

# Multi-locus approaches for the characterization of the diversity of the *Rickettsiales Ehrlichia ruminantium*



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## INTRODUCTION

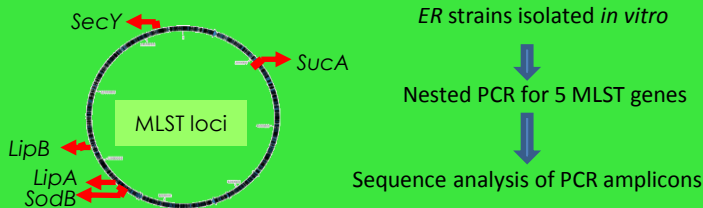
- ✓ *Ehrlichia ruminantium*, ER: agent of heartwater, a tropical fatal disease of ruminants
- ✓ Present in Sub-Saharan Africa, in Indian ocean islands & Caribbean islands
- ✓ Lack of effective vaccines due to high genetic diversity
- ✓ Genetic characterization using single genes: *pCS20* & *map-1*

## OBJECTIVES

- ✓ To develop new multi-locus approach based on VNTRs (Variable Number of Tandem Repeats) for ER
- ✓ To characterize the ER genetic diversity by VNTRs & MLST (Multi-Locus Sequence Typing)

## METHODS

- ✓ For MLST typing: 5 genes *LipA*, *LipB*, *SecY*, *SodB* & *SucA*



- ✓ For VNTRs typing:



- Design of primers & optimization of PCR conditions for single & nested PCRs

- Obtention of allelic profile with several VNTRs
- Determination of index of discrimination (I.D.):

$$I.D. = 1 - \frac{1}{N(N-1)} \sum_{j=1}^s nj(nj-1)$$

*N* = total number of strains  
*n<sub>j</sub>* = number of strains with same allelic profile

- ✓ ER strains from different areas analysed by VNTRs & MLST

Isolate/Strain	Name	Passages	Origin	Geographical area
Gardel	ERGA p18	18		Caribbean
	ERGA p237*	237	Guadeloupe	
Blonde	BLONDE p8	8		
Bekuy 255	ERBE p9	9		West Africa
Bankouma 421	ERBAK p4	4	Burkina Faso	
Banan 112	ERBA p7	7		
Cameroon	ERCA p9	9	Cameroon	
Sankat 430	ERSA p4	4	Ghana	
Senegal	ERSE p5	5	Senegal	
	ERSE p64*	64		
Lutale	ERLU p2	2	Zambia	South Africa
Mara	ERMA p1	1	South Africa	
Umpala	ERUM p2	2	Mozambique	
Welgevonden	ERWE p11	11	South Africa	

\* Attenuated strain

## DEVELOPMENT OF VNTRs USING SINGLE & NESTED PCRS

- ✓ Based on ER genome data from Gardel and Welgevonden strains 21 VNTRs were chosen using the tandem repeats database (<http://minisatellites.u-psud.fr>)
- ✓ 16 / 21 single PCRs & 7 / 21 nested PCRs targeting VNTRs were successfully developed when tested on 17 ER strains
- ✓ The global ID is 0.97 either for 16 or 7 VNTRs

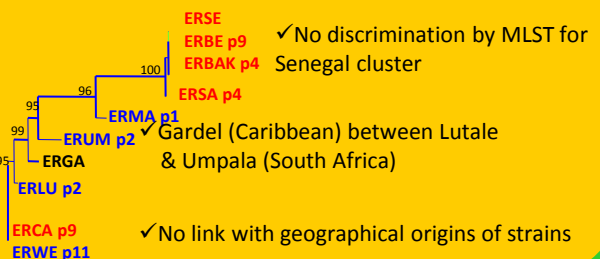
## ER GENETIC CHARACTERIZATION BY MLST & VNTRs

### Allelic profiles using 7 VNTRs (RU)

ER strain	RU-6	RU-11	RU-12	RU-13	RU-14	RU-15	RU-19	Origin
ERGA p18								Caribbean
ERGA p237*								
BLONDE p5								
ERBE p9								West Africa
ERBAK p4								
ERBA p7								
ERCA p9								
ERSA p4								
ERSE p5								
ERSE p64*								South Africa
ERLU p2								
ERMA p1								
ERUM p2								
ERWE p11								
Nb of alleles	3	4	5	3	5	3	4	

- ✓ There was no difference of VNTR profiles between:
  - Virulent & attenuated strains
  - Gardel & Blonde isolated in the same area 19 years apart
- ➔ Good stability of VNTRs
- ✓ 3 to 5 different alleles per VNTR

### MLST Neighbor Joining tree



## CONCLUSION

- ✓ Successful development of ER VNTRs using nested PCRs
- ✓ Similar global profiles obtained by MLST & VNTRs
- ✓ But VNTR more discriminatory than MLST
- ✓ Good epidemiological molecular tools
- ✓ Extension of the study to larger panel of ER strains for further phylogenetic study