Transcriptomic analysis of *Ehrlichia ruminantium* by micro-arrays

**Introduction**

Rocky Mountain spotted fever (RMSF), caused by *Ehrlichia ruminantium* (ER), is an obligate intracellular pathogen. This characteristic explains that little is known about the genetic determinants and molecular mechanisms of ER pathogenesis. Recently a method of selective capture of transcribed sequences (SCOTS) (2) was successfully adapted to ER. This step was essential for any future transcriptomic study of obligate intracellular pathogens. The annotation of 2 genomes of Gaetan and Wolgamouters strain revealed that 30% of the CDS has unknown functions (3). The objectives of this study was to go deeper throughout gene expression analysis through the development of ER microarrays and then identify genes involved in the pathogenesis and attenuation of ER strains and comprehend these mechanisms. An in vitro model using both invariant and attenuated Gaetan strain in bovine endothelial cells allowed us to compare for the first time ER gene expression. In this study, we reported a set of genes differentially expressed between virulent and attenuated strains of ER Gaetan.

**Experimental model & Strategy**

- BAE cells infected with ER Gaetan strain & attenuated 24, 48, 72, 96 & 120 h
- RT & SCOTS
- ER microarrays
- Q-PCR
- Identification of genes (validated)
- Validation gene expression
- Composition of gene expression between virulent & attenuated strains at different stage of development

**ER Micro-arrays validation**

- Genes differentially expressed between virulent & attenuated strains

**Discussion & Perspectives**

The adaptation of SCOTS method to our in vitro model allowed to obtain a large amount of ER transcripts and to further transcriptomic study. For the first time, ER microarrays was validated by hybridization of ER mRNA and SCOTS method allowed the detection of the lowest expressed genes such as cDNA or real. There was a strong increase of detection of transcripts after SCOTS both demonstrated by Q-RT PCR and micro-array analysis. We demonstrated that SCOTS method was crucial for expression analysis during early stationary phase of growth of ER (24 h) with 24% of transcripts detected compared to 1% of transcripts before capture. Preliminary results on ER microarrays at late stage of development demonstrated that 14 genes were strongly modulated depending on virulent or attenuated strain. 19 of these genes were strongly up-regulated for virulent strain. Through comparative genomic study, on 24 genes, 19 had unknown function and 5 were involved in metabolism pathway. Interestingly, 14 genes were identified to be virulent factors for other intracellular pathogens and 3 was hypervirulent virulent factor specific of ER. Those of these genes should be strongly involved in the virulence (CDS_323, CDS_26, CDS_7) up-regulated for virulent strain) and 3 involved in the attenuation (CDS_421, CDS_26, CDS_99) down-regulated. The different gene expression is under validation by Q-RT PCR. Further analysis on ER earlier stage of development using SCOTS method are under process. This strategy paves the way for revealing in pathogenicity of obligate intracellular pathogens.