Identification of genes involved in pathogenicity of *Ehrlichia ruminantium* by a transcriptomic approach



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INTRODUCTION

- \checkmark Ehrlichia ruminantium (ER): agent of heartwater, a tropical fatal disease of ruminants \checkmark To understand the mechanisms of ER pathogenicity by ✓ Lack of efficient vaccines due to high genetic diversity
- ✓ Genomic sequence for 3 virulent strains: Gardel, Senegal & Welgevonden

OBIECTIVE

differential gene expression study at 2 stages of development of the Gardel strain

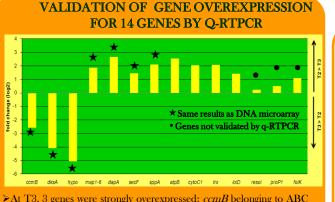
ER MICROARRAY RESULTS COMPARING T2 &T3

>5% (54/950) of ER genes identified by µarrays are differentially expressed

PROTOCOL Gardel virulent strains at 2 stages of development in vitro T2 (72 hpi) T3 (120 hpi) Quantification of gene expression RNA extraction **DNA** extraction 1/10 of sample 9/10 of sample cDNA conversion of ER transcripts & Validation by q-RTPCR on 4 replicates Number of cDNA/µl MEASURE OF DIFFERENTIAL GENE EXPRESSION (R2) between T2 & T3 by q-RTPCR with R1_{Tx} = $\frac{Number\ of\ cDNA/\mu l}{Number\ of\ bacteria/\mu l}$ $R1_{\underline{T2}}$

Quantification of ER by q-PCR Number of bacteria /µl 2 Selective captures µarrays hybridization List of genes differentially expressed between T2 &T3: Choice of 14 genes of interest

between intermediate T2 & late T3 stages of development ➤ Categories of gene functions Mainly unknown function (50%) Higher proportion: •At T2 for co-enzyme transport and metabolism and replication, recombination and DNA repair groups •At T3 for transport of carbohydrates, amino acids, inorganic ions and nucleotides group Selection of genes for q-RTPCR validation Over-Genes ratio log2 **Functions** expression Map1-6 5.87 SecF 5,18 Intracellular trafficking, secretion, and vesicular transport Amino acid transport and metabolism + Cell 1.96 T2>T3 dapAwall/membrane/envelope biogenesis Posttranslational modification, protein turnover, 1.65 sppAchaperones Posttranslational modification, chaperones -3,16 -4,58 cytoC1 Energy production and conversion Energy production and conversion 4.68 -4,91 Posttranslational modification, chaperones ccmb T3>T2 Transport and metabolism of Carbohydrates + Amino proP1 -5,28 acids + Inorganic ions Defense mechanisms lolD Coenzyme transport and metabolism

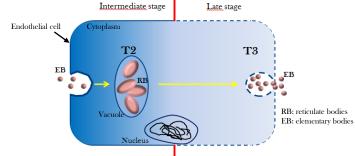


Results expressed in fold change (log2)

Significant difference if | R2|>1

- At T3, 3 genes were strongly overexpressed: *ccmB* belonging to ABC transporter, dksA coding for transcription factor & known as inducer of virulence in Salmonella typhimurium and one with hypothetic function
- At T2, 8 genes were overexpressed: 3 coding for proteins involved in metabolism, 4 in the transport & exchange of nutrients & 1 in resistance to oxydative stress

FUNCTIONS OF OVEREXPRESSED GENES



- -Exchange & transport: secF, lolD, sppA, map1-6
- -Energy production: atpB, cytoC1
- -Metabolism: dapA -Defense mechanism: trx
- -Exchange & transport : ccmB
- -Transcription factor: dksA
- -hypo: Cell wall modification?

CONCLUSION

R2=

R1__{T3}

The overexpression of 11 out of 14 genes were validated by q-RTPCR. Some genes coding for proteins involved in the development of the bacteria were identified for Gardel strain. The gene expression results will be compared to the results of ER genome sequencing & proteomic projects in order to understand the behavior of ER in its biological cycle.