INTERACTION BETWEEN THE DOG GENETIC BACKGROUND AND DISTINCT GENOTYPES OF *Leishmania infantum*

[1,3]Luís Fábio Batista, [2]Juliana G. Mariotti, [3]João L. Reis-Cunha, [1]Frederico M. Ferreira, [1]Vânia Lúcia da Matta, [1]Islam H. Chouman, [1]Thaise Y. Tomokane; [2]Patricia Sayuri S. Matsumoto; [4]Valéria M. Camprigher, [4]Virgínia B. R. Pereira, [5]Elisa Cupolillo, [2]José Eduardo Tolezano; [3]Daniel C. Jeffares & [5]Mariana C. Boité & [1]Márcia D. Laurenti.

[1]Laboratorio de Patologia de Moléstias Infecciosas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil; [2] Centro de Parasitologia e Micologia do Instituto Adolfo Lutz, São Paulo, São Paulo, Brazil; [3]Department of Biology, University of York, United Kingdom; [4]Centro de Laboratórios Regionais II Bauru, Instituto Adolfo Lutz, São Paulo, Brazil; [5]Laboratório de Pesquisa em Leishmaniose, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil.

INTRODUCTION

Genetic variability of Trypanosomatids may impact the outcome of infection, drug resistance or epidemiological traits. We hypothesized that *Leishmania infantum* strain deleted (DEL) for the Miltefosine Sensitivity Locus (MSL) cause a subclinical and less detectable infection in the canine reservoir, which might persist for long-term in endemic areas, contributing to the greater spread of DEL than Non-DEL strain.

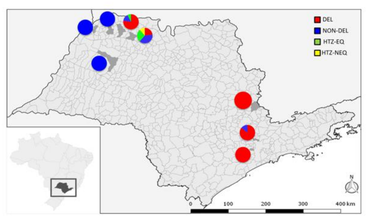
AIM

To better understand the impact of the MSL on the clinical-immunological outcome in dogs naturally infected by *L. infantum*.

METHODS

METHODS

Municipalities	Cases
Bauru	29
Votuporanga	10
Andradina	7
Caieiras	6
Fernandópolis	2
Araçatuba	1
Belo Horizonte	1
Brasília	1
Embu das Artes	1
Jales	1
Itapira	1
Total	**60**



Previously studied municipalities of São Paulo state

+

Bauru – São Paulo

✓ **Diagnosis:**

- Dual-Path Platform (DPP)
- Enzyme-linked immunosorbance assay (ELISA)
- Polymerase chain reaction (PCR)

✓ **Clinical evaluation**

✓ **DNA isolation from popliteal lymph node**

33/60 DEL *L. infantum*

Genotyping (qPCR)

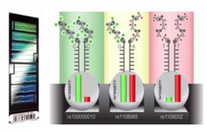
27/60 Non-DEL *L. infantum*

ELISA

anti-*L. infantum* IgG, IgM, IgA, IgE
anti-*Lu. longipalpis* SGL IgG

qPCR

GWAS



Clinical evaluation
Humoral response
Parasite load

GWAS Workflow

Genotyping of 237 samples by 173,662 SNPs CanineHD BeadChip (Illumina)

Samplae structure analysis
Principal componentes analysis (C1 e C2)
1. GENETIC CLUSTER ASSOCIATED TO MSL

Filtration (VCFtools + Plink)
SNP call rate ≥ 95% / MAF 5% / Fisher for HWE p ≥ 1 x 10-5 / sample call rate > 90%

GWAS – LDAK – Linear Mixed Model: y~ TRAIT + kinchip matrix + covariates
Heritability analysis - LDAK – REML (restricted maximum likelihood): y ~ TRAIT + covariates + kinchip matrix

Binary traits: MSL, clinical status

Quantitative traits: clinical staging, parasite load, anti-*L. (L) infantum*IgA, IgE, IgM, IgG, anti – SGL *Lu. longipalpis* IgG

Genome-wide association analysis (234 amostras e 118,786 SNPs)
2. GWAS for ONLY MSL as trait or MSL + covariates → **TO INVESTIGATE SNP ASSOCIATED TO MSL**
3. GWAS for clinical-imunological traits + MSL as covariate → **IF MSL CHANGE THE SIGNIFICANCE**
4. Heritability estimation → **PROPORTION OF VARIATION IN CLINICAL-IMMUNOLOGICAL TRAITS EXPLAINED BY MSL**
- Effect of substructure were examined in Q-Q plots
- Markers with p-value < permuted cutoff (5% percentile) were prioritized for functional analysis

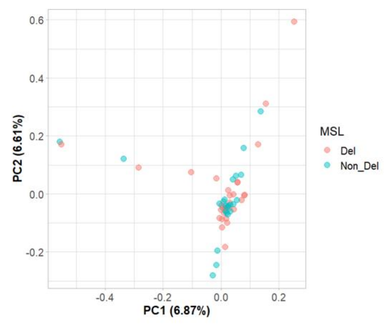
Functional analysis (1Mb)
Candidate gene mapping (Ensembl Genes 64, CanFam 3.1, coordinated by orthology with Non-Dog RefSeqGenes in UCSC Genome Browser v323)

RESULTS

MSL is not linked to the dog genetic background

Principal Component Analysis

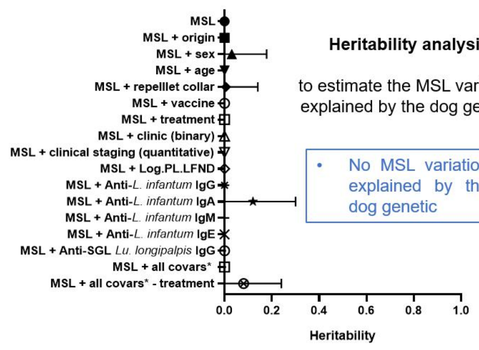
to identify genetic cluster associated to MSL



- No genetic structure associated to MSL

Heritability analysis

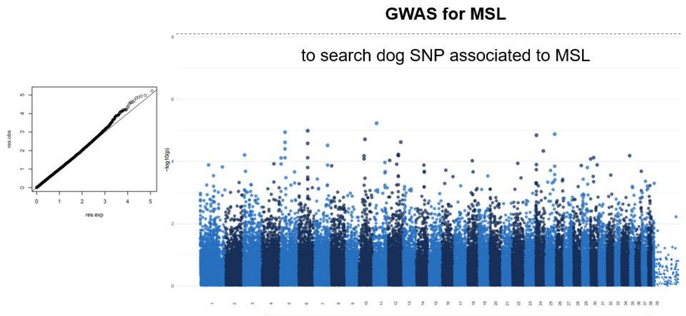
to estimate the MSL variation explained by the dog genetic



- No MSL variation explained by the dog genetic

GWAS for MSL

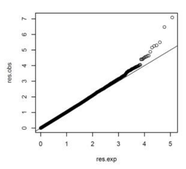
to search dog SNP associated to MSL



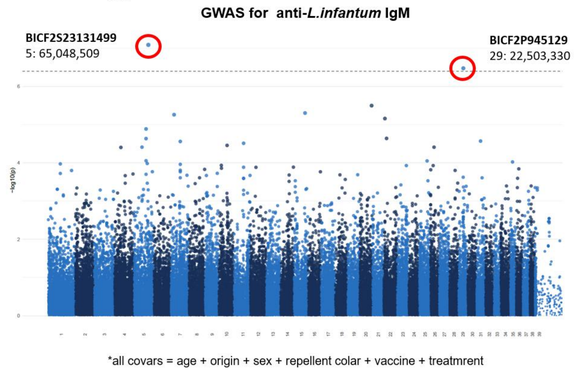
- No significant SNP associated to MSL

DEL strain infection was associated to low levels of anti - *L. infantum* IgA, IgG and increased the significance of SNPs associated to IgM and IgG

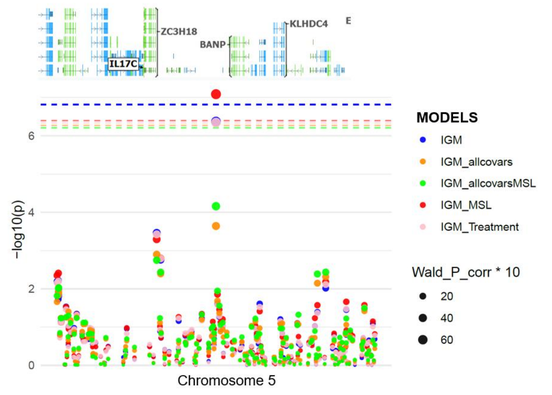
SNP marker	GWAS P-value	Allele associated to ↓ IgG	*L. infantum* genotype	AA	AB	BB	Association P-value	Candidate genes
BICF2G630654815 (IgG SNP)	9,64x10^{-6}	A	DEL	21	7	1	P < 0,0001 chi-square	*RAB38, CTSC, NOX4*
			Non-DEL	3	5	11		



GWAS for anti-*L.infantum* IgM



*all covars = age + origin + sex + repellent colar + vaccine + treatmrent



CONCLUSION

These data suggest that although there is no dog genotype associated to predominant adaptation of DEL or Non-DEL strains during the *L. infantum* infection, distinct MSL genotypes may differently interact with the host's immune system, leading to different clinical-immunological outcomes and effect of their genetic basis, potentially impacting the epidemiology of the canine visceral leishmaniasis.

Supported by:

FAPESP · INSTITUT PASTEUR · FIOCRUZ Fundação Oswaldo Cruz · LIM HC · FMUSP