

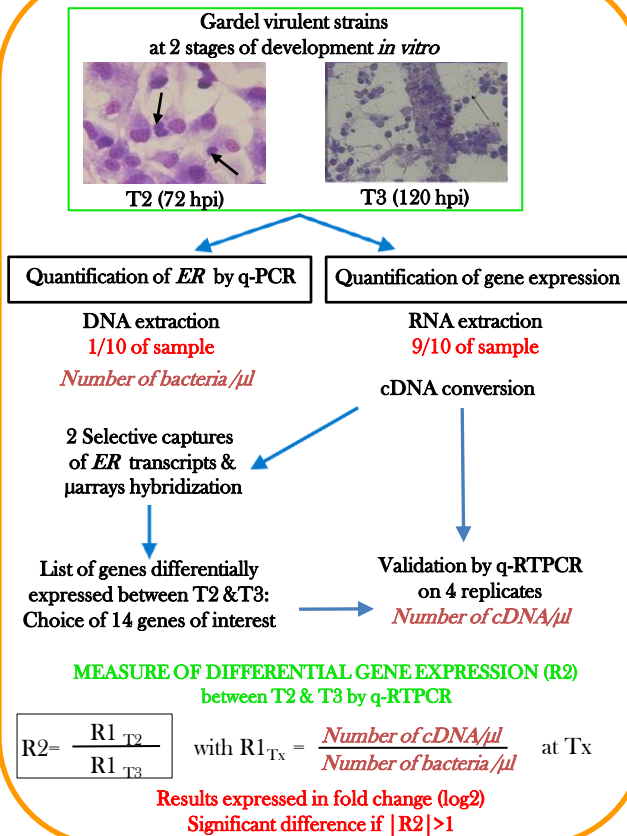
INTRODUCTION

- ✓ *Ehrlichia ruminantium* (ER): agent of heartwater, a tropical fatal disease of ruminants
- ✓ Lack of efficient vaccines due to high genetic diversity
- ✓ Genomic sequence for 3 virulent strains: Gardel, Senegal & Welgevonden

OBJECTIVE

- ✓ To understand the mechanisms of ER pathogenicity by differential gene expression study at 2 stages of development of the Gardel strain

PROTOCOL



ER MICROARRAY RESULTS COMPARING T2 & T3

➤ 5% (54/950) of ER genes identified by arrays are differentially expressed between intermediate T2 & late T3 stages of development

➤ Categories of gene functions

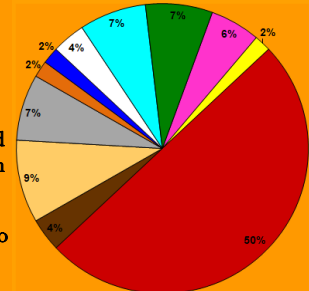
Mainly unknown function (50%)

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-expression	Genes	ratio log2	Functions
T2>T3	<i>Map1-6</i>	5.87	Map1-related protein
	<i>SecF</i>	5.18	Intracellular trafficking, secretion, and vesicular transport
	<i>dapA</i>	1.96	Amino acid transport and metabolism + Cell wall/membrane/envelope biogenesis
	<i>sppA</i>	1.65	Posttranslational modification, protein turnover, chaperones
T3>T2	<i>trx</i>	-3.16	Posttranslational modification, chaperones
	<i>cytoC1</i>	-4.58	Energy production and conversion
	<i>atpB</i>	-4.65	Energy production and conversion
	<i>resol</i>	-4.68	Replication, recombination and repair
	<i>ccmB</i>	-4.91	Posttranslational modification, chaperones
	<i>proP1</i>	-5.28	Transport and metabolism of Carbohydrates + Amino acids + Inorganic ions
	<i>lolD</i>	-5.54	Defense mechanisms
	<i>folK</i>	-6.16	Coenzyme transport and metabolism
	<i>Hypo</i>	-8.17	Hypothetical protein
	<i>dkSA</i>	-8.23	Signal transduction mechanisms

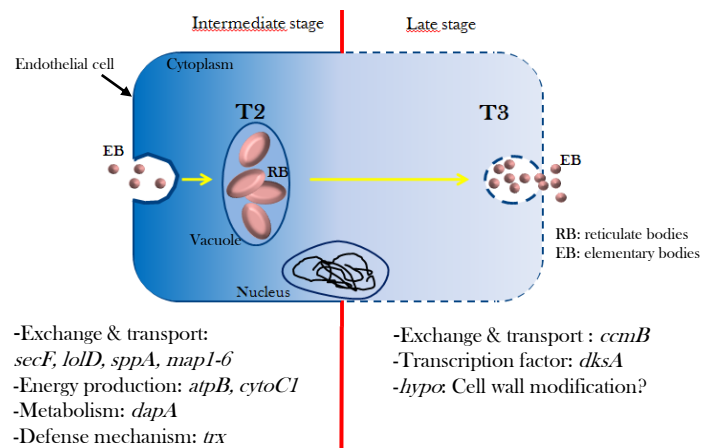
VALIDATION OF GENE OVEREXPRESSION FOR 14 GENES BY Q-RTPCR



➤ 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 oxidative stress

FUNCTIONS OF OVEREXPRESSED GENES



CONCLUSION

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.