New High Resolution Melting PCR assay for detection and differentiation of several *Babesia* spp. infecting humans and animals

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Babesiosis is an emerging vector-borne disease caused by protozoa of the genus *Babesia*. Although microscopy can be used for the detection of *Babesia* in red blood cells, the sensitivity of the method is too low to detect infections with low levels of *Babesia* spp. parasitemia. Furthermore, *Babesia* can be confused with other parasites in blood smear examination. Moreover, microscopy is a very time-consuming tool for detecting the presence of *Babesia* in ticks. The aim of the study was to design a single-tube PCR test for the detection and differentiation of *Babesia* species known to infect humans and animals, using DNA samples obtained from such vertebrate tissues as blood, and their invertebrate hosts (ticks).

The positive controls were DNA samples from vertebrates infected with *Babesia*: *B. canis*, *B. divergens*, *B. microti* and *B. venatorum*. The negative controls comprised DNA from a healthy human, DNA from a human infected with *Plasmodium falciparum*, DNA from a *Babesia*-uninfected *Microtomus oeconomus*, DNA from the blood of a dog, DNA of *Toxoplasma gondii* and tick DNA. New primers for amplification of short polymorphic regions of *Babesia* 18S rRNA gene were designed to match the DNA of multiple *Babesia* spp. and to have a low level of similarity to DNA sequences of other intracellular protozoa and *Babesia* hosts. A multiplex, single-tube PCR test was designed to detect the DNA of *Babesia* spp. including *B. microti*, *B. divergens*, *B. venatorum* and *B. canis*. The PCR products amplified from DNA *Babesia* were subjected to High Resolution Melting analysis for *Babesia* species identification.

The designed test allowed detection and differentiation of four *Babesia* species: three zoonotic species (*B. microti*, *B. divergens*, *B. venatorum*) and one not (*B. canis*). It was possible to detect and identify all tested *Babesia* species by analyzing the HRM curves of the PCR products derived from the human, dog, rodent and tick samples. The assay is the first PCR-based test capable of detecting and differentiating between several *Babesia* spp. of medical and veterinary importance using a single-tube reaction without the need for sequencing PCR products or the use of molecular probes.

The results of the study show that the designed assay for *Babesia* detection and identification could be a practical, versatile and inexpensive tool for diagnostics and screening studies.