Detection and Differentiation of Babesia microti and Other Pathogenic Babesia Species by a Single Amplicon, Dual-Probe Real-Time PCR Assay

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Abstract

Background: Babesiosis is an emerging zoonotic disease caused by infections with white blood parasites. Babesia microti is most frequently acquired through bites with infected ticks but may also be transmitted through blood transfusion or vertical infection. In the U.S., most human infections are caused by Babesia microti, but infections with Babesia duncani (previously WA-1) and the unnamed strain MO-1 are increasingly recognized. Microscopic examination of blood films, the reference method for diagnosis of Babesiosis, is labor intensive, only suitable for general-level discrimination, and may pose difficulties distinguishing Babesia spp. from Plasmodium. Our recently validated real-time PCR only detects Babesia microti. Thus, we developed a multiplex, real-time PCR for detection and differentiation of Babesia microti (strain MO-1) and other human pathogenic Babesia spp. (strain MO-1).

Methods: A 190bp segment of the 18S rRNA gene (18S) was amplified with a common set of PCR primers and detected by two different probes: (1) Babesia microti, (2) other Babesia spp. of human relevance. Test performance was established with 39 whole blood specimens from patients with known B. microti infections diagnosed by microscopy (n=8) and PCR (n=31). Whole blood was also spiked with B. duncani organisms (AN5C 98-1402) and plasmids covering the 18S target of MO-1, B. divergens, and EU-1.

Results: Analytical sensitivity for both channels was between 240–450 copies/μl (3-10 copies/μl). All positive whole blood samples produced the expected results. Positive patient specimens had a range of 1,668–970 copies (range 2,100–1,120–1). No cross-reactivity was observed with 56 other human pathogens, including the 5 relevant Plasmodium spp., Trypanosoma cruzi, and Leishmania infantum.

Conclusions: The incidence of Babesiosis has increased in recent years and previously unrecognized species have been detected as novel human infections. We describe the first multiplex, real-time PCR assay for sensitive detection of all Babesia species of known human relevance using an innovative single-amplicon, dual-probe design. Given the highly conserved 18S target, this assay will enable us to identify and monitorevolution of unusual Babesia species by subsequent 18S sequencing.

Introduction

Babesiosis is caused by the protozoan, Babesia, that infects red blood cells and is spread by certain ticks. There are over 100 species identified, but relatively few Babesia species have caused documented human infection. Babesiosis caused by Babesia microti, or B. microti, is most common in the United States. Infection by other species such as Babesia divergens-like (MO-1), Babesia duncani (WA-1) is rare. In Europe, Babesiosis is largely caused by infection with Babesia venatorum (EU-1) and Babesia divergens. Most infections are asymptomatic and self-limiting, although in aplastic, immunocompromised, or elderly individuals, symptoms may be severe. Symptoms can take 1-9 weeks to appear after the initial tick bite. Patient symptoms are easily confused for Lyme disease and may include: fever (intermittent), chills, extreme fatigue, severe anemia, back and abdominal pain, nausea, vomiting and diarrhea.

Diagnosis is based on clinical and laboratory findings. Traditionally, peripheral blood smears (Giemsa-stained) are examined to look for ring-like forms of Babesia. Trained personnel are needed to read the blood smear as the ring forms can be easily confused for Plasmodium falciparum. Serology testing is useful to determine if there is past exposure to Babesia, but may not determine if an infection infection. Many PCRs are sensitive and specific but may only pick up Babesia microti and may not detect other possible pathogenic Babesia.

We developed a real-time PCR assay that will detect the major species of Babesia (Babesia microti, Babesia duncani, Babesia spp. MO-1, Babesia divergens-like (MO-1), Babesia sp. EU-1, Babesia divergens) that cause Babesiosis in humans.

The real-time assay was designed to detect the 18S RNA of Babesia and will detect Babesia microti and other Babesia species (B. duncani, B. divergens, Babesia spp. MO-1, Babesia spp. EU-1). There is one amplicon of ~190 bp region of the 18S with the ability to bind two probes. One probe is specific for Babesia microti and one probe that will detect all Babesia spp. of human relevance using an innovative single-amplicon, dual-probe design. Given the highly conserved 18S target, this assay will enable us to identify and monitorevolution of unusual Babesia species by subsequent 18S sequencing.

Background and Methods

FIGURE 1: Important Babesia species that cause disease in humans

- B. microti: Detected by smear microscopy and PCR
- B. duncani (WA-1): Detected by smear microscopy and PCR
- B. divergens (MO-1): Detected by smear microscopy and PCR
- B. venatorum (EU-1): Detected by smear microscopy and PCR

FIGURE 2: Timeline for positive results

Seasonality of positives
- Detected
- Not Detected

FIGURE 3: Babesia results within age groups

FIGURE 4: Patient locations and positivity rates by state

FIGURE 5: Babesia results within age groups

FIGURE 6: Babesia microti and other Pathogenic Babesia Species by a Single Amplicon, Dual-Probe Real-Time PCR Assay

Results

- Babesiosis is a reportable disease in 22 states, CDC detected positives in 14 of 22 states
- We detected Babesia microti positives in 2 additional states (Texas and Florida) for which Babesiosis is not reportable
- Total of 512 patients tested from 11/30/2013 – 9/22/2014
  - Females n=234 (Pos n=15)
  - Males n=277 (Pos n=25)
- Majority of positive specimens were sent from Massachusetts (n=10), New Jersey (n=11), New York (n=12)
- Seasonality is mostly in the summer months, however some Babesia microti positives were found in Dec, Jan, Mar which may be due to transfusion infection
- Positives were detected in the older age range (60-79)
- Our assay of one amplicon with dual probe is very sensitive and specific

Conclusions