The use of transfection technology as a *Babesia bovis* vaccine development tool
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Babesia bovis is an intraerythrocytic pathogen responsible for one of the most prevalent and costly tick borne diseases of cattle worldwide. The parasite is transmitted by *Rhiphicephallus* (*Boophilus*) ticks, and have the ability to evade the immune system of the vertebrate hosts, causing persistent disease. Acute babesiosis can be prevented quite effectively by combining tick control and vaccination with living attenuated organisms. However these methods of control have numerous limitations and improved approaches are needed. A new research toolbox that includes *B. bovis* full genome sequencing combined with the improved ability to genetically modify the organism is enhancing our understanding of their biology and allows the use of recently developed *Babesia* transfection methods for functional gene characterizations and for vaccine development. However, significant knowledge gaps on the role of key parasite molecules involved in cell invasion, adhesion, asexual and sexual reproduction, tick transmission, and evasion of the immune system, remain. Overall, a better understanding of the biology of these organisms will positively contribute toward the goal of developing improved immunological and pharmacological interventions against *B. bovis*. While developing *Babesia* subunit vaccines might still be an achievable goal, transfection tools will allow for the production of improved genetically modified live vaccines to be developed under the framework of the current effective live vaccines. For instance, an important limitation of current vaccines is the lack of a simple method to discriminate between vaccinated and naturally infected animals. One approach toward addressing this limitation is the use of marker vaccines using recombinant approaches. In addition, because attenuated parasites also establish persistent infection, expression of exogenous genes by stably transfected, attenuated *B. bovis* provides an opportunity to explore the possibility of combining antigens from multiple infectious agents and parasites into a single live vaccine. The ability to persistently infect the host makes this approach particularly promising when immune protection requires frequent boosting, for example, with other hemoparasites and ticks. However, to serve as efficient live vectors for vaccination, recombinant attenuated *B. bovis* vaccines would be required to establish mild acute infection leading to persistence, express exogenous antigens throughout infection, and remain genetically stable. A *Babesia bovis* line stably transfected with the *gfp-bsd* gene was shown to be able to establish acute and persistent infections with a pattern that was undistinguishable of its parental wild type strain. Furthermore, exogenous transgenes can be expressed and remain stable throughout acute and persistent infection in calves, which remained protected of clinical disease upon challenge with a virulent strain. New transfection strategies are been currently developed using bidirectional promoters that provide the ability to express more than a single transfected gene, thus increasing the potential of transfected attenuated parasites as vaccine delivery systems. Overall, the use of genome data in conjunction with novel transfection and genomic related approaches will be instrumental for closing our current knowledge gaps toward a better understanding of the biology of these organisms and for formulating new vaccines and other therapeutic interventions. Together with a better understanding of the protective immune responses, these new experimental approaches will likely be the keys leading to the developing of improved methods of control of this devastating cattle disease.