recipients (Homer et al., 2000; Leiby, 2006). Second, infected hosts may suffer from long-lasting subclinical parasitemia that is hard to detect and infected donors may be at risk for transmitting babesia for extended periods of time (Krause et al., 1998). Lastly, based on data obtained from the analysis of transfusion-transmitted infections, parasites can survive for 21–35 days under standard blood storage conditions (Mintz et al., 1991; Eberhard et al., 1995; Leiby and Gill, 2004). So far, about 60 cases of transfusion-transmitted babesiosis have been reported in the literature (Leiby, 2006). Transfusion-transmitted cases involved blood recipients ranging in age from neonate to 79 years and most of the cases were attributed to B. microti (Herwaldt et al., 1997; Kjemtrup et al., 2002; Pantanowitz et al., 2002; Della-Giustina et al., 2005; Leiby, 2006; Hildebrandt et al., 2007). Observed incubation periods for these cases ranged from 1 to 9 weeks. The exact number of cases, however, is difficult to assess because there are no mandatory reporting systems and frequently, cases of new infections are not published because they are not considered novel or noteworthy (Leiby, 2006). Few reliable estimates of transmission risk are currently available with regard to transfusion-acquired babesiosis. In two recent studies from endemic areas in Connecticut the risk of acquiring B. microti from a unit of red blood cells was reported to range between 1 in 601 and 1 in 1800 and the risk after transfusion of a unit of platelets was 0 in 371 patients investigated (Gerber et al., 1994; Leiby, 2006). Increasing evidence suggests that the geographical range of distribution of Babesia spp. appears to be greater than initially thought and on a worldwide scale progressively more transfusion-transmitted cases are reported from areas where such infections were not known to occur before (Herwaldt et al., 1997; Saito-Ito et al., 2000; Kjemtrup et al., 2002; Leiby, 2006; Hildebrandt et al., 2007). Moreover, newly recognised organisms such as B. duncanii in the US and B. microti-like parasites in Canada, Japan, and Germany are increasingly implicated in transfusion-transmitted cases, particularly, in highly susceptible patients who are immunocompromised, asplenic, and have received large amounts of blood (Saito-Ito et al., 2000; Kain et al., 2001; Leiby, 2006; Hildebrandt et al., 2007, in press). Clearly, the successful detection and characterization of such blood-derived parasites depends on medical awareness and the availability of sophisticated diagnostic methods such as PCR technology, serological tests, and culture techniques (Saito-Ito et al., 2000; Kain et al., 2001; Hildebrandt et al., 2007, in press). As such the currently reported distribution and frequency of transfusion-acquired cases of babesiosis is probably not representative and may instead be regarded as the tip of the iceberg when assessing the true dimension of the problem (Hildebrandt et al., in press).

8. Prevention and vaccines

With increasing outdoor activities more people are exposed to ticks. However, I. ricinus nymphs can be easily overlooked and most infested persons do not remember a tick bite (Leiby, 2006; Hildebrandt et al., in press). Unfortunately, antibiotic prophylaxis for human babesiosis has not been established and live vaccines are available for animal babesiosis only (de Waal and Combrink, 2006). There is a need for effective recombinant vaccines against apicomplexan pathogens including Babesia, Plasmodium, or Theileria spp. but development has proved unsuccessful so far (de Waal and Combrink, 2006). Therefore, prevention of babesia infection mainly relies on wearing appropriate clothing, application of repellents (DEET, permethrin), and the prompt removal of any ticks (Gray and Weiss, 2008). With regard to prevention of transfusion-acquired babesiosis no licensed techniques are currently available to prevent or reduce transmission of Babesia spp. (Zavizion et al., 2004; Leiby, 2006). PCR- or antibody screening of donors is not yet established because molecular and serological diagnostics are not readily available. Moreover, regular blood donor screening is not cost-effective even in the known geographical areas of high endemicity in the US (Leiby, 2006). In summary, protective and preventive measures against zoonotic babesia, to date, are limited and must focus on clinical awareness in susceptible populations after tick exposure or after transfusion of blood products (Hunfeld and Brade, 2004; Leiby, 2006; Hildebrandt et al., in press) to readily diagnose and treat babesia infections.

9. Conclusions

The diversity of Babesia spp. and the ubiquity of ticks point to a high potential for Babesia spp. to further emerge as zoonotic pathogens on a worldwide scale. Concordantly, several new babesia parasites have recently been recognised and cases of human babesiosis have been increasingly reported in geographical areas where the presence of Babesia spp. in enzootic cycles was obvious for decades but where the risk for humans of acquiring Babesia spp. either from ticks or from human blood products was not known before (Hildebrandt et al., in press). Most importantly, human babesiosis may become progressively more important for the steadily increasing population of highly susceptible immunocompromised individuals and should be regularly considered in the differential diagnosis of infection or fever of unknown origin, especially, in splenectomized patients and in individuals with recent transfusion of blood products. The need for better diagnostics is obvious and laboratories require broad access to reliable tests such as IFAT and PCR for timely diagnosis in suspected cases.
Better molecular detection and strain typing of parasites is also necessary to clarify the epidemiology of zoonotic Babesia spp. and whether their virulence or enhanced transmissibility is potentially strain-dependent. Further studies are therefore urgently needed to better characterize the distribution and medical relevance of these pathogens in many parts of the world.

References


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