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Babesia sp.: Emerging Intracellular Parasites in Europe

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Abstract

The emergence of Lyme borreliosis as the most prevalent arthropod disease of humans in the temperate northern hemisphere has resulted in renewed interest in human babesiosis, transmitted by the same tick vectors. The advent of new molecular tools has made possible a reappraisal of the main parasites involved (*Babesia divergens* in Europe and *Babesia microti* in the USA). *B. divergens* is probably restricted to European cattle, though there are several nearly identical species. *B. microti* occurs as a world-wide species complex rather than as a single species, and although both phenotypic and genotypic features lend support to suggestions that zoonotic *B. microti* may occur in Europe, convincing medical evidence is lacking. Comparative biology should support genetic data in taxonomic studies of these parasites.

Key words: human babesiosis, *B. divergens* and *B. microti* taxonomy

Introduction

Babesiosis is caused by tick-borne intraerythrocytic protozoan parasites of the genus *Babesia* and more than 100 species have now been recorded in mammal hosts (Telford *et al.*, 1993). Although these parasites are best known as a cause of disease in animals, particularly in the tropics and subtropics, increasing attention is being paid to zoonotic human babesiosis. Most human cases are thought to be caused by *B. microti*, a rodent parasite (in the USA), and *B. divergens*, a bovine parasite (in Europe). However, several other species are involved, mostly of unknown host origin (Kjemtrup and Conrad, 2000; Homer *et al.*, 2000), and it is only now, with the increasing availability and use of molecular tools, that real progress is being made in characterising zoonotic *Babesia* species. This review will consider *B. divergens* and *B. microti* in relation to their taxonomic identity and their possible roles in human disease in Europe.

Babesia divergens

Biology and pathology. *B. divergens* probably occurs wherever cattle and the vector, *Ixodes ricinus*, coexist. The precise humidity requirements of *I. ricinus* restrict it to areas with a moisture-saturated microhabitat at the base of permanent herbage in forest woodland, on rough hill-land and damp low-lying meadows. Since most of the continental-European habitat for *I. ricinus* is woodland and cattle make limited use of such habitat, the distribution of *B. divergens*-infected ticks shows incomplete overlap with the overall distribution of the vector. Countries with the highest incidence of the disease appear to be those where significant tick populations occur in open hill-land or meadows, for example Ireland (Gray and Harte, 1985), and where woodland frequently abuts cattle pasture, for example France (L'Hostis and Seegers, 2002). The parasite is transmitted transovarially and all active feeding-stages, larva, nymph and adult female, are infective. Only adult females can acquire the infection from the host. Ticks are important reservoirs of infection because the infection can persist, even in the absence of cattle, for more than one generation. Cattle are also reservoirs and subclinical infections may occur for more than two years. Although the Mongolian gerbil, *Meriones unguiculatus,* is a highly susceptible laboratory host, there is no evidence that wild rodents can become infected and serve as reservoir hosts (Zintl *et al.*, 2003).

Many cases of bovine babesiosis are relatively mild, but case fatality rates may be as high as 10% despite treatment (Gray and Harte, 1985). Severe cases present with high fever, anaemia, anorexia, depression,

weakness, cessation of rumination and an increase in respiratory and heart rate. Parasitaemias may rise to between 30 and 45% causing extensive erythrocyte destruction. The resulting haemoglobinuria, which gives the disease the colloquial name of redwater fever, is frequently the first clinical sign detected and may manifest at parasitaemias as low as 3%. Fatal cases usually result from cardiac failure, or hepatic and renal insufficiency. Human patients show most of the symptoms of the acute bovine disease, including haemoglobinuria. All the recorded cases (>30) have occurred in splenectomised individuals, frequently with further immunocompromising conditions, and are characterised by rapid fulmination (Zintl *et al.*, 2003). Current recommended treatment consists of exchange transfusion (2 or 3 exchanges) followed by treatment with clindamycin (sometimes with quinine) (Gorenflot *et al.*, 1998). Though the anti-malarial atovaquone appears to be a much more effective drug against *B. divergens* (Pudney and Gray, 1997), it has not yet been used in human cases.

Identity of *B. divergens.* In recent years DNA detection methods have shown that closely related but hitherto uncharacterised parasites can cause symptoms characteristic of *B. divergens* infection. Two European cases, one Italian and one Austrian, were found to have been caused by a babesia (EU1) that on phylogenetic analysis of the sequenced 18S rRNA gene, clusters with *B. odocoilei,* a parasite of American white-tailed deer (*Odocoileus virginianus*). *B. odocoilei* has not been recorded in Europe, but another babesia, also apparently related to *B. odocoilei* and differing from EU1 by only one base was reported from *I. ricinus* in Slovenia (Duh *et al.*, 2001) (Table I). Conversely, although *B. divergens* has been regarded as exclusively European, very similar parasites have been identified in three acute cases of human babesiosis in the USA. In a fatal case that occurred in Missouri, the parasite (MO1) was found to cluster with *B. divergens.* A small fragment (144 bases) of the 18S rRNA gene was sequenced initially and showed 100% homology with *B. divergens*, but the authors nevertheless concluded that this parasite was probably not identical to *B. divergens.* Subsequent sequencing of the whole gene supported this view, though it is clearly very closely related (Table I). The second case occurred in Kentucky and was described as "*B. divergens*" since the 18S rDNA sequence only differed by 3 bases (99.8% homology) (Beattie *et al.*, 2002). A third case from Washington State (Herwaldt *et al.*, 2004) was found to differ from *B. divergens* by 8 bases (99.5% homology, though comparison with the partial sequence of the Purnell strain U13670 gives a 6-base difference *i.e.* 99.6% homology – Table I). In this case the authors refrained from designating the parasite *B. divergens.* This latter research group established, by resequencing the whole 18S rRNA gene of the three original GenBank depositions (U13670, Z48751 and U07885), that all are in fact identical with respect to this gene (100% homology) (Slemenda *et al.*, unpublished, in Herwaldt *et al.*, 2004). The only accurate sequence for the 18S rRNA gene among these original depositions is for the Purnell strain (U16370). This finding puts the identity of some parasites isolated from human cases and described as *B. divergens* (*e.g.* from Portugal, Centeno-Lima *et al.*, 2003; from Madeira, Olmeda *et al.*, 1997; from Kentucky, Beattie *et al.*, 2002) in serious doubt (Table I).

A few *B. divergens*-like parasites from animals other than cattle and humans have been described. A parasite isolated from Scottish red deer (*Cervus elaphus*) was morphologically and serologically (IFA) identical to *B. divergens*, but did not infect splenectomised cattle and was therefore described as *B. capreoli* (Adam

Table I
Babesia divergens 18S rRNA gene homology (EMBL-EBI ClustalW analysis) of selected isolates estimated from GenBank sequences, with reference to the Purnell strain (GenBank Accession number U16370)

Strain	Accession No.	Base pairs compared	% homology	Source	Author
Madeira	AF435415	309	99.7	Human	Olmeda *et al.*, 1997
MO1, Missouri, USA	AY048113	1724	99.7	Human	Slemenda *et al.*, 2001
Washington State, USA	Y274114	1724	99.6	Human	Herwaldt *et al.*, 2004
Portugal	AY048113	1712	99.2	Human	Centeno-Lima *et al.*, 2004
Reindeer, UK	AY098643	1712	99.8	Reindeer	Langton *et al.*, 2003
Deer, Slovenia	AY572456	1724	99.6	Red, roe deer	Duh et al., unpubl.
Rabbit, Nantucket, USA	AY144688	1166	99.7	Cottontail rabbit	Goethert and Telford, 2003
EU1 Slovenia	AY553915	1727	98.1	*I. ricinus*	Duh *et al.*, 2004
EU1 Austria, Italy	AY046575	1723	98.2	Human	Herwaldt *et al.*, 2003
Purnell-2 *B.divergens*	AY046576	1664	100.0	bovine	Herwaldt *et al.*, 2003

et al., 1976). Similarly a parasite isolated from sika deer (*Cervus nippon*) in Ireland (Gray *et al.*, 1990) was shown by the same criteria to be identical to *B. divergens,* but failed to infect splenectomised cattle or gerbils (*Meriones unguiculatus*), a laboratory host that is highly susceptible to bovine isolates of *B. divergens* (L'Hostis and Chauvin, 1999). It is tempting to conclude that these parasites are the same as a parasite sequenced from red and roe deer (*Capreolus capreolus*) in Slovenia (Duh *et al.*, unpublished), which is not identical to *B. divergens* (Table I). A similar babesia was isolated from reindeer (*Rangifer tarandus*) in the UK (Langton *et al.*, 2003) and this too was uninfective to gerbils, though indistinguishable from *B. divergens* morphologically and serologically. In this case the 18S rRNA gene was found to differ from the Purnell isolate GenBank sequence by 4 bases (99.8% homology); a very small difference but possibly enough, together with non-infectivity to gerbils, to differentiate it from bovine isolates of *B. divergens.* The sequence also shows small differences from that obtained from roe and red deer in Slovenia (Duh *et al.*, unpublished). The EU1-like parasite detected in Slovenian ticks (Duh *et al.*, 2001 differs from that obtained from red/roe deer and from the UK reindeer by at least 27 bases (98.4% homology), so is certainly a separate species. Lastly, an intriguing paper (Goethert and Telford, 2003) on transmission of "*B. divergens*" among American cottontail rabbits (*Sylvilagus floridanus*) describes a parasite reported by the authors as showing 100% 18S rDNA homology with the Kentucky human isolate (Beattie *et al.*, 2002) and only 3 base differences with the bovine *B. divergens* Purnell strain (99.8% homology). It seems likely that this parasite can be infective for humans, but in view of even the small difference shown from the type-bovine *B. divergens* isolate (Purnell strain), together with lack of information on its infectivity to cattle or gerbils, it is perhaps too early to synonymise it with European bovine *B. divergens*. Its apparent identity with the Kentucky isolate (for which no sequence has yet been deposited in GenBank) allows the Kentucky, Washington and MO1 isolates to be compared. The Kentucky and Washington isolates are not identical (99.6% homology), but there is 100% 18S rDNA homology between the partial sequence from the cottontail rabbit isolate (and therefore the Kentucky isolate) and the MO1 isolate from Missouri. Although this finding is based on partial 18S rDNA sequences there may be two rather than five *B. divergens*-like species in the USA.

Babesia microti

Biology and pathology. Although assigned to the same genus, *B. divergens* and *B. microti* appear to be only distantly related, and *B. microti* may be nearer to the genus *Theileria* (Homer *et al.*, 2000). *B. microti* has long been regarded as a parasite of small rodents but morphologically identical species have been reported from many hosts throughout the world (Goethert and Telford, 2004). Human infections caused by these parasites are much less widespread. The majority of cases occur on the north-eastern seaboard of the USA (Kjemtrup and Conrad, 2000) but the disease has also been reported from Japan (Saito-Ito *et al.*, 2000). Infected rodents occur throughout Europe but so far no confirmed human cases have been recorded; this is addressed in more detail below.

Unlike cases of *B. divergens*, *B. microti*-infection can give rise to disease in spleen-intact patients, manifesting as malaise, myalgia, anorexia and mild fever. In splenectomised and elderly patients the disease is likely to be more severe, resembling cases of *B. divergens* infection and characterised by haemoglobinuria, splenomegaly, hepatomegaly and jaundice (Kjemtrup and Conrad, 2000). Even in mild cases parasitaemia may persist for months or even years after treatment (Krause *et al.*, 1998). The infection is transmitted transstadially by ticks of the *I. ricinus* complex and human infections are acquired in habitats where both small rodents and ticks are present, for example mixed woodland.

B. microti is much less susceptible than *B. divergens* to antibabesials, reflecting the taxonomic differences referred to earlier. For example, atovaquone, the drug of choice (in combination with azithromycin) for *B. microti*, (Krause *et al.*, 2000) was estimated to have an ED_{50} more than 18 times that for *B. divergens* (Gray and Pudney, 1999). Co-infection with *B. microti* is thought to exacerbate Lyme borreliosis (caused by the spirochaete *Borrelia burgdorferi* sensu lato, which shares the same habitat and is transmitted by the same vectors) (Krause *et al.*, 1996). Treatment problems perceived to arise from co-infections of *B. microti* and *B. burgdorferi* s.l. appear to be behind the recent use of the anti-malarials artemesia and mefloquine in "chronic" LB patients (Horowitz *et al.*, 2000), despite the fact that no anti-babesial activity for these two drugs was evident in earlier laboratory studies (Marley *et al.*, 1997).

There is no firm evidence that any cases of human babesiosis have resulted from infection with European *B. microti.* One suggested explanation is that the main vector is the rodent-specific tick, *I. trianguliceps* (Homer *et al.*, 2000; Kjemtrup and Conrad, 2000). However, at least one strain has been shown to be transmitted by

I. ricinus (Gray *et al.*, 2002), and *B. microti* has been detected in *I. ricinus* specimens collected from vegetation (Duh *et al.*, 2001; Skotarczak and Cichocka, 2001; Foppa *et al.*, 2002; Kalman *et al.*, 2003). Serological evidence for human infection with *B. microti* exists (Krampitz *et al.*, 1986; Hunfeld *et al.*, 2002; Foppa *et al.*, 2002) but no isolations of parasites have been made from human patients despite speculation that *B. microti* infection may underlie atypical presentations of Lyme borreliosis. The establishment of *B. microti* as a European zoonosis is possibly only a matter of time (see next section) and indeed a recent publication (Meer-Scherrer *et al.*, 2004) claims to report the first autochthonously acquired case in Europe. However, there are many unconvincing aspects of this case. The clinical presentation was persuasive, but immunofluorescence assay titres were very low, some positive PCR results were obtained but the product was not sequenced, the PCR was negative at a time when parasites were reportedly observed in blood smears, and objects identified in photographs as parasites appear to be platelets.

The zoonotic status of *B. microti* in Europe must therefore remain open to question. Future attempts to identify human cases of *B. microti* infection in Europe should include blood transfer to susceptible laboratory hosts, such as hamsters or gerbils, and also the sequencing of any PCR products obtained.

Identity of *B. microti.* *B. microti* has long been regarded, based on morphological and host characteristics, as a single species. However, molecular techniques have recently provided new insights and it is evident that *B. microti* consists of a genetically diverse species complex. Goethert and Telford (2004) analysed the 18S rDNA and beta-tubulin genes of isolates of *B. microti*-like parasites from the USA (including Alaska), Switzerland, Spain and Russia, from a variety of vertebrate hosts (humans, voles, woodmice, shrews, foxes, skunks, raccoons, and dogs) and from ticks. The 18S rRNA gene proved the most discriminatory and resulted in identification of three clades, only one of which (Clade 1) contained strains thought to be zoonotic. All of these are probably parasites of rodents, but rodent babesias are also present in Clade 3. Clade 2 appears to consist entirely of carnivore parasites. Interestingly, Clade 1 includes Swiss and Russian isolates, supporting the possibility of the occurrence of zoonotic strains in Europe. It is also notable that the confirmed zoonotic parasite GI from the USA can be transmitted by *I. ricinus* as easily as a German isolate (HK) (Gray *et al.*, 2002). Although not included in the analysis of Goethert and Telford (2004), these parasites evidently both belong to Clade 1. The GI strain, used as a reference strain in Table II, is identical to the Clade 1 Nantucket strain, while HK shows a high level of homology to GI, as do the Clade 1 Swiss and Russian strains (all 99.9%). The Swiss strain and another European strain from Berlin proved to be identical to HK (Table II), further strengthening the case for the occurrence of a zoonotic form of *B. microti* in Europe. The Russian strain showed 99.8% 18S rDNA homology to these European strains. It is also of interest to note that despite sharing vector infectivity and a relatively high degree of genetic homology, GI and HK are of very different appearance in the erythrocytes of laboratory gerbils (*Meriones unguiculatus*) (Gray *et al.*, 2002).

The Japanese Kobe strain (AB032434), despite being zoonotic and of rodent origin, does not appear to belong to Clade 1 (Table II). However, a rodent strain showing 100% beta-tubulin gene homology with the zoonotic US types, including the GI strain, has recently been identified in northern Japan (Zamoto *et al.*, 2004a). Similar genotypes to this potentially zoonotic Clade 1 parasite have also been found on mainland northeastern Eurasia (Zamoto *et al.*, 2004b). The rodent Kobe strain is clearly distinct from another European rodent isolate, 'Munich' (AB071177) (98.2% 18S rDNA homology) (Table II) and the Munich isolate is also separated from Clade 3, the other group containing rodent parasites in Goethert and Telford's study

Table II
Babesia microti 18S rRNA gene homology (EMBL-EBI ClustalW analysis) of selected isolates estimated from GenBank sequences, with reference to the GI strain (GenBank Accession number AF231348)

Strain	Accession No	Base pairs compared	% homology	Source	Author
HK* Hannover	AB085191	1705	99.9	*Clethrionomys*	Tsuji *et al.*, 2002
Berlin*	AF231349	1705	99.9	*I. ricinus*	Zahler *et al.*, 2000
Switzerland*	AY144692	1255	99.9	*I. ricinus*	Goethert and Telford, 2004
Russia	AY144693	1254	99.9	*Clethrionomys*	Goethert and Telford, 2004
Munich	AB071177	1701	89.9	*Mus musculus*	Tsuji *et al.*, 2001
Kobe (Japan)	AB032434	1705	99.5	Human, *Apodemus,*	Saito-Ito *et al.*, 2000
Nantucket (USA)	AY144722	1255	100.0	Human, *I. scapularis*,	Goethert and Telford, 2004

* HK, Berlin and Switzerland strains show 100% 18S rDNA homology

(98.2% 18S rDNA homology compared with the Clade 3 isolate from Maine, USA, AY144690). Goethert and Telford's Clade-2 isolates are all parasites of carnivores, and the recently described *B. microti*-like parasite of dogs in Spain (*Theileria annae*?) (Camacho *et al.*, 2001) also appears to belong to this group since it (AY534602) shows 100% 18S-rDNA homology to a Clade 2 parasite (AY144702) from a fox in Cape Cod, MA, USA. There is no evidence to suggest that *T. annae* is zoonotic.

In addition to the above observations on homology between isolates in respect to the 18S rRNA gene, an unpublished analysis of other genes has shown that UK isolates, transmitted by the rodent tick *I. trianguliceps* rather than *I. ricinus*, are well separated from a group of continental European strains that includes the Berlin strain (M. Zahler, pers. comm.). Clearly, further characterisation of European strains of *B. microti* is necessary.

B. divergens and *B. microti* biology in relation to genotype

The 18S rRNA gene has been analysed by several researchers in order to determine the identity of isolates of *B. divergens* and *B. microti.* While this has proved to be revealing in many respects, it is difficult to determine the degree of homology required for conspecificity. For example, whereas several isolates of *B. divergens*-like parasites showed sufficient homology to be considered the same species, in the few cases where biological data were available obvious discrepancies arose, for example the apparent lack of infectivity of deer '*B. divergens*' for gerbils or cattle (Langton *et al.*, 2003). Similarly, the isolation of *B. divergens*-like parasites from humans in the USA initially suggested that *B. divergens* occurs on that continent, but there is no suggestion that bovine cases have ever occurred there. The evident 100% homology between bovine isolates with respect to the 18S rRNA gene (Slemenda *et al.*, unpublished, in Herwaldt *et al.*, 2004) now suggests that the Kentucky (Beattie *et al.*, 2002), Portuguese (Centeno-Lima *et al.*, 2003), Madeira (Olmeda *et al.*, 1997), cottontail rabbit (Goethert and Telford, 2003) and reindeer (Langton *et al.*, 2003) parasites are not *B. divergens.* There is still no reason to think that the pathogenic bovine parasite, *B. divergens,* is not restricted to Europe.

In the case of *B. microti,* we now know that many strains exist and that *B. microti* should be regarded as a species complex (Goethert and Telford, 2004). Whether these strain differences manifest in significant biological differences remains to be seen, though it is already apparent that there is some variation in host specificity. The occurrence in Europe of strains with a high degree of 18S rDNA homology to zoonotic American strains (GI) suggests that some European strains may also be zoonotic, but there is no conclusive medical evidence to support this. Further genetic and biological characterisation of European *B. microti* isolates are required.

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