Molecular detection and genetic diversity of toxoplasma gondii oocysts in cat faeces from Klang Valley, Malaysia, using B1 and REP genes in 2018

ABSTRACT

The major route for Toxoplasma gondii (T. gondii) infection is through the ingestion of foods contaminated with oocyst from cat faeces. The microscopic detection of T. gondii oocysts in cat faeces is challenging, which contributes to the failure of detecting or differentiating it from other related coccidian parasites. This study aims to detect T. gondii oocysts in cat faeces using two multicopy-target PCR assays and to evaluate their genetic diversity. Cat faecal (200) samples were collected from pet cats (PCs; 100) and free-roaming cats (FRCs; 100) within Klang Valley, Malaysia, and screened for coccidian oocysts by microscopy using Sheather's sucrose floatation. PCR assays were performed on each faecal sample, targeting a B1 gene and a repetitive element (REP) gene to confirm T. gondii oocysts. Additionally, the PCR amplicons from the REP gene were sequenced to further confirm T. gondii-positive samples for phylogenetic analysis. Microscopy detected 7/200 (3.5%) T. gondii-like oocysts, while both the B1 gene and the REP gene detected 17/200 (8.5%) samples positive for T. gondii. All samples that were microscopically positive for T. gondii-like oocysts were also shown to be positive by both B1 and REP genes. The BLAST results sequenced for 16/200 (8.0%) PCR-positive T. gondii samples revealed homology and genetic heterogeneity with T. gondii strains in the GenBank, except for only one positive sample that did not show a result. There was almost perfect agreement (k = 0.145) between the two PCR assays targeting the B1 gene and the REP gene. This is the first report on microscopic, molecular detection and genetic diversity of T. gondii from cat faecal samples in Malaysia. In addition, the sensitivities of either the B1 gene or REP gene multicopy-target PCR assays are suitable for the accurate detection of T. gondii from cat faeces.

Keyword: Oocysts; Toxoplasma gondii; B1 gene; REP gene; Multicopy-target; Cat faeces; Genetic diversity; Malaysia; PCR