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Digital droplet PCR: a novel tool for the diagnosis of *Leishmania* infection

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Background

Droplet digital PCR (ddPCR) is an alternative to "traditional" PCR for the detection of pathogen DNA, particularly when low quantities of target DNA are present in the samples. In comparison with other detecting methods, ddPCR ensures precision, sensitivity, and specificity, also providing absolute quantification of the target without a standard curve. For all these reasons, ddPCR can be considered an ideal tool for the detection of microorganisms such as *Leishmania* spp. [1].
Leishmania infantum is the main agent responsible for canine and human leishmaniasis in the Mediterranean area [2]. In the same area a reptile-associated *Leishmania*, *Leishmania tarentolae*, is also present [3]. Recently, DNA of *L. tarentolae* has been detected in human and dog blood in southern Italy, suggesting the possible exposure of mammals to this parasite [4,5]. Furthermore, *L. infantum* DNA was detected in blood samples taken from reptiles near dog shelters [6]. Tools for the detection and identification of these two species are thus needed.

Materials and methods

Here we present a protocol for the specific detection of *L. infantum* and *L. tarentolae*, based on a multiplex ddPCR targeted on minicircle DNA. Promastigote forms of *L. tarentolae* and *L. infantum* were used in this study. A standard curve for each species was prepared to optimize the protocol and a precise number of cells was resuspended in human blood and treated for DNA extraction using the Qiagen DNeasy Blood and Tissue kit. The obtained DNA was then used to produce a standard curve. The method was then tested on field-collected samples of reptiles and sand flies.

Results

After the amplification, the number of positive droplets was detected and quantified using a droplet reader. Currently, 107 total DNA samples have been tested, including five *Leishmania* laboratory strains and 102 field-collected samples. The obtained results highlighted that the ddPCR is more sensitive than the conventional PCR. The precise number of *Leishmania* cells present in a sample was determined by comparing the starting number of cells and the number of positive droplets in our standard curve, for both *L. infantum* and *L. tarentolae*. The use of two specific fluorescent probes allows *Leishmania* detection of up to 1 and 5 cells, for *L. tarentolae* and *L. infantum* respectively.

Conclusions

The obtained results confirmed the potential of ddPCR for *Leishmania* spp. identification, indicating the high sensitivity and specificity of the method on the tested species. Further analyses on additional collected samples are currently in progress.

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