Standardisation of the PCR-RFLP for the molecular detection of Toxocara cani and Toxocara cati

Main author: Maria Alejandra Vethencourt Ysea (University of Medical Sciences (UCIMED))

Co-authors: Valerio Idalia

INTRODUCTION

Visceral larva migrans (VLM) is a clinical condition that can be caused by immature stages of some helminths, mainly nematodes. Depending on its anatomical location, the infection can range from being asymptomatic to causing serious health problems that compromise the lives of infected patients. Parasites belonging to the genus Toxocara (Toxocara canis and T. cati) are considered to be one of the main causal agents of this clinical presentation, being reported with some frequency in paediatric patients around the world. Being a zoonosis, the reservoirs of the parasites are animals, mainly domestic dogs and cats, whose faeces which contain immature eggs reach the ground, where they reach maturity, entering human beings orally, through contaminated water and food or by geophagy. Diagnosis in humans is carried out with serological methods, however, cross-reactions with other ascarididae are not uncommon, so molecular methods stand out as a more sensitive and specific alternative for their diagnosis, in addition, they would investigate the presence of eggs of these helminths in the environment.

METHODOLOGY

DNA from helminths identified as Toxocara cani 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 standardised to amplify the region of rDNA comprising ITS-1, 5.8 S, ITS-2. The PCR was carried out with the primers NC2 and NC5, described by Zhu et al., 2000. The sensitivity of the PCR was evaluated in terms of the minimum amount of DNA that generated a visible amplified in agarose gel. The characterisation of the parasites was carried out by RFLP (length polymorphism obtained with restriction enzymes) by digesting the amplified with the endonucleases Hinf I, Rsa I and Hae III. The agarose gels were visualised with the aid of a UV transilluminator (Slimline Series; Spectroline), the image was captured with an image digitiser (Enduro TM GDS, Labnet international, Inc.). The RFLPs obtained will be analysed with the help of TotalLab 1D software, version 14.0.

RESULTS

The DNA obtained was between 1.29 and 11.9 ng / uL, with a considerably high degree of degradation. Despite this, PCR amplification was achieved, both for adults samples stored in alcohol or not stored in alcohol, of an amplification of approximately 1180 base pairs (bp), corresponding to the literature. The standardised PCR managed to amplify up to 5 pg / µL of DNA. Through RFLP analysis, it was possible to molecularly differentiate T. canis from T. cati. The analysis of the partial sequence of the region to be amplified for T. cati (Genebank MX309925.1; of 974 bp) and T. cani (Genebank JN617989.1 of 968 bp) by means of RFLP in a Bioinformatic way (http: // www. bioinformatics.org/sms2/rest_digest.html) coincided with the obtained polymorphism. In this regard. For T. cati only cut points for Hae III enzyme (RFLP: 595, 180, 156, 72 and 24 bp) and for Rsa I (RFLP: 652, 278 and 103 bp) were obtained. For T. canis it was possible to obtain RFLP for the 3 enzymes. For Hinf I an RFLP 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.

DISCUSSION

The PCR-RFLP thus standardised can be used for the molecular detection of the species that causes VLM, in addition to serving as a molecular tool for the investigation of eggs of these helminths in soils, waters or vegetables that are consumed raw. On the other hand, this molecular technique could be useful to carry out a first approach in the investigation of Ascarididae that infect fish for human consumption.