

Babesia bigemina Gene Expression during Parasite Development within Adult Female Tick Rhipicephalus microplus

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Abstract

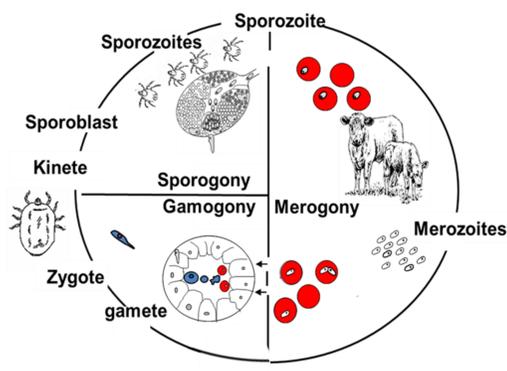
Bovine babesiosis is caused by apicomplexan pathogens of the genus *Babesia* including *B. bigemina*, *B. bovis* and *B. divergens*. These tick-borne pathogens have a complex life cycle with infection of vertebrate and invertebrate hosts for transmission. In the tick midgut, extracellular *Babesia* parasites transform into gametes that fuse to form zygotes. Therefore, intervention at the extracellular *Babesia* tick stages could prevent tick infection and subsequent parasite transmission. We used bioinformatic analysis to identify *B. bigemina* homologs of *Plasmodium falciparum* gamete specific genes. We identified 11 common genes that share amino acid sequence identity ranging from 18 to 58% and contained conserved domains. Those identified genes were transcribed by *B. bigemina* blood and tick stages except BBBOND_0204030. BBBOND_0204030 was transcribed exclusively by *B. bigemina* tick stages and detectable in infected engorged females immediately after ticks dropped off the infected animal. BBBOND_0204030 contains a methyltransferase domain that has been previously shown to be important in gametogenesis. Additionally, we report a new method to induce *B. bigemina* sexual stages in *in vitro* culture using Tris 2_carboxyethyl phosphine. This method did not require secondary induction triggers like temperature reduction or addition of xanthurenic acid. To confirm that this gene is transcribed by early tick stages of *B. bigemina*, we demonstrated BBBOND_0204030 transcription by *in vitro* induced sexual stages using reverse transcription PCR. Our findings improve the understanding of the *B. bigemina* life cycle at the molecular level, and identify a parasite stage specific gene as a potential target for drug intervention of bovine babesiosis.

Introduction

Babesia bigemina life cycle
Bovine host: *B. bigemina* blood stages

Trophozoites
Merozoites

Tick host: *B. bigemina* tick stages
sexual stages
zygote
kinete
sporozoites



Hypothesis

Babesia bigemina specific tick stage genes will be transcribed only during its development within adult female *R. microplus* ticks

Material and Methods

1. *In silico* analysis

National Center for Biotechnology Information

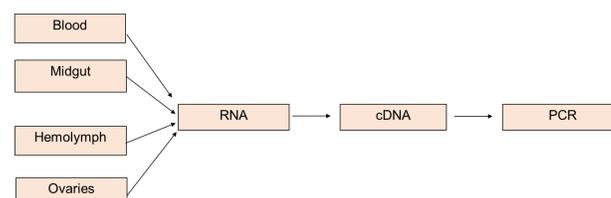
<https://www.ncbi.nlm.nih.gov/>

<http://www.ebi.ac.uk/Tools/msa/clustalo/>

Simple modular architecture research tool

<http://smart.embl-heidelberg.de/>

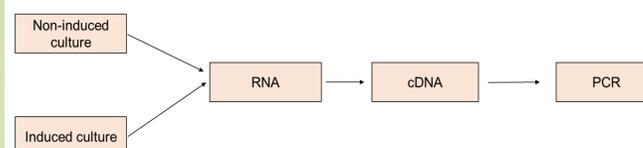
2. Evaluate the transcription of *B. bigemina* homologs of *P. falciparum* gamete specific genes in infected female tick



3. *Babesia bigemina* sexual stages induction

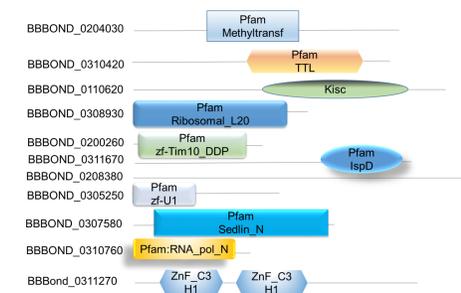
Tris 2_carboxyethyl phosphine (TCEP)

4. Detection of *B. bigemina* tick stage specific genes transcription in induced culture

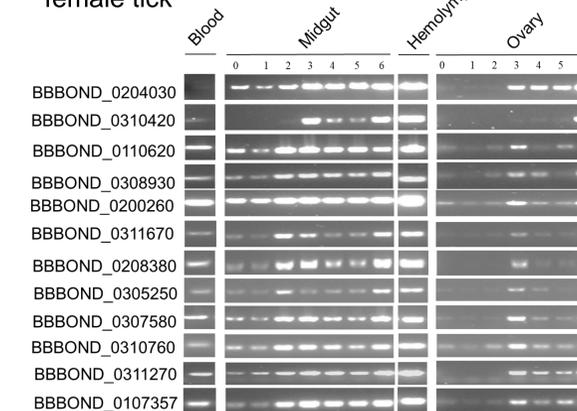


Results

1. Schematics of conserved domains of *B. bigemina* homologs of *P. falciparum* gamete specific proteins



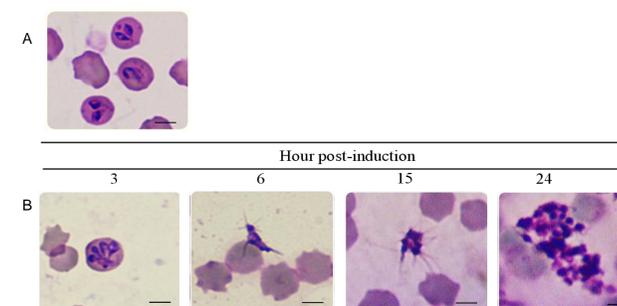
2. The transcription of *B. bigemina* homologs of *P. falciparum* gamete specific genes in infected female tick



3. *Babesia bigemina* sexual stages induction

Morphology of induced sexual stages

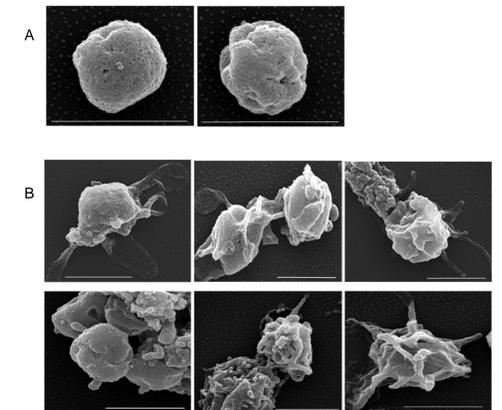
Giemsa smear



Non-induced blood culture (A) and *in vitro* induced blood culture (B), scale bar:5 μ m.

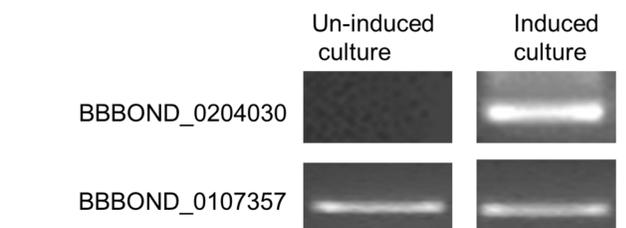
Results

Scan electrograph



Non-induced blood culture (A) and *in vitro* induced blood culture (B), scale bar:2 μ m.

4. Detection of *B. bigemina* tick stage specific genes transcription in induced culture



Conclusion

Bovine babesiosis remains without effective methods to study specific parasite stages due to the lack of consistent approaches to induce and rear *Babesia* stage specific parasites. Herein we developed a new method of inducing *B. bigemina* sexual stages *in vitro* that does not require secondary signals such as temperature changes. Using this method, we identified a gene containing a methyltransferase domain that is transcribed exclusively by *B. bigemina* tick stages. This induction method will allow *in vitro* evaluation of drugs or vaccines targeting sexual stage genes or proteins and subsequent interference with transmission

Acknowledgements

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