Characterization of the genetic diversity of the *Rickettsiales Ehrlichia ruminantium*, at the world scale, using MLVA and MLST

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INTRODUCTION

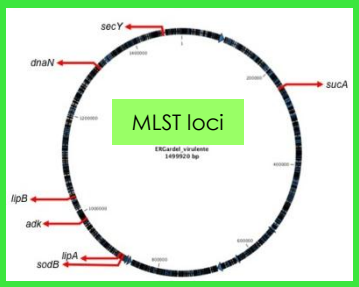
- ✓*Ehrlichia ruminantium*, *ER*: agent of heartwater, a tropical fatal disease of ruminants
- ✓Present in Sub-Saharan Africa, in Indian ocean islands & Caribbean islands
- ✓Lack of efficient vaccines due to high genetic diversity
- ✓Genetic characterization using single genes: *pCS20 & map-1*

OBJECTIVES

- ✓To develop epidemiological molecular tool based on VNTRs (Variable Number of Tandem Repeats) for *ER*
- ✓To characterize the *ER* genetic diversity by MLVA (Multi-Locus VNTR Analysis) & MLST (Multi-Locus Sequence Typing)

METHODS

✓**For MLST typing:** 5 genes *lipA*, *lipB*, *secY*, *sodB* & *sucA*



Reference *ER* strains & field samples

⇩

Nested PCR for 5 MLST genes

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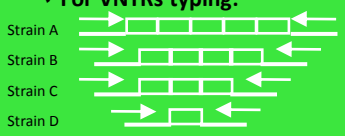
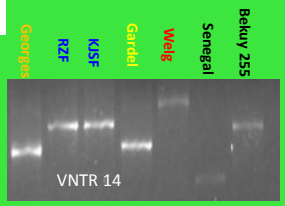
Sequence analysis of PCR amplicons

✓**For VNTRs typing:**



Different sizes of PCR products

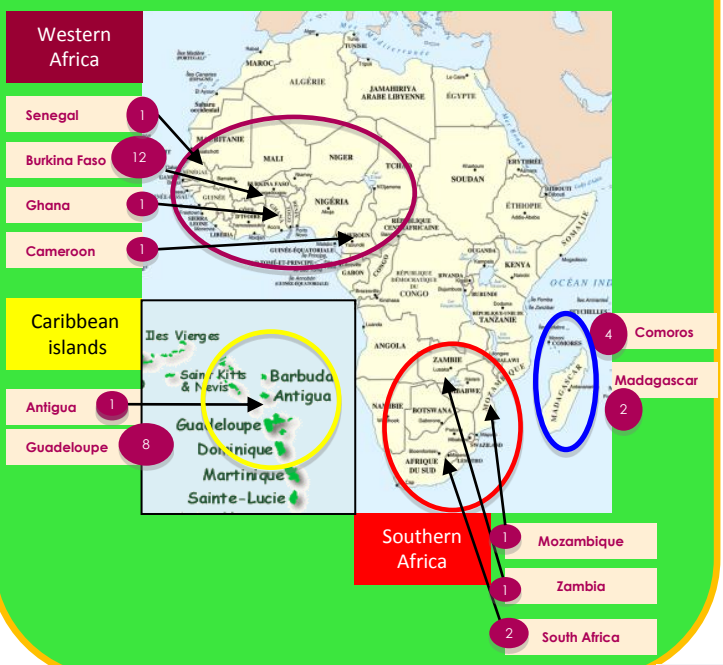


- Design of primers & optimization of PCR conditions for single & nested PCRs on Gardel & Welgevonden *ER* strains
- Obtention of allelic profile with several VNTRs allowing MLVA
- Determination of index of discrimination (I.D.) on 21 strains:

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

N= total number of strains
nj= number of strains with same allelic profile

✓***ER* strains from different areas analysed by MLVA & MLST**



DEVELOPMENT OF VNTRs USING 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)

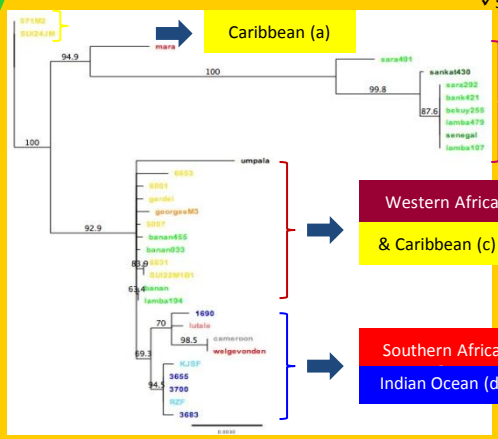
✓7 nested PCRs targeting VNTRs were succesfully developped on 21 *ER* strains: Direct use for field samples ticks & organs

✓Global ID = 0.97 for the 7 VNTRs
3 to 7 different alleles per VNTR
0.59 < individual ID < 0.78 ID > 0.76 for 3 VNTRs

ER GENETIC CHARACTERIZATION BY MLST & VNTRs

MLST NJ tree

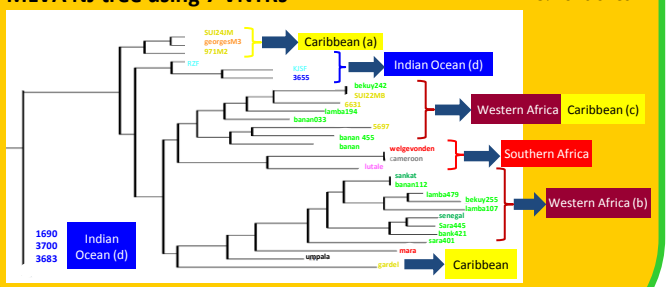
✓51% of clones



✓2 differentiated clusters with Caribbean (a) & Western African strains (b)
✓One cluster with Western African & Caribbean strains (c)
✓Indian ocean strains closely linked to South African strains (d)

MLVA NJ tree using 7 VNTRs

✓25% of clones



✓Similar clusters for Caribbean(a), Western Africa (b), West Africa & Caribbean(c) strains
✓Different clusters with Indian Ocean strains (d) & discrimination within clusters (b) & (c)
✓2 caribbean strains belonging cluster (c) by MLST are strongly different by MLVA

CONCLUSION & PERSPECTIVES

- ✓Successful development of molecular tools for phylogeny studies
- ✓Identification of clusters linked to geographical origins
- ✓Confirmation of the West African origin for the Caribbean strains
- ✓Identification of recent genetic divergence by MLVA for IO & Caribbean strains
- ✓Additional strains to confirm the Indian Ocean clusters
- ✓Current studies at regional level in Caribbean & Indian Ocean regions

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