Simultaneous identification and DNA barcoding of six Eimeria species infecting turkeys using PCR primers targeting the mitochondrial cytochrome c oxidase subunit I (mtCOI) locus


Abstract:

Species-specific PCR primers targeting the mitochondrial cytochrome c oxidase subunit I (mtCOI) locus were generated that allow for the specific identification of the most common Eimeria species infecting turkeys (i.e., Eimeria adenoeides, Eimeria meleagrimitis, Eimeria gallopavonis, Eimeria meleagridis, Eimeria dispersa, and Eimeria innocua). PCR reaction chemistries were optimized with respect to divalent cation (MgCl2) and dNTP concentrations, as well as PCR cycling conditions (particularly anneal temperature for primers). Genomic DNA samples from single oocyst-derived lines of six Eimeria species were tested to establish specificity and sensitivity of these newly designed primer pairs. A mixed 60-ng total DNA sample containing 10 ng of each of the six Eimeria species was used as DNA template to demonstrate specific amplification of the correct product using each of the species-specific primer pairs. Ten nanograms of each of the five non-target Eimeria species was pooled to provide a non-target, control DNA sample suitable to test the specificity of each primer pair. The amplifications of the COI region with species-specific primer pairs from pooled samples yielded products of expected sizes (209 to 1,012 bp) and no amplification of non-target Eimeria sp. DNA was detected using the non-target, control DNA samples. These primer pairs specific for Eimeria spp. of turkeys did not amplify any of the seven Eimeria species infecting chickens. The newly developed PCR primers can be used as a diagnostic tool capable of specifically identifying six turkey Eimeria species; additionally, sequencing of the PCR amplification products yields sequence-based genotyping data suitable for identification and molecular phylogenetics.

Keywords:

Molecular characterization, Turkey coccidia, Diagnostics, Phylogenetic analysis, Species identification

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