ABSTRACT

Amplification of DNA from *Angiostrongylus cantonensis* (Metastrongylida) recovered from wild rats in Jamaica, with an assessment of the diversity of PCR products using SSCP analysis

Cheridah Danique Todd

Angiostrongylus cantonensis is the main cause of eosinophilic meningitis in humans. Outbreaks of angiostrongylosis underscore the importance of practical knowledge of the parasite's life cycle and its distribution in the environment in order to control transmission. Morphological identification and serological tests performed by Enzyme-Linked Immunosorbent Assay (ELISA) are accurate methods of detection. However, morphological tests are tedious and time consuming and tests performed by ELISA are sometimes unavailable.

This study assessed the usefulness of a species-specific Polymerase Chain Reaction (PCR) combined with Single-Strand Conformation Polymorphism (SSCP) analysis. Fifty four worms recovered from trapped wild rats (*Rattus rattus* and *R. norvegicus*) captured from various parishes in Jamaica, and stored in 95% alcohol, were analyzed. Genomic DNA extraction entailed the disruption and lysis of worm tissue followed by the removal of proteins and other contaminants. Purified DNA extracted from adult *A. cantonensis* was subjected to PCR using AC-1/AC-2 primers or CT-1/CT-2 primers.

There was limited success with AC-1/AC-2 primers as it only amplified material from female worms. CT-1/CT-2 primers successfully amplified 54% (29/54) of samples analyzed to yield the expected product. The inability of PCR to amplify DNA from the remaining 46% of the worms could be due to poor lysis, incomplete homogenization or centrifugation, poor binding of DNA to diatom, poor elution or the necessity to rehydrate the worms in water prior to DNA extraction. Single Strand Conformation Polymorphism analysis of all the PCR amplicons gave similar banding patterns on ethidium bromide stained polyacrylamide gels. While PCR-SSCP is able to detect single-nucleotide polymorphisms (SNPs), it did not detect any differences in the PCR amplicons. Despite the level of success, this is the first report of this extent of achievement of amplification of DNA from worms stored in 95% alcohol, and strongly suggests that PCR combined with SSCP can be used for analysis of *A. cantonensis* in Jamaica.

Keywords: *Angiostrongylus cantonensis*; *Rattus rattus*; *R. norvegicus*; Polymerase-chain reaction (PCR); Single Strand Conformation Polymorphism (SSCP); eosinophilic meningitis; Jamaica