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PARASSITOLOGIA

DEVELOPMENT AND APPLICATION OF A HIGH RESOLUTION MELT (HRM)-BASED TEST FOR THE RAPID SCREENING OF LEISHMANIA INFANTUM GENOTYPES

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INTRODUZIONE

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, based on the electrophoretic mobility of several enzymes, is considered the reference method for parasite typing. Through this method, about 45 zymodemes (also termed MON) have been identified in the Mediterranean basin for L. infantum species. 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. Previously, we identified the SNP 390T>G in malic enzyme gene as a potential marker to differentiate the most common L. infantum zymodemes, i.e. MON-1, 72, 201 having genotype 390T, from all others.
The aim of this study was to develop high resolution melt (HRM)-based assays to identify the polymorphism 390T>G and to assess its applicability in canine and human clinical samples.

METODI

A qPCR assay followed by HRM analysis (qPCR-MEint) was optimized to rapidly identify the genotypes 390T or 390G. HRM analysis was performed over the range 85–95 °C. Data were analyzed by the Rotorgene 6000 software. The qPCR-MEint was applied to L. infantum clinical isolates (n=7), human (n=4) and dog (n=39) clinical samples positive for L. infantum collected in the Marche region and Pantelleria island. Four L. infantum reference strains were used for genotypes 390T and 390G. To ensure applicability with clinical samples, a pre-amplification step was introduced. The results of HRM analysis was verified by direct sequencing of DNA amplicons in selected samples.

RISULTATI

The qPCR-MEint showed a sensitivity of 100% and 79.5% in human and canine clinical samples, respectively. Overall, the results indicated that the genotype 390T was found in 96.8% of canine samples confirming the high prevalence of the most common zymodemes. On the other hand, the genotype 390T was found in only 25% of human clinical samples, evidencing a different genotype distribution between humans and dogs. The clinical isolates were all genotype 390T with one exception, isolated from a marten.

CONCLUSIONI

The applicability of our HRM-based rapid typing of L. infantum was demonstrated in human and canine clinical samples, without need for parasite cultivation. This assay could be useful to investigate the role of other mammalian hosts in rapid epidemiological screening.