Assessment of *Trypanosoma rangeli* tissue tropism in mice through bioluminescence imaging

Adriana Corrêa da Silva[1], Laryssa V. de Liz[1], Carime L. M. Pontes[1], Francisco Olmo[2], Amanda F. Francisco[2], Fernanda C. Costa[2], Shiromani Jayawardhana[2], Martin C. Taylor[2] Patrícia H. Stoco[1], John M. Kelly[2], Edmundo C. Grisard[1]

1 – Department of Microbiology, Immunology and Parasitology, Universidade Federal de Santa Catarina – Florianópolis – Brazil.
2 – Department of Infection Biology, London School of Hygiene and Tropical Medicine – London – United Kingdom.

INTRODUCTION

Trypanosoma rangeli is a non-pathogenic parasite of mammals, in which, several aspects of the parasite life cycle such as tissue tropism or replication sites are unknown. Here, we describe a new bioluminescence imaging tool allowing assessment of the infection biology *in vivo*.

RESULTS

Design of the pTRIXrang plasmid

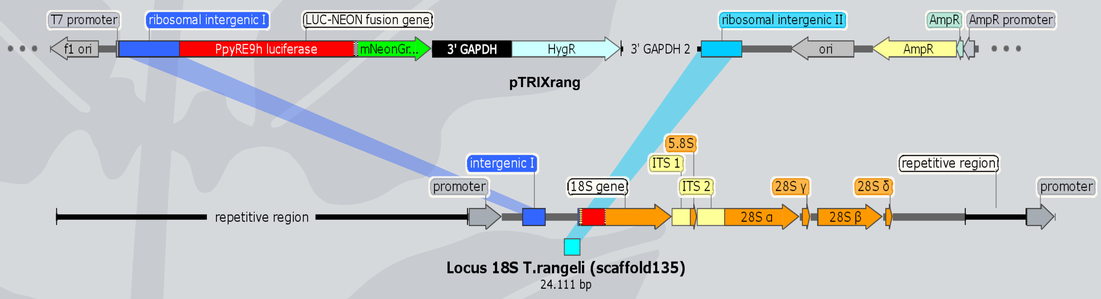
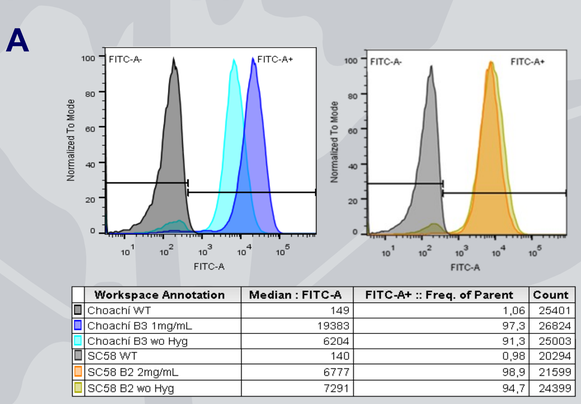


Figure 1: pTRIXrang plasmid was built through substitution of *Trypanosoma cruzi* homology arms present on the pTRIX2 Luc::Neon plasmid (Costa et al., 2018) by the intergenic regions of *T. rangeli* rDNA locus (blue regions). Top scheme: Integration cassete of pTRIXrang. Bottom: *T. rangeli* rDNA locus representation with the pTRIXrang homology arms located upstream of the 18S gene.

pTRIXrang promotes stable expression of Luc::Neon



Workspace Annotation	Median : FITC-A	FITC-A+ :: Freq. of Parent	Count
Choachí WT	149	1,06	25401
Choachí B3 1mg/mL	19383	97,3	26824
Choachí B3 wo Hyg	6204	91,3	25003
SC58 WT	140	0,98	20294
SC58 B2 2mg/mL	6777	98,9	21599
SC58 B2 wo Hyg	7291	94,7	24399

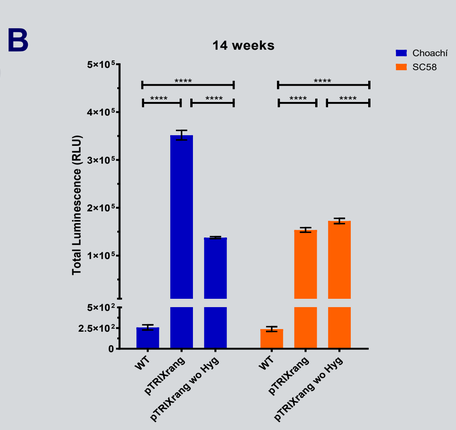
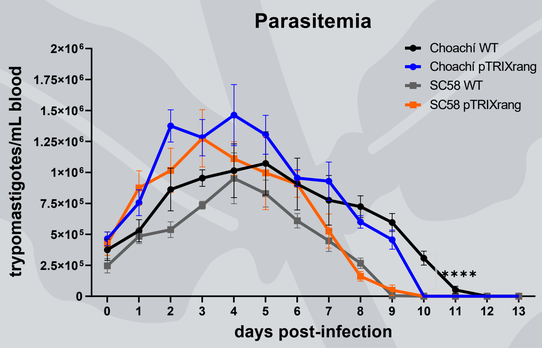


Figure 2: A) Flow cytometry analysis of *T. rangeli* epimastigotes cultivated in the presence (dark colours) or absence (light colours) of hygromycin 14 weeks post-transfection with the pTRIXrang plasmid. Choachí pTRIXrang lineage (blue), SC58 pTRIXrang (orange) and Wild Type strains (grey). Internal legend of each histogram with the median of mNeongreen intensity, fluorescent population rate and cell count. **B)** Luciferase activity *in vitro* of epimastigotes (2x10^6 parasites lysate) after 14 weeks post-transfection. Data expressed as mean and standard error of five replicates. Statistical analysis: Two-way ANOVA with Bonferroni's multiple comparisons test. (***) p<0.001, (****) p<0.0001.

Expression of Luc::Neon does not alter *T. rangeli* biology



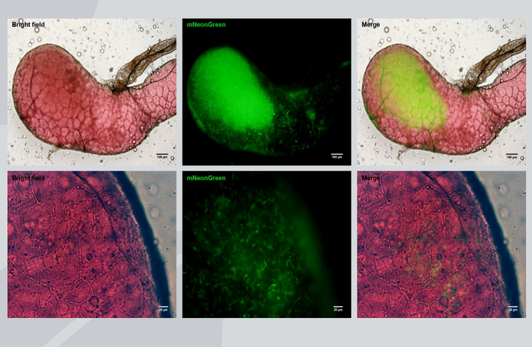


Figure 3: A) Comparative parasitemia of transfected and parental strains in BALB/c mice as determined by the Brener-Pizzi method. Observation on day 0 was carried out 6 h post-infection. Statistical analysis: Two-way ANOVA with Bonferroni's multiple comparisons test. Choachí WT vs Choachí pTRIXrang p=0.325; SC58 WT vs SC58 pTRIXrang p=0.187. (For multiple comparison (****) p<0.0001). **B)** *Rhodnius prolixus* salivary gland infected by Choachí pTRIXrang 2 months after intracoelomic infection with epimastigotes.

T. rangeli infection dynamics by *in vivo* bioluminescence

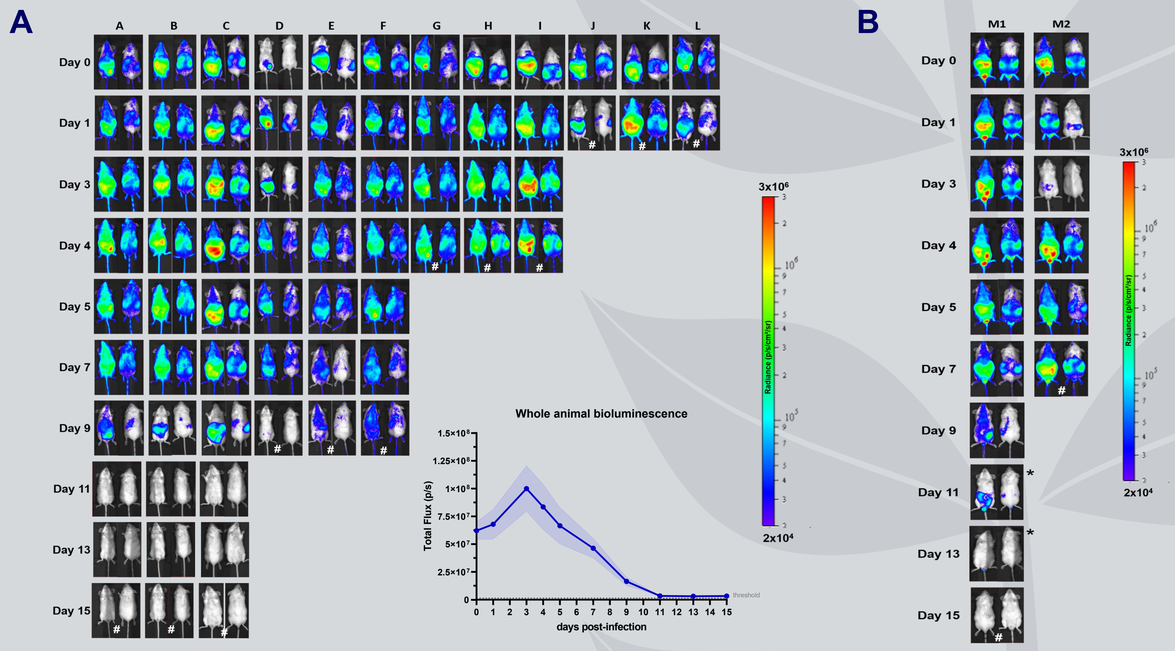


Figure 4: A) BALC/c female mice infected by intraperitoneal injection of 1x10^7 Choachí pTRIXrang trypomastigotes. # indicates culled mice at each time points for *ex vivo* analysis. Internal graph: Total bioluminescence data (sum of ventral and dorsal) as mean and SEM. Threshold establish by control luminescence. **B)** Infection course of BALC/c male mice. *Imagens acquired in different binning (radiance scale 3x10^3 to 3x10^4).

T. rangeli accumulates in the lungs and spleen during the acute phase of infection

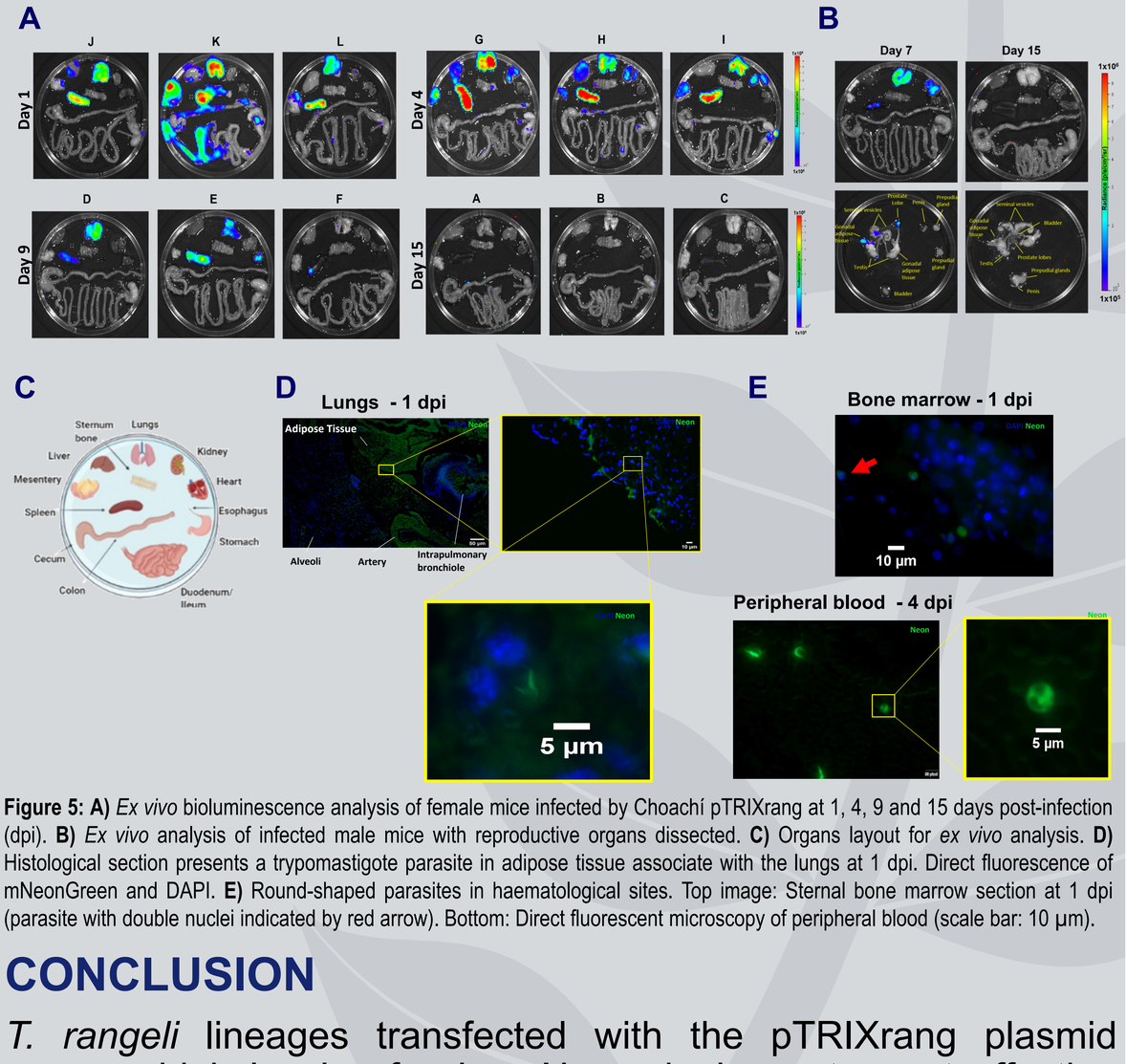


Figure 5: A) *Ex vivo* bioluminescence analysis of female mice infected by Choachí pTRIXrang at 1, 4, 9 and 15 days post-infection (dpi). **B)** *Ex vivo* analysis of infected male mice with reproductive organs dissected. **C)** Organs layout for *ex vivo* analysis. **D)** Histological section presents a trypomastigote parasite in adipose tissue associate with the lungs at 1 dpi. Direct fluorescence of mNeonGreen and DAPI. **E)** Round-shaped parasites in haematological sites. Top image: Sternal bone marrow section at 1 dpi (parasite with double nuclei indicated by red arrow). Bottom: Direct fluorescent microscopy of peripheral blood (scale bar: 10 µm).

CONCLUSION

T. rangeli lineages transfected with the pTRIXrang plasmid express high levels of a Luc::Neon dual-reporter, not affecting the parasite life cycle progression in the vector as well as in the mammal host and allowing detection of parasites in distinct tissues. During the acute phase of infection, the highest parasite burden was detected in the spleen and the lungs.

SUPPORT:

CAPES · Print UFSC · CNPq · Finep INOVAÇÃO E PESQUISA