

Ehrlichia ruminantium - Amblyomma variegatum tolerant interaction: towards a peaceful life together

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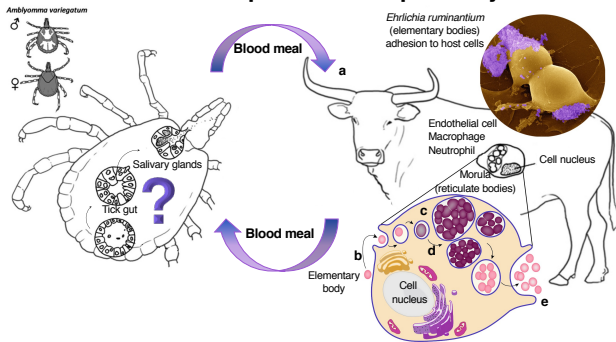


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Heartwater is a fatal disease of ruminants caused by a tick-borne obligate intracellular bacterium named *Ehrlichia ruminantium*. This pathogen is transmitted by several ticks of the genus *Amblyomma*, and more particularly by *Amblyomma variegatum* which is originated from Africa. Heartwater is present in many countries of sub-Saharan Africa and Caribbean islands but recently, many studies suggest the risk possibility of spreading to non-endemic regions. Thus, heartwater may become a high economic and health burden for America mainland and the United States Department of Agriculture (USDA) has classified *E. ruminantium* and its vector *Amblyomma variegatum* on the list of the High-consequence foreign animal diseases and pests. In order to understand the infection mechanisms of *E. ruminantium* inside its vector tick, this project aims i) to assess the vector competency of *A. variegatum* for this pathogen and ii) to study the molecular determinants associated with the bacterial pathogenesis and required for intracellular replication.

E. ruminantium biphasic development cycle in hosts

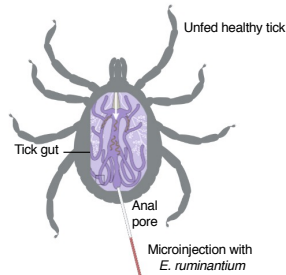


***E. ruminantium* infection.** After infected tick bite, bacteria are spread to ruminants (a). Following adhesion of infectious elementary bodies (b) to host cells, *E. ruminantium* is capable to perform its intracellular biphasic lifestyle (c). Then, reticulate bodies (replicative/vegetative form) fill a morula (cytoplasmic vesuole) within mammalian or tick cells (d). Formation of this vacuole is critical and subsequent lysis of host cell is essential for establishment of heartwater disease (e) (from V. Rodrigues & D. Meyer unpublished, 2022).

Experimental infection is a golden tool to study the vector competency of *A. variegatum* for *E. ruminantium*

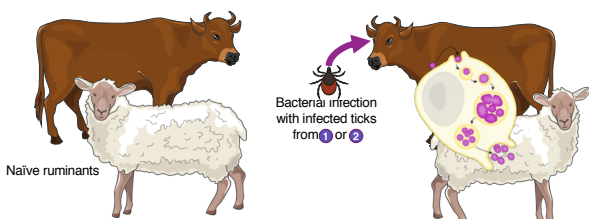


1 In vitro tick feeding system. With the aim of improving tick attachment and efficiency in acquiring pathogens, the system can simultaneously deliver at least four different conditions (R. Vimonish *et al.* 2017).



2 Microinjection of live *E. ruminantium* into *A. variegatum* anal pore for example. Microinjection of the bacteria will allow generation of *E. ruminantium*-infected ticks (V. Taank *et al.* 2020).

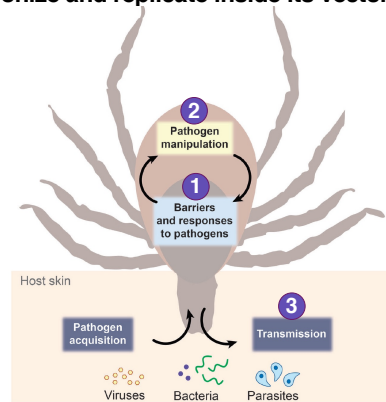
Pathogenicity assays on naïve ruminants (test for viability and infectivity)



Tick experimental infection through inoculation of *E. ruminantium* to naïve hosts.

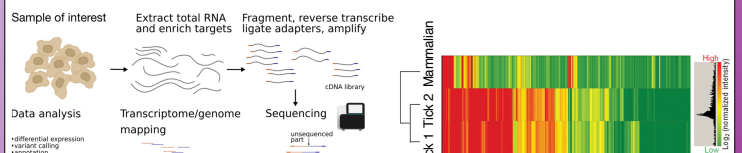
- Following tick infection with *E. ruminantium* using the *in vitro* feeding systems, ticks will be used to infect naïve hosts
- Infected animals will be monitored for clinical signs, the multiplication of *E. ruminantium*, and the immune responses.
- In parallel, the development of *E. ruminantium* inside ticks will be investigated

What are mechanisms allowing *E. ruminantium* to survive, colonize and replicate inside its vector tick?



Schematic representation of three aspects of tick-microbe interactions. The vector-side of tick-borne disease is open for discovery. Question marks represents topics in the field that remain incompletely understood: (1) tick barriers to colonization and responses to pathogens, (2) how microbes manipulate the arthropod to colonize and survive within the tick, and (3) deepen knowledge about the role and composition of salivary secretions are during pathogen transmission dynamics (adapted from J.M. Park *et al.*, 2021).

Analysis of the transcriptomes of mammalian and tick infected cells will highlight crucial genes for pathogenesis and immune responses



Overview of the experimental steps in RNA-seq approach (K. Van den Berge *et al.*, 2018).

Gene expression profiles from *Ehrlichia*-infected mammalian and tick cell lines. Heat map with gene expression in eukaryotic cells; red, up-regulated; yellow, normal; green, down-regulated. (adapted from J.A. Kuriakose *et al.*, 2011).

Conclusion & Perspectives

- In order to replicate and survive, *E. ruminantium* use mechanisms to cross barriers and evade the immune response of eukaryotic hosts. We will show how *E. ruminantium* manipulates its vector tick.
- We will use several artificial techniques to assess *A. variegatum* vector competency for *E. ruminantium*
- We expect to determine what are virulence factors that are expressed in the tick compartments
- We will further compare the differential gene expression between mammalian and tick cells

References

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