



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

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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 meleagridis*, *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

Published In:

Parasitology Research , DOI 10.1007/s00436-015-4361-y , 1-8