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Investigating disease severity in an animal model of concurrent babesiosis and Lyme disease

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Abstract

The incidence of babesiosis, Lyme disease and other tick-borne diseases has increased steadily in Europe and North America during the last five decades. *Babesia microti* is transmitted by species of *Ixodes*, the same ticks that transmit the Lyme disease-causing spirochete, *Borrelia burgdorferi*. *B. microti* can also be transmitted through transfusion of blood products and is the most common transfusion-transmitted infection in the U.S.A. *Ixodes* ticks are commonly infected with both *B. microti* and *B. burgdorferi*, and are competent vectors for transmitting them together into hosts. Few studies have examined the effects of coinfections on humans and those have had somewhat contradictory results. One study linked coinfection with *B. microti* to a greater number of symptoms of overall disease in patients, while another report indicated that *B. burgdorferi* infection either did not affect babesiosis symptoms or decreased its severity. Mouse models of infection that manifest pathological effects similar to those observed in human babesiosis and Lyme disease offer a unique opportunity to thoroughly investigate the effects of coinfection on the host. Lyme disease has been studied using the susceptible C3H mouse infection model, which can also be used to examine *B. microti* infection to understand pathological mechanisms of human diseases, both during a single infection and during coinfections. We observed that high *B. microti* parasitaemia leads to low haemoglobin levels in infected mice, reflecting the anemia observed in human babesiosis. Similar to humans, *B. microti* coinfection appears to enhance the severity of Lyme disease-like symptoms in mice. Coinfected mice have lower peak *B. microti* parasitaemia compared to mice infected with *B. microti* alone, which may reflect attenuation of babesiosis symptoms reported in some human coinfections. These findings suggest that *B. burgdorferi* coinfection attenuates parasite growth while *B. microti* presence exacerbates Lyme disease-like symptoms in mice.

Graphical Abstract

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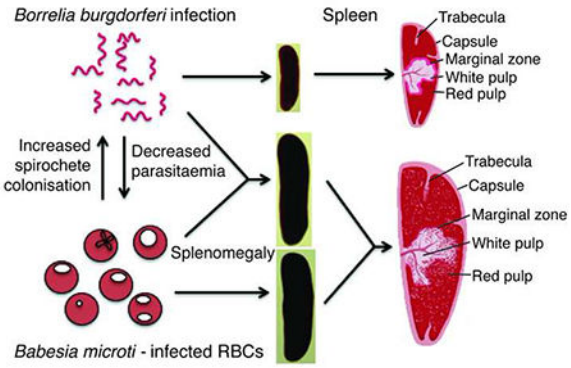

Keywords

Babesia microti; Babesiosis; *Borrelia burgdorferi*; Coinfections; Tick-borne diseases

1. Introduction

Babesia microti and *Borrelia burgdorferi* cause two of the most prominent tick-borne diseases in the U.S.A., human babesiosis and Lyme disease, respectively. Transmission of these pathogens is primarily through species of *Ixodes* ticks. In addition to the shared tick vector, *B. microti* and *B. burgdorferi* have common animal reservoirs and overlap in their epidemiology and transmission cycles (Spielman et al., 1985; Oliver et al., 1993; Swanson et al., 2006). The white-footed mouse is the primary reservoir host for both pathogens and the white-tailed deer has contributed to expansion of the endemic regions for both diseases in the U.S.A. (Telford et al., 1996; Levin et al., 2002; Thomas et al., 2009; Ismail et al., 2010; Magnarelli et al., 2010; Rikihisa, 2010). *Babesia* species were identified as infectious organisms in 1893 and babesiosis was first detected in humans in the U.S.A. in 1969 (Western et al., 1970; Ouhelli and Schein, 1988). In 1991, one of 13 babesiosis cases in Connecticut, U.S.A. was transmitted through a blood transfusion (Anderson et al., 1991). Soon after *B. burgdorferi* identification as the causative agent of Lyme disease (Burgdorfer et al., 1982), it was shown to cause coinfections with *B. microti* in hamsters through *Ixodes dammini/scapularis* ticks (Piesman et al., 1987). Both pathogens were recovered concurrently from rodents, *Peromyscus leucopus* and *Microtus pennsylvaticus*, in northeastern U.S.A., indicating coinfection of reservoir hosts in the field (Anderson et al., 1986, 1987; Stafford et al., 1999; Magnarelli et al., 2013). *Babesia microti* is transmitted less efficiently by ticks relative to *B. burgdorferi* (Krause et al., 2006). However, acquisition of *B. microti* from mice by the tick vector improves when mice are coinfected with a highly infectious strain of *B. burgdorferi* (Dunn et al., 2014). The prior presence of *B. burgdorferi* in a geographical region and its coinfection enhances the expansion in range and establishment of *B. microti* in that region (Dunn et al., 2014), especially when larvae and nymphs feed together on a reservoir host. The higher incidence of babesiosis in long-established *B. burgdorferi* endemic regions relative to those where infection of ticks is more recent is likely due to underreporting of babesiosis in the latter (Diuk-Wasser et al., 2014).

Thorough investigations of coinfection with *B. microti* and *B. burgdorferi* in humans have started only in the last decade. Patients were considered coinfected based on serological diagnostic tests, although serological results cannot always distinguish between prior exposure and an ongoing infection. Nearly 10% of patients in southern New England (U.S.A.) reporting tick bites exhibited evidence of infections with Lyme spirochetes and *B. microti* as early as the 1990s. Several Lyme disease and babesiosis symptoms overlap and are non-specific. Patients with exposure to both pathogens as determined serologically, with or without testing and evidence of spirochetal DNA in their blood, showed significantly more intense flu-like symptoms such as fatigue, chills, nausea, fever and headache that persisted for longer periods than patients infected only with *B. burgdorferi* (Krause et al., 1996, 2002, 2003). The same studies reported that coinfected patients showed either no difference or displayed less severe symptoms compared with patients infected with *B. microti* alone. There is a clear need for further studies on the effects of *B. microti-B. burgdorferi* coinfections to determine the pathogenic mechanisms that exacerbate or mitigate disease symptoms. Here, we review infection of hosts with *B. microti* and *B. burgdorferi* and the impact of infection on the host immune system and disease manifestations inflicted by each pathogen. Using a mouse model of coinfection, we will discuss insights that can be gained into the pathogenesis of coinfections by *B. microti-B. burgdorferi.*

2. Babesiosis; an emerging parasitic disease

Babesia species belong to intracellular apicomplexan protozoa that multiply in the red blood cells (RBCs). *Babesia* undergoes repeated cycles of infection and asexual replication within erythrocytes. Their intra-erythrocytic multiplication causes cell lysis and results in hemolytic anemia. *Babesia microti*, and to some extent *Babesia duncani*, causes infection in humans in the U.S.A. while *Babesia divergens* is responsible for most cases of human babesiosis in Europe. According to the Centers for Disease Control and Prevention (CDC) of the U. S.A., 97% of cases of babesiosis in the USA in 2011 were caused by *B. microti.* These were reported primarily in the northeastern United States (Massachusetts, Connecticut, New York, New Jersey and Rhode Island) and Great Lakes region of Wisconsin and Minnesota (Herwaldt et al., 2003; Joseph et al., 2011). Immunocompetent people often remain asymptomatic or experience mild flu-like symptoms that include fever and aching muscles while immunocompromised, elderly or splenectomized individuals experience severe, acute and sometimes fatal babesiosis (Genda et al., 2016). The disease is very likely under-reported since initial symptoms are non-specific and testing requires a high index of suspicion from the clinician. Typically, physicians recommend testing for *Babesia* only after observing hemolytic anemia.

Transmission of *B. microti* through transfusion of blood products was recognized in 1994 (Gerber et al., 1994). Since babesiosis is the most common infection transmitted through blood transfusion in the U.S.A, the United States Food and Drug Administration (FDA) recently recommended screening blood and/or blood donors for *Babesia* infection (Lobo et al., 2013; https://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/TransfusionDonationFatalities/UCM598243.pdf). Individuals with fully competent immune systems can establish *Babesia* carriage states for prolonged durations without exhibiting infection-associated clinical manifestations and often donate blood. Since

these parasites survive during cold storage of donated blood, transfusion of tainted blood products can result in babesiosis in immunocompromised or splenectomized patients (Hunfeld et al., 2008; Herman et al., 2010; Chiang and Haller, 2011; Herwaldt et al., 2011; Sinski et al., 2011; van Vugt et al., 2011; Cushing and Shaz, 2012; Holler et al., 2013; ; Poisnel et al., 2013; Cursino-Santos et al., 2014; Fang and McCullough, 2016). Rare examples of transplacental transmission of *Babesia* have also been reported. In the cases of congenital babesiosis through mothers infected with either *Babesia* alone or coinfected with Lyme spirochetes, infants suffer from jaundice, anemia, thrombocytopenia and neutropenia (Joseph et al., 2012; Luckett et al., 2014; Wormser et al., 2015; Saetre et al., 2018). A recent report showed a significant decrease in transfusion-transmitted babesiosis when donated blood was prescreened for *B. microti* DNA presence by quantitative PCR (qPCR) and for *anti-Babesia* antibodies by Arrayed Fluorescence Immunoassay (Moritz et al., 2016). Although not yet FDA approved for widespread application or commercially available, this type of screening, if employed universally in regions endemic for tick-borne diseases, can eventually eliminate the hazard of transfusion-transmitted babesiosis.

Age is a major risk factor for babesiosis in humans. Manifestations in elderly patients include low and unstable blood pressure, chills, pain, severe hemolytic anemia, disseminated intravascular coagulation and vital organ failure, and can even result in mortality (Krause et al., 2003; Joseph et al., 2011; Martinez-Balzano et al., 2015). It is likely that age-related weakening of the immune system prevents clearance of this parasite in the elderly. The spleen plays a critical role in the resolution of human babesiosis since asplenic/splenectomized people have a heightened risk of the disease (Krause et al., 2008; Raffalli and Wormser, 2016).

3. Lyme disease

Lyme disease is the most predominant tick-borne infectious disease. The CDC estimates that ~300,000 individuals are infected by *B. burgdorferi* every year in the U.S.A. (Kuehn, 2013) but only 10% of them are reported (Moore et al., 2016). Lyme disease starts with an early localized stage manifested as erythema migrans in up to 80% of infected individuals , followed by dissemination of spirochetes to different tissues, often manifested as multiple skin lesions with neuronal, joint and heart involvement. Late stages of infection display chronic arthritis, acrodermatitis and neuroborreliosis (Steere, 2001).

Similar to other pathogenic infections, Lyme disease pathogenesis is multifactorial. Both bacterial and host factors affect the severity of disease symptoms. The major outer surface protein A (OspA) and OspB of *B. burgdorferi* facilitate colonisation of the midgut in unfed ticks (Pal et al., 2000; Fikrig et al., 2004; Pal et al., 2004; Neelakanta et al., 2007). Down-regulation of OspA post-blood meal is concurrent with induction of OspC, a lipoprotein critical for initiation of mammalian infection (Grimm et al., 2004; Tilly et al., 2006). Interestingly, OspC is dispensable for later stages of mammalian infection, i.e., once the adaptive immune response is established in the infected mammal. After initial infection, several *B. burgdorferi* proteins interact with extracellular matrix (ECM) components to enable colonisation of a variety of mammalian tissues. Interaction of *B. burgdorferi* with endothelial cells (Leong et al., 1998; Ebady et al., 2016) is followed by dissemination to

various tissues, where colonisation occurs through recognition of fibronectin, glycosaminoglycan (GAG) and proteoglycan, decorin present on the host cell surface in ECM components, and is facilitated by spirochete proteins including BB0347, BBK32, Bgp and DbpA and DbpB in mice (Parveen and Leong, 2000; Parveen et al., 2006; Seshu et al., 2006; Weening et al., 2008; Saidac et al., 2009; Benoit et al., 2011; Hyde et al., 2011; Lin et al., 2012, 2015; Schlachter et al., 2018). Only P66 and BBB07 of *B. burgdorferi* have been shown to recognize integrins present in the host cell cytoplasmic membrane (Behera et al., 2008; Ristow et al., 2015). Some of these *B. burgdorferi* proteins that participate in cell adherence also contribute to the long-term survival of spirochetes in tissues. Knockout mutants of these adhesins often result in attenuated Lyme disease in mice (Parveen et al., 2006; Parveen and Leong, 2006; Shi et al., 2006; Blevins et al., 2008; Weening et al., 2008; Hyde et al., 2011; Schlachter et al., 2018).

4. Host immune response against *B. microti* and *B. burgdorferi* infections

The immune response during intracellular multiplication of *B. microti* was defined by a case study that was followed by a proposed model depicting the specific cell involvement (Shaio and Lin, 1998; Homer et al., 2000). In these reports, innate immune responses facilitated by both macrophage and natural killer (NK) cells were implicated in the control of this parasite in humans in the acute phase, and was likely facilitated by production of IL-2, IL-12, TNF-α and IFN-γ by these cells. The mechanisms involved in inhibition of the intra-erythrocytic cycle of *B. microti* are still not well understood. In some mouse models, macrophages and NK cells, together with IL-12 and IFN-γ, were found to play important roles in the resolution of *B. microti* parasitaemia and protection from future infections (Igarashi et al., 1999; Chen et al., 2000; Aguilar-Delfin et al., 2003). In addition to the host genotype, the strain of *B. microti* affects the anti-parasitic immune response. In mice, IFN-γ is required but not essential for the control of infection by some but not all *B. microti* strains. Several previous studies suggested that depending on the protozoan strain used for infection, IFN-γ could be required but not essential for control of *B. microti* parasitaemia in mice (Matsubara et al., 1993; Igarashi et al., 1999; Clawson et al., 2002; Skariah et al., 2017).

Borrelia burgdorferi induces protective, albeit proinflammatory, immune responses in the host, which contribute to Lyme arthritis. The innate response, by modulating Toll like receptor (TLR) signaling, enables host defense against *B. burgdorferi* but also increases inflammation and disease severity. TLR2 signaling pathways activated by *B. burgdorferi* lipoproteins play pivotal roles in the control of spirochetes in joints, the induction of pro-inflammatory cytokines by host macrophages, and in increasing Lyme arthritis (Wooten et al., 2002; Wang et al., 2008; Dennis et al., 2009; Salazar et al., 2009; Iliopoulou and Huber, 2010). Interestingly, a TLR1 polymorphism causes an increase in Th1 immune responses and poses a risk of antibiotic-refractory Lyme arthritis in humans (Strle et al., 2012). TLR1/TLR2 heterodimers are also important for stimulating immune responses against *B. burgdorferi* such that both TLR2 and MyD88 knockout mutants showed increased tissue colonisation and severe arthritis (Alexopoulou et al., 2002; Yoder et al., 2003; Marre et al., 2010). Activation of TLR8 by *B. burgdorferi*-derived RNA in monocytes also induces Type I interferon and IFN-responsive host genes and contributes to the severity of Lyme arthritis (Miller et al., 2010). Increased production of IFN-γ, resulting from induction of a Th1

response, as reported in *B. burgdorferi*-infected patients relative to uninfected controls, was found to contribute to increased Lyme disease pathogenesis. This immune response also correlates with potential autoimmune reactions depending on the age or genotype of the host. Genetic constitution (HLA-DR haplotypes) and associated autoimmune responses may underline susceptibility of humans to post-treatment Lyme syndrome. Attempts to model antibiotic-refractory Lyme arthritis in mouse models have had mixed results (Iliopoulou et al., 2009; Steere et al., 2011). Therefore, factors contributing to chronic/treatment refractory inflammatory Lyme disease remain poorly understood.

5. Impact of *B. microti-B. burgdorferi* coinfections on disease manifestation

As early as 1985, serological testing in endemic regions demonstrated coinfections with *B. burgdorferi* in 54% of patients with babesiosis, and with *B. microti* in 64% of Lyme disease patients (Benach et al., 1985). Since then, high levels of coinfections with *Borrelia* and *Babesia* spp. continue to be reported in Australia (Mayne, 2011, 2015), northeastern and midwestern U.S.A. with a reported rate of 32–40% in patients examined in New England (Anderson et al., 1991; Krause et al., 1991; Mitchell et al., 1996; Belongia, 2002), which is similar to a coinfection rate of 38.5% we observed in New Jersey (Primus et al., 2018). Some studies have found *B. burgdorferi-B. microti* to be the most common coinfection representing as high as 81% of tick transmitted coinfections in New England regions of the U.S.A. (Swanson et al., 2006). Long-term clinical outcomes of coinfections are likely influenced by the genetic compositions of pathogenic strains of *B. microti* and *B. burgdorferi*, differential immunological responses in patients, and the potential contribution of additional coinfecting tick-borne pathogens.

Symptoms such as chills, fever, fatigue, headache and general malaise occur in both Lyme disease and babesiosis (Pruthi et al., 1995). Coinfections can have serious consequences and have complex clinical manifestations including those associated with cardiac involvement. A patient who showed persistent fever, chills, myalgias, erythematous skin lesions and 3% *B. microti* parasitaemia displayed pericarditis prior to death (Marcus et al., 1985). An autopsy revealed the presence of *B. burgdorferi* spirochetes in the myocardium, suggesting that the severity of Lyme carditis was responsible for this death (Marcus et al., 1985). In contrast to cardiac manifestations, the number and persistence of musculoskeletal or neurological symptoms in patients with simultaneous infections with both pathogens were not reported to be higher than in patients infected only with *B. burgdorferi* (Krause et al., 1996), but there is one report of severe transverse myelitis in a coinfected patient (Oleson et al., 2003). While coinfections with *B. microti* worsen some acute symptoms of Lyme disease, long-term (on average 6 months post-exposure) outcomes of Lyme disease were the same in coinfected and only *B. burgdorferi*-infected patients (Wang et al., 2000). One confounding feature of human coinfections is the challenge of distinguishing concurrent infections from previous exposure to each pathogen.

6. Mouse models of infections and *B. microti-B. burgdorferi* coinfections

Mice are natural hosts for both *B. microti* and *B. burgdorferi.* Selected laboratory mouse strains infected with *B. microti* and *B. burgdorferi* exhibit pronounced disease manifestations, enabling the development of mouse models of Lyme disease and human babesiosis. The experimental accessibility of mouse models of coinfection can provide valuable information on pathogenic mechanisms and host immune responses during concurrent or sequential infections with the two pathogens. The ability to control infectious doses, timing of infection, together with host and pathogen genotypes, provide a unique opportunity to obtain insights relevant to human diseases.

Host gender is an important variable in the outcome of infectious diseases. One study found the incidence of babesiosis to be significantly higher in men than women (Menis et al., 2015). The data in mice is somewhat conflicting. Infection of several strains of moderately susceptible mice with the highly infectious *B. microti* strain, WA1, caused higher mortality in female than male mice (Aguilar-Delfin et al., 2001). However, infection with *B. microti* Munich strain lead to higher peak parasitaemia and greater anemia in males of several strains compared with respective strains female mice (Sasaki et al., 2013). These varying results highlight the need to consider the effects of both the host and *B. microti* genotypes on the course of infection in mice of both sexes.

Age is a well-known risk factor in human babesiosis. In addition, DBA/2 mice displayed an age-related increase in susceptibility to *B. microti* strain RM/NS. Early peak parasitaemia were higher in older DBA/2 mice compared with younger ones (Vannier et al., 2004). Older mice were also compromised in clearing the parasite and, after resolution of the initial peak, displayed persistent low-level parasitaemia for a longer time as compared with younger mice (Vannier et al., 2004).

There are only two published reports on *B. microti-B. burgdorferi* coinfections in mice (Moro et al., 2002; Coleman et al., 2005) and these provided inconsistent results. One study examined coinfection and infections with each pathogen individually, in BALB/c and C3H/HeN mice (Coleman et al., 2005). The impact of coinfection, relative to either *B. microti-* or *B. burgdorferi-associated* symptoms, was not found to be statistically significant. Thus, coinfection did not exacerbate *B. microti* parasitaemia, associated splenomegaly, decrease in hematocrit, haemoglobin levels and platelet count since all parameters were similar to *B. microti*-infected, normal, aged and splenectomized mice (Coleman et al., 2005). A puzzling result in this study is the lower peak parasitaemia in old versus young C3H/HeN mice infected only with *B. microti* (Coleman et al., 2005) since it is at odds with an age-related increase in susceptibility reported in humans after *B. microti* infection and in other strains of mice (Vannier et al., 2004). Thus, the results of Coleman and colleagues suggested that the course of *B. microti* infection is unaffected by concomitant infection with *B. burgdorferi.* Interestingly, coinfected mice displayed similar *B. burgdorferi* burdens in tissues and ankle swelling compared with mice infected with *B. burgdorferi* alone in their study.

The second study (Moro et al., 2002) found increased Lyme arthritis in coinfected BALB/c mice compared with mice infected exclusively with *B. burgdorferi*. Increased ankle swelling was attributed to simultaneous reduction in IL-10, produced by splenocytes and localized lymph nodes at approximately 4 weeks p.i., in coinfected mice compared with *B. burgdorferi-infected* BALB/c mice (Moro et al., 2002). The inconsistent outcomes of coinfections reported in these two studies (Moro et al., 2002; Coleman et al., 2005) highlight the need for further development of a murine model of *B. microti-B. burgdorferi* coinfection.

To fill the gap in understanding of coinfections in mice, we recently began studies in C3H mice. To evaluate the effect of coinfections by these pathogens, young (4 weeks old), female C3H/HeJ mice were infected through i.p. injection of *B. microti* Gray strain-infected RBCs (1×10^4 per mouse) and s.c. injection of the infectious *B. burgdorferi* N40 strain (1×10^3 per mouse), either singly or together. We observed that *B. burgdorferi* colonisation diminished in female mice at 3 weeks p.i., while coinfected mice continued to show significantly higher colonisation of joints and brain (unpublished data). As a consequence, coinfected mice demonstrated increased inflammatory Lyme arthritis compared with mice infected only with *B. burgdorferi*. Our results are in agreement with a previous report on Lyme disease patients with concurrent babesiosis that showed exacerbation and persistence of acute Lyme disease symptoms compared with patients inflicted with Lyme disease alone (Krause et al., 1996, 2002). Interestingly, unlike the case in humans, carditis in coinfected mice was indistinguishable from mice infected with *B. burgdorferi* alone. These differences point to the need for additional studies using mouse models of coinfection that replicate different disease manifestations observed in humans. Despite these differences, understanding of human illness during simultaneous infection with *B. burgdorferi* and *B. microti* can be facilitated using the mouse models of coinfection that replicate different disease manifestations observed in humans.

Coinfection with *B. burgdorferi* did not affect the rate of growth of *B. microti* but the peak *B. microti* parasitaemia in coinfected mice was significantly lower than in mice infected only with *B. microti*. Giemsa-stained blood smears at peak parasitaemia during *B. microti* infection display pleomorphic, intracellular forms of *B. microti* and a significant reduction in erythrocytes resulting in anemia (Fig. 1). Following this peak parasitaemia, there was a marked reduction in haemoglobin levels in both *B. microti*-infected and coinfected mice, with mice infected with *B. microti* only displaying slightly lower haemoglobin levels than coinfected mice (Fig. 1B and C). Resolution of *B. microti* parasitaemia was associated with rapid restoration of haemoglobin levels in both sets of mice, suggesting that long-term effects of the *B. microti* infection cycle are minimal in this mouse model. Our results are consistent with reports of Lyme disease patients coinfected with *B. microti* displaying less severe symptoms of babesiosis that patients infected with *B. microti* alone (Krause et al., 2002; Diuk-Wasser et al., 2016).

The spleen is proposed to be the most important lymphoid organ in antibody production during protozoan infections (Lundqvist et al., 2010; Bermejo et al., 2011). The major impact of *B. microti* infection in C3H mice, in the presence or absence of *B. burgdorferi*, was splenomegaly with the spleen weight of *B. microti*-infected mice 4-5 times that of *B. burgdorferi*-infected mouse spleens, underlining the central role played by this organ in

clearance of infected RBCs. The architecture of the spleen is significantly altered by *B. microti* infection (Fig. 2). The marginal zone merges with red and white pulp zones and the clear demarcation of these zones observed in uninfected or *B. burgdorferi*-infected mice disappears. Unlike in other infectious diseases, the spleen has been proposed to be the most important lymphoid organ playing a role in antibody production during protozoan infections (Lundqvist et al., 2010; Bermejo et al., 2011). We observed a significant decrease in both splenic T and B cells in *B. microti*-infected and coinfected mice relative to those in only *B. burgdorferi*-infected mice. A more dramatic effect was observed on the B cell population. These results are consistent with observations in other protozoan diseases including Chagas disease and malaria, where specific B-cell responses against parasites are delayed or abrogated due to B cell apoptosis and their depletion in the spleen (Radwanska et al., 2008; Bockstal et al., 2011; Obishakin et al., 2014; Liu et al., 2015). Interestingly, specific antibody responses against both of these tick-borne pathogens in our study were significantly lower in coinfected mice compared with mice infected with either pathogen individually. The decreased antibody response in coinfected mice could explain the increased burden of Lyme spirochetes in tissues of coinfected mice. In addition, these results potentially imply a relatively minor role for antibodies in clearance of the parasite since *B. microti* parasitaemia was lower in coinfected mice compared with mice infected with *B. microti* alone. Coinfected mice also demonstrated a significant increase in splenic macrophage numbers (unpublished data). Overall, our results suggest that a thorough investigation of the immune response is warranted to fully understand the pathogenesis of each disease during coinfection.

To summarize, various studies suggest that *B. microti* enhances *B. burgdorferi* colonisation and Lyme disease manifestations while *B. burgdorferi* attenuates *B. microti* parasitaemia. In addition, innate immune responses stimulated by *B. burgdorferi*, probably due to its large number of lipoproteins, could diminish *B. microti* growth and enhance parasitic resolution. The diminished cellular and humoral immune responses could be responsible for a higher *B. burgdorferi* burden in organs and tissues but these did not seem to affect resolution of babesiosis in mice. The spleen appears to be critical for elimination of *B. microti* infection in both humans and mice. Overall, all of these studies indicate that mouse coinfection models will improve the understanding of human infections with *B. burgdorferi* and *B. microti* separately or simultaneously.

7. Ethics Statement

Data generated from animal studies conducted by the laboratory of the corresponding author is included in this article. Designated members of Rutgers New Jersey Medical School, Newark Institutional Animal Care and Use Committee (IACUC), U.S.A., reviewed and approved the protocol number D-14011-A1 under which experiments were conducted following guidelines of the Animal Welfare Act, The Institute of Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals, and Public Health Service Policy, U.S.A.

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Highlights

- Incidence of babesiosis and Lyme disease is high in the U.S.A. and Europe
- Coinfection by *Babesia microti* and *Borrelia burgdorferi* shows diverse and persistent symptoms in patients
- *Babesia microti* infection reduces hemoglobin and hematocrit levels, and results in anemia
- The spleen, as a lymphoid organ, plays an important role in clearance of *B. microti* during infection
- Coinfection with *B. burgdorferi* and *B. microti* attenuates parasite growth while exacerbating Lyme disease symptoms in mice

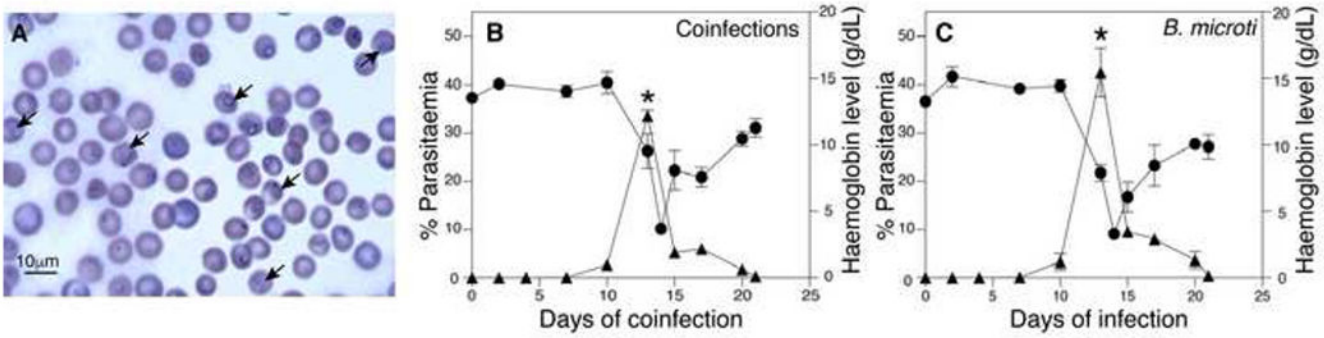

Fig. 1.

Babesia microti peak parasitaemia was followed by a pronounced decrease in haemoglobin levels. (A) Giemsa-stained blood smears reveal intra-erythrocytic pleomorphic forms at peak parasitaemia in *B. microti*-infected mice (40 × magnification). (B and C) Asterisk indicates that *B. microti-Borrelia burgdorferi* coinfected mice (B) display significantly lower peak parasitaemia (marked by triangles) compared with mice infected with *B. microti* alone (C). Furthermore, increase in *B. microti* parasitaemia was accompanied with sharp declines in haemoglobin levels (circles). Haemoglobin returned to normal levels after resolution of parasitaemia. Significance was determined by a student's *t* test for unequal variance (*statistically significant difference in parasitaemia, $P<0.05$).

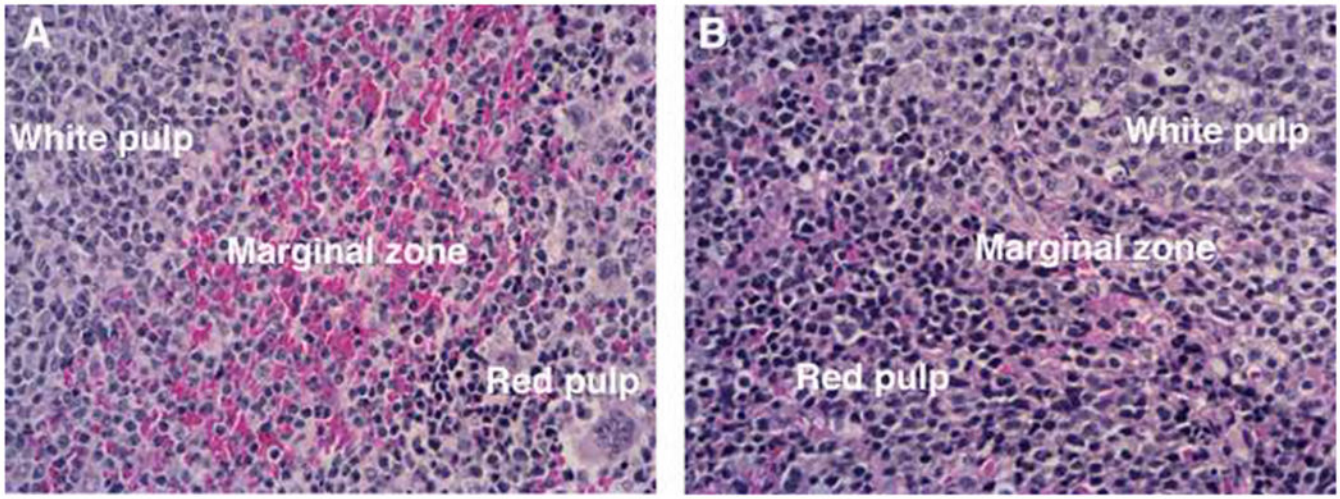

Fig. 2.

Babesia microti stimulates changes in splenic architecture irrespective of the presence of *Borrelia burgdorferi.* (A) Spleens in *B. burgdorferi*-infected C3H/HeJ mice have well-demarcated red pulp, marginal zone and white pulp regions. (B) Spleens of coinfected mice display enlargement and merging of red pulp, marginal zone and white pulp regions.