XLVIII Congresso Nazionale AMCLI
Palacongressi di Rimini
9 - 12 Novembre 2019

Development and Application of a High Resolution Melt (HRM)-based test for the Rapid Screening of *Leishmania infantum* Genotypes

Gloria Buffi[1], Aurora Diotallevi[1], Marcello Ceccarelli[1], Francesca Andreoni[1], Daniela Bencardino[1], Michelealberto Abruzzese[1], Germano Castelli[2], Federica Bruno[2], Fabrizio Vitale[2], Mauro Magnani[1], Luca Galluzzi[1]

[1]Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Italy
[2]Istituto Zooprofilattico Sperimentale of Sicily "A Mirri", Italy

INTRODUCTION

Leishmaniasis includes anthropo-zoonotic infectious diseases caused by a protozoan of the *Leishmania* genus, associated with different clinical manifestations, affecting both humans and other vertebrates, including dogs. The Mediterranean basin, including Italy, is considered an endemic area for both visceral and cutaneous leishmaniasis caused by *L. infantum*. The multi-locus enzyme electrophoresis (MLEE), based on the electrophoretic mobility of several enzymes from promastigotes cultures, is considered the reference method for parasite typing. Through this method, about 45 *L. infantum* zymodemes (also termed MON) have been identified in humans in the Mediterranean basin [1]. Among these, *L. infantum* MON-1 is the most widespread, representing about 70% of all identified strains. In Italy, canine infections showed a high prevalence of MON-1 (91%) with the remaining composed almost exclusively of MON-72 [2]. Since MLEE technique is cumbersome, time-consuming and requires parasites isolation, several biomolecular approaches have been developed. In particular, we identified the SNP 390T>G in malic enzyme (ME) gene as a potential marker to differentiate the most common *L. infantum* genotype, i.e. 390T (corresponding to zymodemes MON-1, 72, 201) from all others [3].

AIMS

This study aimed to develop a Rapid Genotype Screening (RGS) assay for *L. infantum* genetic characterization in clinical samples using high resolution melt (HRM) analysis, exploiting the polymorphism 390T>G in the ME gene.

METHODS

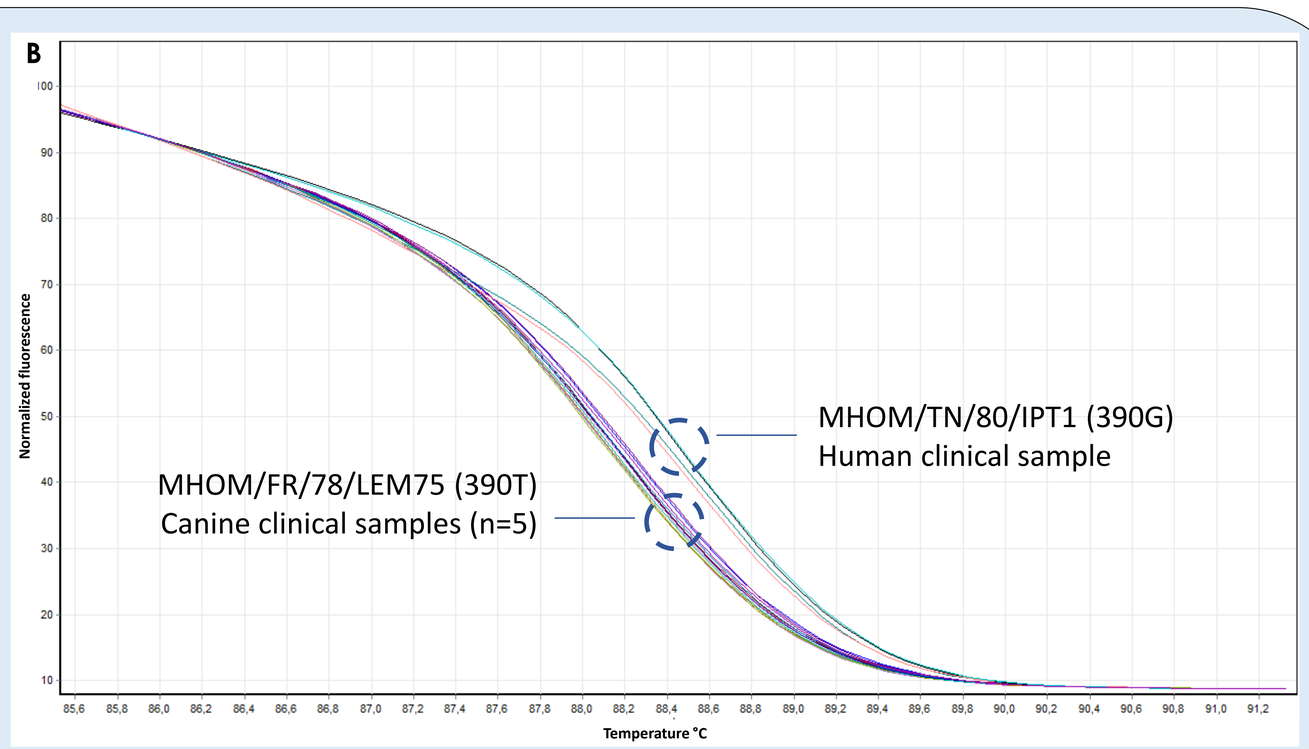
A qPCR assay followed by HRM analysis (qPCR-MEint) was optimized to rapidly identify the genotypes 390T or 390G. The qPCR-MEint was applied to 6 *L. infantum* clinical isolates, 4 human and 41 dog clinical samples positive for *L. infantum* collected in the Marche region and Pantelleria island. Four *L. infantum* strains were simultaneously processed as references for 390T and 390G genotypes. To ensure applicability on clinical samples, a pre-amplification step in conventional PCR, was introduced. Genotype attribution in clinical samples was performed by the Rotorgene 6000 v.1.7. software. The results of HRM analysis was verified by direct sequencing of DNA amplicons in selected samples.

A

L. infantum strain	Zymodeme	Sanger Sequence	qPCR MEint Genotype
MHOM/FR/78/LEM75	MON-1	390T	390T
MHOM/TN/80/IPT1	MON-1	390G	390G
MHOM/DZ/82/LIPA59	MON-24	390G	390G
MHOM/IT/86/ISS218	MON-72	390T	390T

(A) *Leishmania infantum* strains used as references for 390T and 390G genotypes
(B) HRM analysis example of qPCR-MEint amplicons with genotypes 390T and 390G. The HRM profiles allowed the assignation of human clinical sample and canine clinical samples to genotypes 390G and 390T, respectively. Strains MHOM/TN/80/IPT1 (genotype 390G) and MHOM/FR/78/LEM75 (genotype 390T) were used as references.

B



RESULTS

The qPCR-MEint showed a sensitivity of 100% and 80.5% in human and canine clinical samples, respectively. Overall, the results indicated that the genotype 390T was found in 97.3% of canine samples, confirming the high prevalence of the most common zymodemes (i.e. MON-1, MON-72) in dog population. Despite the limited number of human samples, it is noteworthy that the genotype 390T was only 25%, evidencing a different genotype distribution between humans and dogs and confirming that dogs could not be the only *reservoir* of infection for humans.

46 Canine Samples

41 Clinical samples (Cs)
- **30** lymph node
- **5** conjunctival swab
- **3** peripheral blood
- **3** bone marrow

5 Clinical isolates (Ci)

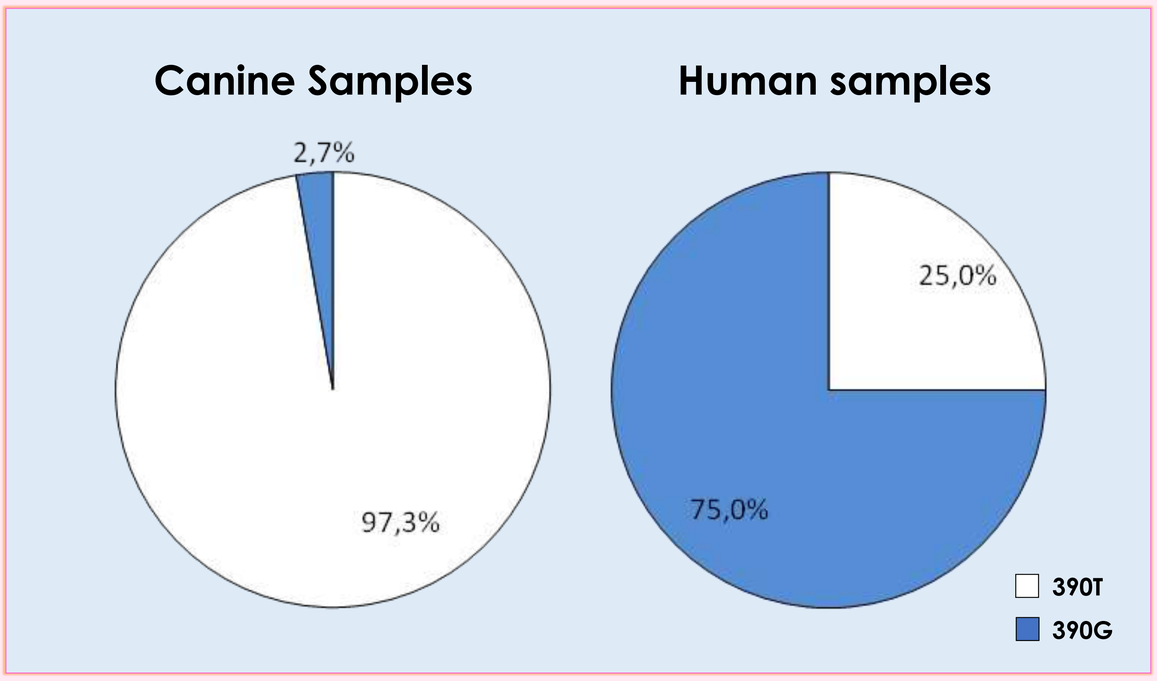
Genotyping:
- **8** Cs **not detectable**
- **33** Cs + **4** Ci **390T**
- **1** Ci **390G**

5 Human Samples (4 patients)

4 Clinical samples (Cs)
- **P1:** peripheral blood
- **P2:** biopsy
- **P3:** lesion swab
- **P4:** biopsy

1 Clinical isolate (Ci)
- **P4**

Genotyping:
- **P1: 390T**
- **P2-P3-P4: 390G**



CONCLUSIONS

The results demonstrated the applicability of our RGS assay for *L. infantum* in human and canine clinical samples from different sources, without the need for parasite cultivation. This assay could be useful to investigate the role of other mammalian hosts as infection *reservoirs* and could be exploited for rapid epidemiological screening.
In order to improve the genotyping discrimination, more polymorphisms in protein-coding genes can be exploited to design new HRM-based assays.

REFERENCES

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3.Ceccarelli M. et al. Parasit Vectors. 2018 Nov 1;11(1):572.