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First description of the pathogenicity of *Babesia vogeli* in experimentally infected dogs

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ABSTRACT

Babesiosis is a tick-borne disease that occurs worldwide with the most recognized *Babesia* species that infect dogs being Babesia canis, *B. vogeli*, *B. rossi* and *B. gibsoni*. While *B. vogeli* is reported in dogs worldwide, clinical and laboratory data on infections is based on reports of naturally infected dogs. To provide reliable data on the clinical and laboratory abnormalities associated with acute and more chronic infections in healthy dogs free of other tick-borne diseases, we experimentally infected dogs with a Chinese strain of *B. vogeli*. All of the six infected Beagles, three of which were splenectomized, became infected with *B. vogeli* detected in blood smears taken the day following infection and the organism detected by FRET-qPCRs in most blood samples (77/86; 90%) collected about every 4 days until the end of the experiment on day 95. All the infected dogs showed fever, partial anorexia and malaise that was more severe in the splenectomized dogs that did not gain weight for three weeks in the period after initial infection. Regenerative anemia, thrombocytopenia and decreased white blood cell counts were seen in all dogs but were more severe in the splenectomized dogs, of which two had life threatening infections and had to be removed from the study for treatment. Following re-infection on day 66, none of the dogs showed clinical signs and copy numbers did not change significantly although all the dogs were negative by FRET-qPCR on at least some of the subsequent sampling days. Laboratory values in the non-splenectomized dogs were relatively unchanged while in the splenectomized dog there was a temporary small increase in the platelet and white blood cell counts and a temporary slight worsening of the anemia. In summary, our study shows dogs experimentally infected with a *B. vogeli* strain from China develop only mild clinical signs that are followed by asymptomatic infections that can last for least 95 days. In splenectomized dogs, however, severe life threatening signs may develop.

1. Introduction

Babesiosis is a tick-borne disease that occurs worldwide (Bai et al., 2002; Criado-Fornelio et al., 2003; Uilenberg 2006; Criado-Fornelio et al., 2009) and is caused by protozoal hemoparasites belonging to the genus *Babesia* (Homer et al., 2000). There are a number of *Babesia* species that infect dogs, the most recognized being *Babesia canis*, *B. vogeli*, *B. rossi* and *B. gibsoni* (Köster et al., 2015). *B. rossi* is endemic in Southern Africa (Köster et al., 2015) while *B. canis* has been reported in Europe and Asia (Rar et al., 2005). *B. gibsoni* appears to occur worldwide (Solano-Gallego and Baneth, 2011) as does *B. vogeli* (Table 1).

The widespread distribution of *B. vogeli* probably reflects the worldwide distribution of its major reservoir and vector, *Rhipicephalus sanguineus* sensu lato (Zahler et al., 1998).

B. vogeli is regarded as being only mildly pathogenic causing subclinical or only mild disease (Solano-Gallego & Baneth, 2011; Köster et al., 2015). Signs that may occur are typical of those seen with other *Babesia* and include depression, weakness, anorexia, palor, fever, lymphadenomegaly, splenomegaly, anemia, thrombocytopenia, jaundice

Abbreviations: FRET, fluorescence resonance energy transfer; PCR, polymerase chain reaction; ALT, alanine aminotransferase; AST, aspartate aminotransferase

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Table 1
Worldwide occurrence of *B. vogeli* in dogs.

Continent	Country	References
Africa	Angola, Egypt, Namibia Nigeria, Sudan and Tunisia	Oyamada et al. (2005), M'ghirbi and Bouattour (2008), Adamu et al. (2014), Salem and Farag (2014), Cardoso et al. (2016), Penzhorn et al. (2016), Rjeibi et al. (2016)
Asia	Cambodia, China, India, Palestine, Philippines, Qatar, Thailand	Piratae et al. (2015), Xu et al. (2015), Alho et al. (2017), Augustine et al. (2017), Azmi et al. (2017), Ybañez et al. (2017)
America	Argentina, Haiti, Brazil, Costa Rica, Colombia, Grenada and USA	Birkenheuer et al. (2005), Yabsley et al. (2008), Vargas-Hernández et al. (2012), Wei et al. (2014), Inpankaew et al. (2016), Mascarelli et al. (2016), Starkey et al. (2016), Malheiros et al. (2016)
Europe	Croatia, Cyprus, France, Italy, Malta, Portugal, Romania, Serbia, Slovenia, Turkey	Duh et al. (2004), Gülanber et al. (2006), Beck et al. (2009), Ionita et al. (2012), Gabrielli et al. (2015), René-Martellet et al. (2015), Attipa et al. (2017), Annoscia et al. (2017), Licari et al. (2017)
Oceania	Australia	Shapiro et al. (2017)

and pigmenturia. Laboratory abnormalities reported include anemia, thrombocytopenia, hypoalbuminemia, hyperbilirubinemia, and elevated liver enzymes (Solano-Gallego et al., 2008; Solano-Gallego & Baneth, 2011 and Solano-Gallego et al., 2016). Although not yet reported in dogs with *B. vogeli* infections, severe complications can develop in dogs infected with other species including systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), refractory hypotension and septic shock (Matijatko et al., 2009), acid-base and ion imbalances (Zygner et al., 2012b), and hormonal and renal abnormalities (Schoeman et al., 2007; Zygner et al., 2015).

Data on the clinical and laboratory findings in dogs infected with *B. vogeli* are derived from naturally infected dogs, in different stages of infections and often with concurrent tick-borne diseases (Vial and Gorenflot 2006; Solano-Gallego et al., 2008; Carli et al., 2009; Kelly et al., 2013; Loftis et al., 2013). To provide more defined data on the pathogenicity of *B. vogeli*, we experimentally infected Beagles under controlled conditions and monitored the clinical and laboratory effects of infection and re-infection with a Chinese strain of *B. vogeli*.

2. Materials and methods

2.1. Babesia vogeli

During ongoing studies on tick-borne diseases in China, we identified *B. vogeli* by FRET-qPCR (see below) in the blood of an apparently healthy adult Border Collie in a commercial dog breeding facility in Taixing, Jiangsu Province of China. The dog was negative in previously described PCRs (Xu et al., 2015) for *Anaplasma* spp., *Dirofilaria immitis*, *Ehrlichia* spp., *Hepatozoon* spp., *Leptospira* spp., *Leishmania* spp., *Theileria* spp. and *Rickettsia* spp.

2.2. Dogs for experimental infections

This study was reviewed and approved by the Institutional Animal Care and Use Committee of Yanghzou University College of Veterinary Medicine. Female Beagles aged 7–8 months (n = 9) were purchased from the Yangzhou Sifang Experimental Animals Company (Baoying, Jiangsu, China), and kept individually in cages in the tick-free Containment Animal Facility at Yangzhou University College of Veterinary Medicine. The dogs were maintained under standard tick-free management conditions and fed a commercial dog food (Nature Bridge, Bridge Petcare Co., Ltd., Shanghai) according to the manufacturer's recommendations. For the infection studies, the dogs were randomly divided into three groups with three dogs per group (Group-1: control; Group-2: infected; Group-3: splenectomized and infected). Splenectomies were performed using standard anesthesia and surgical techniques two weeks before the dogs were infected with *B. vogeli*.

2.3. Experimental infections with Babesia vogeli

Five milliliter aliquots of fresh whole blood collected into EDTA from the index case, an apparently healthy adult Border Collie (see 2.1), were used to intravenously inoculate the experimental Beagles in Group-2 and Group-3 on day 0 (initial infection). Blood (5 ml aliquots in EDTA) was collected again from the apparently healthy adult Border Collie on day 65 to re-infect the experimental Beagles in Group-2 and Group-3 on day 65.

Aliquots (200 µl) of the inocula collected on day 0 and day 65 were tested for the presence of *B. vogeli* by FRET-qPCR as described below.

2.4. Monitoring of experimentally infected dogs

Every day each experimental dog had a full physical examination by one of the attending veterinarians who recorded the findings. About every four days, blood was collected for routine hematology (18 parameters) (BC-2800 Vet, Shenzhen Mindray Bio-medical Electronics CO., LTD. China) and biochemistry screens (23 routine tests evaluating renal, liver, pancreas, and muscle status and electrolyte, lipid and protein levels) (AU480, Olympus Corporation, Germany) in the Clinical Pathology Laboratory of the Animal Hospital of Yangzhou University College of Veterinary Medicine. Aliquots (200 µl) of the inocula were tested for the presence of *B. vogeli* by FRET-qPCR as described below.

2.5. FRET-qPCR of B. vogeli

DNA was extracted from whole blood samples (200 µl) with the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) as described before (Zhang et al., 2014). The Chinese *B. vogeli* was initially detected with a FRET-qPCR as described previously (Li et al., 2015) before being further characterized with an *18S rRNA*-based PCR (upstream: 5′-TGCGAATGGCTCATTACAACA GTT-3′; downstream: 5′-TTAGCAGGTTAAGGTCTCGTTCGTTA-3′; amplicon: 1,071-bp). All PCR amplicons were sequenced (GenScript biotechnology Co., Ltd., Nanjing) and representative nucleotide sequences deposited in GenBank.

2.6. Statistical analysis

Data were compared with the Chi Square test or the Fisher exact test where appropriate ($P \leq 0.05$) and confidence intervals were calculated based on the exact binomial distribution using statistical analysis software (Statistica, StatSoft, Tulsa, USA).

3. Results

3.1. Index case infected with the Chinese strain of B. vogeli

The infected Border Collie had been born and bred in Taixing and had no history of contact with foreign dogs or foreign ticks. The kennel had no tick prevention routine and we found no ticks on the Border Collie when blood samples were collected for the study. The kennels had no health records so we could not determine if the animal had any illnesses in the past. The 1,071 *18S rRNA* sequence amplified from *B.*