Standardization of PCR-RFLP for molecular detection of *Toxocara canis and Toxocara cati*

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Introduction

Visceral larva migrans (VLM) is a clinical condition that can be caused by immature stages of some helminths, mainly nematodes (1). Parasites belonging to the genus *Toxocara* (*Toxocara canis* and *T. cati*) are considered the main causal agents of this clinical presentation (1, 2). Being a zoonosis, the reservoirs of the parasitosis are animals, mainly domestic dogs and cats (Fig. 1), whose feces with immature eggs reach the ground, where they reach maturity, entering the human being orally, through contaminated water and food or geophagy (2, 3). The purpose of this work was standardize the molecular detection techniques of *T. canis* and *T. cati*, by means of PCR-RFLP (Polymerase Chain Reaction-Restriction Fragments of Polymorphic Length), which would allow investigating the presence of eggs of these helminths in the environment.

Methodology

DNA from helminths identified as *T. canis* or *T. cati,* stored in the presence or absence of 70% ethanol at -20 °C, was extracted with the commercial Machery-Nagel kit, after overnight digestion at 56 °C. The quality of the extracted DNA was evaluated in terms of concentration and integrity. PCR was standardized to amplify the region of rDNA comprising ITS-1, 5.8 S, ITS-2, carried out with the primers NC2 and NC5, described by Zhu *et al*. (4). The sensitivity of the PCR was evaluated in terms of the minimum amount of DNA that generated a visible amplified in agarose gel. The characterization of the parasites was carried out by RFLP by digesting the amplified with the endonucleases *Hinf* I, *Rsa* I and *Hae* III**.**

Results

The DNA obtained was between 1.29 and 11.9 ng/µL, with a considerably high degree of degradation. An amplified 1180 base pairs (bp) for genus *Toxocara* spp. was achieved. The sensitivity of the PCR was 5 pg/µL of DNA (Fig. 2). After RFLP, it was possible to molecularly differentiate both species. For *T. cati* an RFLP for the *Hae* III enzyme of 595, 180, 156, 72 and 24 bp were obtained and for *Rsa* I an RFLP of 652, 278 and 103 bp. For *T. canis* an RFLP with *Hinf* I of 550, 395, 94 and 54 bp was obtained; for *Hae* III an RFLP of 850, 198, 57 and 16 bp and for *Rsa* I an RFLP of 500 and 288 bp (Fig. 3).

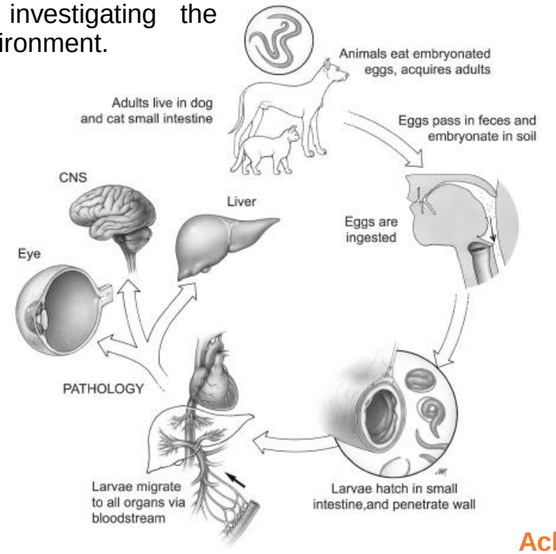

Figure 1. *T. canis and T. cati*, the main causal agents of VLM (1)

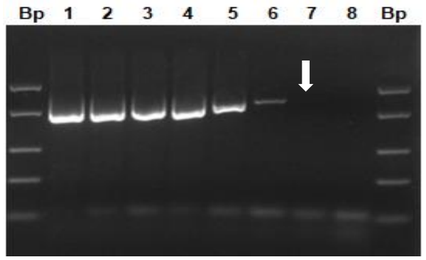

Figure 2. Sensitivity of the PCR that amplifies the region ITS-1, 5.8 S, ITS-2 for *Toxocara* spp. Lines: 50 ng/µL; 2. 10 ng/µL; 3. 5 ng/µL; 4. 0,5 ng/µL; 5. 0,05 ng/µL; 6. 50 pg/µL; 7. 5 pg/µL. Bp. base pair marker (# SM1113; GeneRuler ™, ThermoScientific, USA). Arrow points to limit of detection.

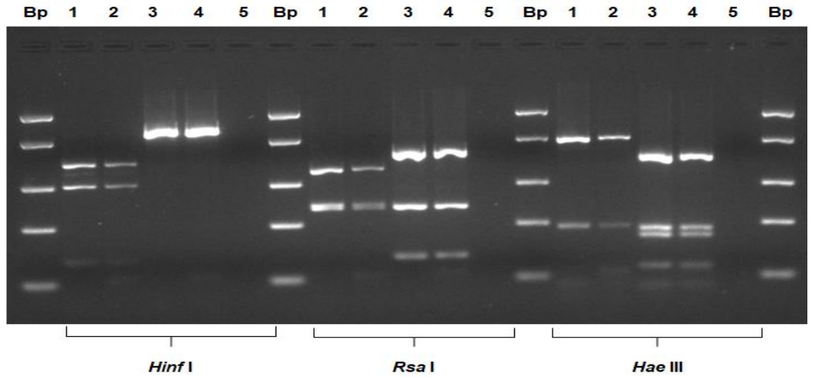

Figure 3. Molecular identification of *T. canis* and *T. cati*, by RFLP with the enzymes *Hinf* I, *Rsa* I, *Hae* III. Lines: 1 and 2. *T. canis´* RFLP; 3 and 4. *T. cati´*s RFLP Bp. base pair marker (# SM1113; GeneRuler ™, ThermoScientific, USA).

Conclusions

The PCR amplified a DNA segment of the ITS-1, 5.8 S, ITS-2 region of approximately 1180 bp, with a sensitivity of 50 pg/µL. The RFLP obtained allowed discriminating between *T. canis* and *T. cati*, which coincided with the polymorphism found by the Bioinformatics pathway. The PCR-RFLP thus standardized can be used for the molecular detection of *T. canis* and *T. cati* that causes VLM.

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Further information

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