DETECTION OF THE LARVAL STAGES OF FASCIOLA HEPATICA AND CALICOPHORON DAUBNEYI IN THE GALBA TRUNCATULA MOLLUSC BY MULTIPLEX PCR

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The aim of this study was to develop and validate a multiplex PCR technique using mitochondrial DNA (mtDNA) for the accurate and early detection of F. hepatica and C. daubneyi in G. truncatula, the mollusc intermediate host of both parasites. A total of 6208 G. truncatula specimens collected at 6 locations in Galicia (NW Spain) were examined under the stereo- and microscope to know if they harboured trematode larvae and to carry out their morphological identification. It was observed that molluscs were infected with F. hepatica (4.42%), C. daubneyi (1.70%) and other Digenea. All the infected and some non-infected molluscs were frozen in liquid nitrogen and stored at -85°C. Moreover, monospecific experimental infections of G. truncatula were carried out using 2-5 miracidia of F. hepatica and C. daubneyi and the molluscs were periodically slaughtered, microscopically examined for larval stage identification and then frozen until DNA extraction. In order to develop a multiplex PCR for simultaneous and specific detection of larval stages of F. hepatica and C. daubneyi in the intermediate host, we first designed primers which amplified mtDNA fragments specific for each parasite and of different sizes (425 pb for F. hepatica and 885 pb for C. daubneyi). The technique showed a high specificity when tested in molluscs infected with other Digenea larvae (Notocotyliidae, Plagiocchiidae, Schistosomatidae) which are usually found in the same intermediate host. Moreover, the PCR designed in this study showed great sensitivity in the early detection of the larval stages of F. hepatica and C. daubneyi in G. truncatula, as infection by both parasite species could be detected from the second day after the experimental infection.

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Palabras clave: Fasciola hepatica, Calicophoron daubneyi, Galba truncatula, PCR, mitochondrial DNA