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Short communication

High seroprevalence of *Babesia* antibodies among *Borrelia burgdorferi*-infected humans in Sweden

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ABSTRACT

In northern Europe, tick-borne diseases such as Lyme borreliosis (LB) and tick-borne encephalitis (TBE) are well known. The actual incidence of *Babesia* infections, however, has remained elusive. In this study, the prevalence of antibodies against two *Babesia* spp. was investigated in a cohort of patients that were seropositive for *Borrelia (B.) burgdorferi* sensu lato (s.l.). Data were compared to a control group of healthy individuals. Sera were collected from 283 individuals residing in the southernmost region of Sweden, Skåne County. Almost one third of the sera were from patients with a confirmed seropositive reaction against *B. burgdorferi* s.l. All sera samples were assessed for IgG antibodies against *Babesia (Ba.) microti* and *Ba. divergens* by indirect fluorescent antibody (IFA) assays. Seropositive IgG titers for at least one of the *Babesia* spp. was significantly more common ($p < 0.05$) in individuals seropositive for *Borrelia* (16.3%) compared to the healthy control group (2.5%). Our findings suggest that *Babesia* infections may indeed be quite common among individuals who have been exposed to tick bites. Furthermore, the results indicate that human babesiosis should be considered in patients that show relevant symptoms; particularly for splenectomized and other immunocompromised individuals. Finally, the data challenges current blood transfusion procedures and highlights the current lack of awareness of the parasite in northern Europe.

1. Introduction

Babesia spp. are parasites from the phylum Apicomplexa and more than 100 different species have been described (Hunfeld et al., 2008). *Babesia* spp. are estimated to be the second most commonly occurring parasite in mammalian blood and are also present in birds. These parasites have been discovered on all inhabited continents, and are most commonly spread by ixodid ticks but can also be spread through blood transfusions (Schnittger et al., 2012). *Babesia* parasites invade erythrocytes, and the symptoms of the host range from general symptoms such as fever, chills, headache and myalgia to cases with fulminant life threatening infections including severe hemolysis (Hunfeld et al., 2008).

In Europe, human babesiosis is primarily caused by *Ba. divergens* and *Ba. microti*; but *Ba. venatorum* has also been implicated. Typically, the disease presents in splenectomized or otherwise immunocompromised patients, and individuals suffering from hematological malignancies. Sporadic cases have been reported from most regions of Europe and Scandinavia (Hildebrandt et al., 2013) including two cases in Sweden (Bläckberg et al., 2018; Uhnoo et al., 1992). Depending on cohort selection and local epidemiology, the prevalence of *Babesia* antibodies in Europe ranges from 2% to 23% (Chmielewska-Badora et al., 2012; Granström, 1997; Hunfeld et al., 2002). The seroprevalence among humans in Sweden, however, remains unclear. Data has shown that 4% of the ticks in southern Sweden carry *Babesia* spp. that can infect humans (Karlsson and Andersson, 2016). This clearly indicates that more attention should be given to understanding and treating infections caused by the *Babesia* parasite.

Borrelia burgdorferi sensu lato is a tick-borne pathogen that manifests as Lyme Borreliosis (LB). In south-eastern Sweden, LB has an annual incidence among humans of 0.5% (Bennet et al., 2006). Studies have shown that approximately 20% of ticks (above 30% in adult ticks, 15% in nymphs) are infected with *B. burgdorferi* s.l. (Wilhelmsson et al., 2013, 2010). One study has shown that if ticks are already infested with *Borrelia*, then the possibility of establishing a co-infection with *Ba. microti* increases (Dunn et al., 2014).

Abbreviation: B, *Borrelia*; *Ba*, *Babesia*; BBAP, *Borrelia burgdorferi* sensu lato antibody positive; IFA, Indirect fluorescent antibody assay; IgG, Immunoglobulin G; IgM, Immunoglobulin M; LB, Lyme Borreliosis; TBE, Tick-borne encephalitis

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To further investigate whether the seroprevalence of *Babesia* antibodies in humans can be used as an indicator of possible *Babesia* infection, an epidemiological study was performed on samples from *B. burgdorferi* s.l. antibody positive individuals from southern Sweden. In these patients, seropositivity was considered a surrogate marker for previous tick exposure. Findings were then compared to healthy individuals from the same area; but without knowledge about tick exposure in their recent medical history.

2. Material and methods

2.1. Serum samples

The serum samples in this study were collected from 2014 to 2015 and stored at −80 °C. All sera were from patients residing in southern Sweden (Skåne County).

2.2. B. Burgdorferi s.l. Antibody positive (BBAP) group

Samples from patients seropositive for *B. burgdorferi* s.l. were obtained through the Biological Specimen Bank of Clinical Microbiology, Skåne University Hospital, Lund (86 samples). Individuals were informed by letter and could decline participation, however, all 86 agreed to inclusion in the study. The patients had been treated in primary health care centers and also as hospital inpatients. Regardless of where in Skåne County the blood was drawn, samples from across the entire region were sent to the specimen bank.

2.3. Control group

Plasma samples were collected from healthy volunteers from different parts within Skåne County (197 samples). No data was available concerning tick exposure. No assay for *B. burgdorferi* s.l. was performed on this group.

2.4. Ethics

This study was ethically approved by the Regional Ethical Board in Lund, Sweden (reference 2014/659) and by the Regional Board for Quality Register (S-KVB).

2.5. Assay for B. Burgdorferi s.l. Antibodies

The samples that were seropositive for *B. burgdorferi* s.l. had all been assessed for both IgG and IgM against *B. burgdorferi* s.l., and had either an elevated IgG concentration (≥30 AU/mL, chemiluminescent immunoassay, LIAISON® *B. burgdorferi*, DiaSorin) or a clearly elevated IgM index (> 1.1). An immunoblot assay (EUROLINE-WB Euroimmun AG) was also performed on the samples with elevated IgM index and confirmed them as *Borrelia* positive. This two-tier protocol is in accordance with current standard recommendations (Dessau et al., 2018). The samples were assessed in the routine laboratory and were not reassessed in this study.

2.6. Assay for Babesia antibodies

The tests used to detect the presence of antibodies against *Ba. divergens* and *Ba. microti* were performed with indirect fluorescent antibody (IFA) assays. A commercially available kit was used for *Ba. microti* (*Ba. Microti* IFA immunoglobulin G/M (IgG/IgM), MRL Diagnostics, Cypress, California). The kit includes golden hamster (*Mesocricetus auratus*) erythrocytes that have been infected with a strain that was originally isolated from a human patient. The test for determining *Ba. divergens* IgG/IgM was an internal IFA assay that has been used in previous studies (Hunfeld et al., 2002; Kampen et al., 2002). The method has been adapted for human sera from an assay for infected cattle in Germany, and antigens were prepared from the blood of jirds (*Meriones unguiculatus*). All sera were titrated on different days (in triplicate) and the results were the mean of the three experiments. Positive and negative controls provided by the manufacturer were used in all assays. The cut-off values for IgG directed against *Ba. divergens* and *Ba. microti* were ≥1:128 and ≥1:64, respectively; and the evaluated specificities for anti-*Ba. microti* and anti-*Ba. divergens* IgG antibody detection were 98.6% and ≥97.5%, respectively (Hunfeld et al., 2002). If the IgG result was > 1:32, IgM was also assessed.

2.7. Statistics

The Chi-squared statistical test was used to compare the results of the seropositive individuals in the BBAP group and the control group.

3. Results

3.1. Description of study cohort samples

The mean ages of the patients in the BBAP and the control groups were 57 and 40 years, respectively. A few children were included in the BBAP group; while the healthy, control group consisted of adults only. The age ranges were 5–87 years and 18–66 years in the BBAP and healthy, control groups, respectively. In the BBAP group there was a majority of men (58%), while among the healthy controls there were more women (62%). In the BBAP group, 65 of the 86 samples were obtained from patients treated in primary health care centers; and 21 samples were from hospital inpatients.

3.2. Babesia antibody positive samples among the BBAP patients

From the 86 samples in the BBAP group, 73 and 13 were seropositive for IgG and IgM against *B. burgdorferi* s.l., respectively. From these patients, the number of *Babesia* seropositive (IgG) individuals was 11 and 3, respectively. This gives a total seroprevalence of 16.3% for *Babesia* antibodies among the BBAP patients (Table 1). Twelve of the 65 samples obtained from primary healthcare centers were positive for *Babesia* antibodies, as were 2 of the 21 inpatient samples (from infectious diseases and neurological clinics).

From the 14 samples that were positive for *Babesia* antibodies in the BBAP group, 5 and 8 were also positive for *Ba. divergens* IgG and *Ba. microti* IgG, respectively. One individual had a positive reaction in both assays (Table 1), and 1 patient also had a positive *Ba. microti* IgM titer (Table 1). For all individuals in the BBAP group, the positive titers for *Ba. divergens* were 1:128; while the positive titers for *Ba. microti* showed a larger variation with one titer reaching 1:512 (Table 1). From the 14 samples that were positive for *Babesia* antibodies in the BBAP group, 6 were from females (3 positive for *Ba. divergens* and 3 positive for *Ba. microti*).

The geographical distribution of the *Babesia* antibody positive individuals was relatively evenly distributed throughout the Skåne County (Fig. 1).

3.3. Babesia antibody positive samples among healthy individuals

In the healthy control group, 5 of the 197 individuals were seropositive for *Babesia* antibodies. Two and 3 were positive for *Ba. divergens* and *Ba. microti*, respectively (Table 1). Thus, the total seroprevalence for *Babesia* antibodies among the healthy individuals was 2.5%. All 5 seropositive individuals in the group were female.

3.4. Comparison of the BBAP group with the healthy individuals

Comparison of the BBAP group with the healthy volunteers revealed that 16.3% and 2.5%, respectively, of the individuals had a positive titer for at least one of the *Babesia* spp. This is statistically significant

Table 1
Results from individuals with positive Babesia antibody titers (left), and for groups in total (far right column).

Sample ID	Group	*Ba. divergens* antibody titer		*Ba. microti* antibody titer		Reactivity in assays for *B. burgdorferi* s.l.		Total number of individuals with *Babesia* IgG divided with total individuals per group (%)
		IgG	IgM	IgG	IgM	IgG (AU/mL)	IgM (index)	
1	BBAP	-	x	1:128	-	69	x	
4	BBAP	1:128	-	-	x	-	1.27	
16	BBAP	-	x	1:128	1:16	119	x	
19	BBAP	-	x	1:64	-	>240	x	
20	BBAP	1:128	-	-	x	81	x	
26	BBAP	1:128	-	1:64	-	73	x	
35	BBAP	1:128	-	-	x	121	x	14/86 (16.3%)
36	BBAP	1:128	-	-	x	-	>6.0	
38	BBAP	-	x	1:512	-	128	x	
44	BBAP	-	x	1:64	-	>240	x	
65	BBAP	-	x	1:256	-	191	x	
77	BBAP	-	x	1:64	-	364	x	
78	BBAP	-	x	1:128	-	>240	x	
83	BBAP	1:128	-	-	x	-	1.26	
2002	healthy control	1:128	x	-	x	x	x	
2036	healthy control	-	x	1:512	-	x	x	
2071	healthy control	-	x	1:256	-	x	x	5/197 (2.5%)
2095	healthy control	-	x	1:128	1:16	x	x	
2100	healthy control	1:128	1:32	-	x	x	x	

'–' negative result.
'x' assay not performed.

with a *p*-value of 0.000022.

4. Discussion

In Sweden to date, human babesiosis is a disease with little to no awareness within the general population. As the infection is rarely taken into consideration, the actual seroprevalence is unclear; although a small sample collection among previously *Borrelia* infected patients did reveal a seroprevalence in 2 of the 15 sera specimens (Uhnoo et al., 1992). *Ixodes ricinus* is the main transfer vector for the *Babesia spp.*, and in Sweden, it has increased in both geographical distribution and number during the last decades. This could also have resulted in augmentation of human seropositivity against *Babesia* (Jaenson et al., 2012).

In other parts of Europe, the prevalence of *Babesia* antibodies in people at risk have been more thoroughly investigated. Tick-exposed individuals in Germany has reached a seroprevalence of 11.5% for *Ba. microti* and *Ba. divergens* (Hunfeld et al., 2002); and a 5% seroprevalence for *Ba. microti* has been reported for forestry workers in Poland (Chmielewska-Badora et al., 2012).

Coinfection with *Babesia* spp. and other tick-borne infections, such as *B. burgdorferi* s.l., have been investigated in areas where *Ba. microti* is

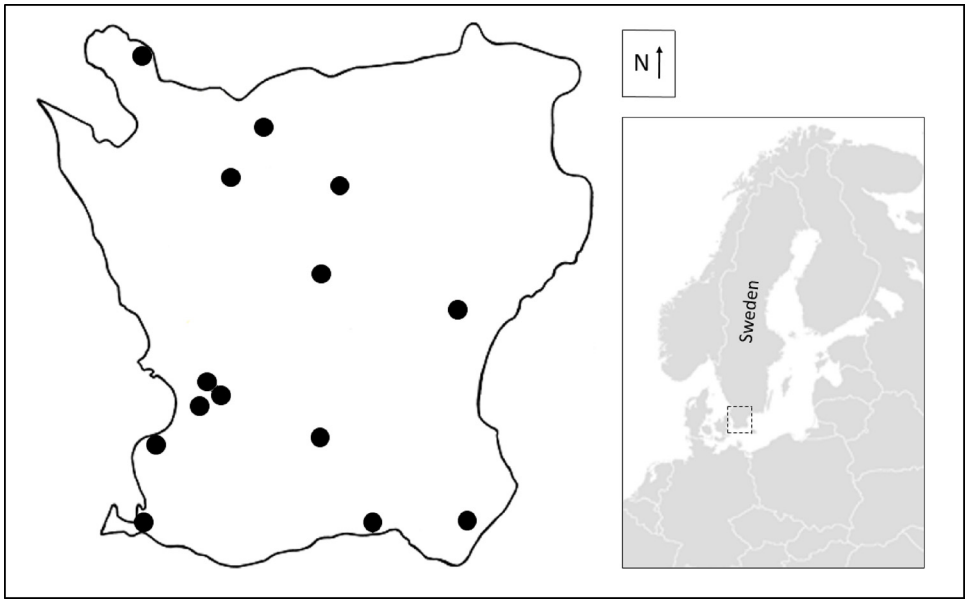

Fig. 1. Geographical distribution of seropositive *Babesia* samples in the BBAP group. The data is based on the referring clinic for the *B.burgdorferi* s.l. assay in Skåne County, the southernmost region of Sweden.

considered endemic. This includes sections of the north-eastern region of the United States; in particular, coastal counties extending from Massachusetts to New Jersey. As many as 19% of LB patients were diagnosed with a *Babesia* spp. coinfection. Approximately 2000 cases of human babesiosis are reported annually in the United States, while the incidences of LB is in the order of 30,000 (Diuk-Wasser et al., 2017).

Babesiosis in cattle has a broader impact in Europe. The disease is well known in Swedish veterinary medicine (Sw: "Sommarsjuka") and is routinely treated by veterinarians. Symptoms include hematuria, spontaneous abortion and death. Through the application of molecular methods, a recent study on domestic cattle in southern Sweden detected *Ba. divergens* in more than 53% of the samples (38 infections from a total of 71 animals) (Andersson et al., 2017). In addition, other mammals such as deer and dogs can also be infected. In a study from southern Norway, the seroprevalence of *Ba. divergens* in cattle was 27% (Hasle et al., 2010). Another study on roe deer from central Sweden, revealed the presence of the *Babesia* spp. antigen (using molecular detection) in 40 of the 77 assessed animals (52%, whereof 44% was due to *Ba. venatorum* which is phylenogenetically closely related to *Ba. divergens*) (Andersson et al., 2016). In our study, the cases covered the entirety of Skåne County (Fig. 1). Reservoir hosts such as cattle and roe deer probably facilitate the spread of *Babesia* spp., which may subsequently result in a comparatively greater presence in rural areas.

The results in this manuscript illustrate the prevalence of possible pathogenic *Babesia* organisms among individuals exposed to ticks and tick-borne diseases in Sweden. The clinical impact of the acquisition of a disseminated infection in vulnerable individuals that are immunocompromised or splenectomized is obviously substantial. Such an effect has been shown in two previously described cases from Sweden where long hospitalization was required (Bläckberg et al., 2018; Uhnoo et al., 1992). Sporadic fatal cases have also been reported elsewhere in Europe (Asensi et al., 2018). Today in Sweden, however, routinely performed diagnostic investigation of the pathogen are very rare. In other parts of the world, such as the north-east of the United States, awareness of *Babesia* spp. and the clinical ramifications is much more predominant.

In earlier studies, antibody levels against *Babesia* spp. appear to decline during the first months following primary infection. In some cases, however, the antibody may still be present after more than a year (Bloch et al., 2016). Our methods have been successfully used in seroprevalence studies of *Ba. divergens* and *Ba. microti* in Germany and France (Hunfeld et al., 2002; Rigaud et al., 2016); and also to investigate the presence of *Ba. microti* in North America (Krause et al., 2003). The cut-off values for our methods were established to provide a specificity of 98.6% in the detection of IgG antibodies against *Ba. microti* and ≥97.5% for *Ba. divergens*. In general, these values are better than most of the other available methods that are used to detect *Borrelia* infections. IFAs were evaluated by including sera from patients with active or recent toxoplasmosis, malaria and syphilis. The results showed primarily negative reactions (Hunfeld et al., 2002).

Microscopy is usually the preferred method of choice in diagnosing acute *Babesia* infections. Nevertheless, it is our belief that serology is still an excellent means of discovering persistent infections; and thus, is suitable for screening purposes. *Babesia* seroreactivity in our patients (16%) was higher than the prevalence of *Babesia* in ticks (4%) in southern Sweden (Karlsson and Andersson, 2016). There are some considerations, however, that need to be taken into account. For example, frequently tick-exposed individuals commonly suffer from several tick-bites a year and also, after latent infection antibodies usually remain for longer periods of time. Most importantly, *Babesia* prevalence in ticks can vary at the local epidemiological level. When only southern Skåne was taken into account, the percentage of *Babesia* positive ticks was as high as 12% (Karlsson and Andersson, 2016). Such local variability has also been previously reported by other molecular epidemiological studies (Foppa et al., 2002).

Today, blood transfusions in Sweden are not routinely assessed for *Babesia* spp. To limit the risk of infection for susceptible, immunocompromised recipients of blood transfusions, such screening could potentially be considered in the future. At minimum, there should be at least an increase in awareness of the pathogen when a patient develops fever following a blood transfusion (WHO, 2012). Unfortunately, for both hospital staff and the public, the level of knowledge concerning *Babesia* infections is often rudimentary. With this study, our hope is to be able to increase awareness of the disease.

5. Conclusions

Our results indicate a significant number of individuals with *Babesia* antibodies among patients in southern Sweden seropositive for *B. burgdorferi* s.l. (16.3% in the BBAP group compared to 2.5% in a healthy control group). These findings reveal that there is an increasing need to broaden awareness of the prevalence of *Babesia* spp. in northern Europe. This is especially relevant in the differential diagnosis of patients that show specific symptoms; particularly in connection with blood transfusions.

DECLARATION OF INTEREST

All authors report no conflicts of interest.

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