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Molecular Confirmation of *Taenia solium* Taeniasis in Child, Timor-Leste

Appendix

Additional Methods and Data

PCR

For molecular confirmation, we amplified a 491 bp fragment of the *Taenia solium cox-1* gene (*1*). The PCR primers used were T1F (5′-ATA TTT ACT TTA GAT CAT AAG CGG-3′) and T1R (5′-ACG AGA AAA TAT ATT AGT CAT AAA-3′) (*2*).

We used AccuPower Hotstart PCR PreMix tubes (Bioneer Co., Ltd, https://www.bioneer.co.kr) for PCR. The reaction mixture contained 1 μL of template DNA (100 ng/μL) and 10 μmol/L of each primer, and diethyl pyrocarbonate–treated water was added to reach a total volume of 20 μL. PCR entailed an initial denaturation phase at 94°C for 3 minutes, succeeded by 40 cycles of denaturation (94°C for 1 minute), annealing (52°C for 1 minute), and extension (72°C for 1 minute), culminating in a final extension at 72°C for 10 minutes.

Phylogenetic analysis

The evolutionary history was inferred by using the neighbor-joining method (*3*). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) were calculated (*4*). The tree was drawn to scale; branch lengths were in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed by using the Kimura 2–parameter method (*5*) and are designated as the number of base substitutions per site. The analysis involved 20-nt sequences. Codon positions were first + second + third + noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). A total of 480 positions were in the final dataset. Evolutionary analyses were conducted by using MEGA11 (*6*).

Sequence Amplified by Using *Taenia* spp. *cox-1* Restriction Fragment Length Polymorphism–PCR Primers

GCGTCATTAACTTTGTTGGGTTTGTGGTCAGGTTTTGTAGGTTTAAGTTTTAGT
TTATTAATTCGTGTTAATTTTTTAGAACCTTATTATAATGTGATTTCTTTGGATTGTTA
TAAATTTTTGATTACTAATCATGGTATAATAATGATTTTCTTTTTTTTAATGCCTATTT
TAATTGGAGGTTTTGGTAAATACTTAATTCCATTGATAGGAGGATTATCGGATTTGA
ATTTACCACGTTTGAATGCTTTAAGTGCATGGTTGTTAGTTCCATCAATTGTTTTTCTT
TTAATTAGGATGTGTTTAGGCGCTGGTATAGGGTGGACTTTTTACCCACCTTTATCAT
CTTCGTTGTTTTCAAGAGGAAGTGGGGTAGATTTTTTAATGTTTTCTTTACATTTAGC
TGGTGTATCAAGTATTTTTAGTTCTATTAATTTTATATGTACATTATATAGAGTTTTTA
TGACTAATAATTTTTTCTCGTA

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