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

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We report a case of *Taenia solium* taeniasis in a 10-year-old child in Timor-Leste, confirmed by molecular analysis, suggesting *T. solium* transmission to humans is occurring in Timor-Leste. Proactive measures are needed to improve public understanding of prevalence, geographic spread, and health implications of human taeniasis and cysticercosis in Timor-Leste.

The pork tapeworm, *Taenia solium*, causes human taeniasis and cysticercosis, which are considerable health problems in many developing countries (*1*). In Southeast Asia, *T. solium* infections are considered endemic, but epidemiologic data remain scarce (*2*). We report a case of *T. solium* taeniasis in Timor-Leste, confirmed by molecular methods.

In March 2019, as part of routine monitoring by the Timor-Leste Ministry of Health's national control program targeting soil-transmitted helminthiasis, in collaboration with the World Health Organization's country office, 1,121 fecal samples from school children in Timor-Leste were examined by using the Kato-Katz method. *Taenia* spp. eggs were identified in 4 samples. Subsequently, we conducted home visits for each affected child and administered a single dose of 10 mg/kg praziquantel (Shin Poong Pharmaceutical Co. Ltd, https://shinpoong.co.kr). We were able to collect expelled worm segments on the same day of treatment from a 10-year-old girl residing in Dili, the capital of Timor-Leste. Throughout most of her life, the child had remained in good health and had not manifested symptoms indicative of human taeniasis. Also, she had not traveled outside of the country.

The retrieved worm segments exhibited a flat, creamy white appearance, aligning with the typical macroscopic characteristics associated with *Taenia* spp. (Figure 1). Microscopic analysis of the segments revealed ≈50 gravid, 20 mature, and 20 immature proglottids of *T. solium*.

To determine the species through molecular analysis, we isolated genomic DNA from 1 segment by using the DNeasy Blood & Tissue Kit (QIAGEN, https://www.qiagen.com), according to the manufacturer's instructions. We performed PCR of genomic DNA to detect the parasite mitochondrial *cox-1* gene that encodes cytochrome c oxidase subunit I (Appendix, https://wwwnc.cdc.gov/EID/article/30/9/22-0154-App1.pdf) (*3*). We purified the PCR products by using DNA Clean & Concentrator-5 (Zymo Research, https://www.zymoresearch.com), according to the manufacturer's protocol. Sanger sequencing was subsequently performed by Bioneer Co., Ltd. (https://www.bioneer.co.kr), which used an ABI3730XL instrument (Applied Biosystems/Thermo Fisher Scientific,

Figure 1. Proglottids of *Taenia solium* collected from a patient in Dili, Timor-Leste, in case study of molecular confirmation of taeniasis in a child. We collected the expelled worm segments from a 10-year-old girl on the same day she was treated with 10 mg/kg praziquantel.

whttps://www.thermofisher.com). We deposited the derived sequence in GenBank (accession no. PP837933.1) and compared it with other *cox-1* sequences in GenBank by using BLAST (https://blast.ncbi.nlm.nih.gov). The sequence showed 98.85%–100% identity with the *T. solium* mitochondrial *cox-1* gene. We used *cox-1* sequences for phylogenetic reconstruction (Figure 2; Appendix). We aligned DNA sequences by using ClustalW (http://www.clustal.org) and conducted evolutionary analyses by using MEGA11 (*4*). The sequence isolated in this study was shown to be most homologous with an isolate from Tulear (also known as Toliara), Madagascar (GenBank accession no. FM958316.1) (*5*). Consequently, molecular evaluation confirmed the infection was caused by *T. solium*.

We report documented human *T. solium* taeniasis in Timor-Leste, an area where previous records of the parasite have been nearly absent (*6*). Clinically diagnosed neurocysticercosis in persons from Timor have been reported in Australia and Indonesia, suggesting the presence of *T. solium* in Timor-Leste (*7*,*8*). However, the only documentation of human taeniasis/cysticercosis within Timor-Leste is a case of oral cysticercosis in a person originally from Timor-Leste reported in Northern Ireland in 2015 (*9*). That particular patient exhibited symptoms of oral submucosal swelling and had relocated from Timor-Leste in 2006. Because no alternative sources of cysticercosis were identified, it is likely that the patient acquired the infection in Timor-Leste before migrating to Northern Ireland, a region where cysticercosis is not endemic (*9*). Similarly, a high probability exists that the child's infection in this case study originated within Timor-Leste, because she had not traveled outside of the country before the worm was detected. Through interviews, we found that she had regular interactions with confined pigs in her backyard and with free-ranging pigs within the village where she lived previously. However, the presence of *T. solium* cysticerci in those pigs and potential infection status remains undetermined.

The *cox-1* sequence from the worm isolated in Timor-Leste was closely related to sequences collected in Toliara in southern Madagascar. According to a previous study conducted in Madagascar, specimens from Toliara had diverged from parasites of the African/South American genotype (*5*). However, the lack of data limits what we can infer about *T. solium* in Timor-Leste. Further epidemiologic studies are needed to determine the extent of *T. solium* infection in pigs and to guide the implementation of control programs.

In conclusion, *T. solium* infections have been identified as endemic in Timor-Leste, a nation previously devoid of documented cases. Considering the widespread practice of backyard pig farming and the presence of free-roaming pigs across much of the country (*10*), veterinarians and clinicians should be vigilant in suspecting this emerging zoonotic parasite as a cause of taeniasis, not only in pig populations but also in humans. Furthermore, we urge health authorities in Timor-Leste to take proactive measures to enhance public understanding of the prevalence, geographic spread, and health implications of human taeniasis and cysticercosis within the nation.

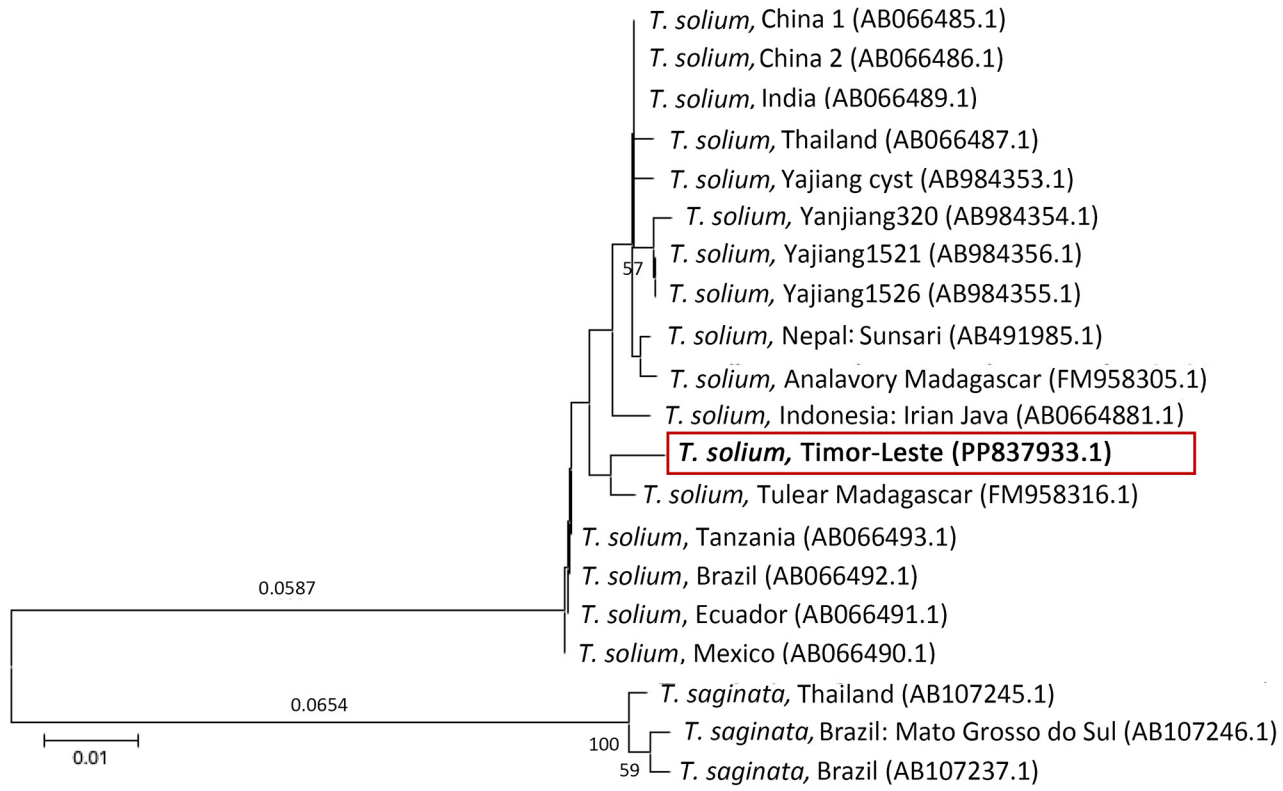

Figure 2. Phylogenetic analysis of the *cox-1* gene in case study of *Taenia solium* taeniasis in a child, Timor-Leste. Evolutionary history was inferred by using the neighbor-joining method and analysis was conducted by using MEGA11 (*4*). Red box and bold text indicates the sequence from this study. GenBank accession numbers are indicated in parentheses. Percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown below the branches. Tree is drawn to scale; branch lengths (above the branches) are 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. Analysis involved 20-nt sequences. Codon positions included 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. Scale bar indicates nucleotide substitutions per site.

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Optimizing Disease Outbreak Forecast Ensembles

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On the basis of historical influenza and COVID-19 forecasts, we found that more than 3 forecast models are needed to ensure robust ensemble accuracy. Additional models can improve ensemble performance, but with diminishing accuracy returns. This understanding will assist with the design of current and future collaborative infectious disease forecasting efforts.

Real-time collaborative forecast efforts have become the standard to generate and evaluate forecasts for infectious disease outbreaks (*1*,*2*). Individual forecasts are aggregated into an ensemble prediction that has historically outperformed individual models and is the primary external communication used (*3*–*5*). Because of the focus on the singular ensemble model and the costs associated with producing individual forecasts, public health officials starting or maintaining a forecast hub face 2 key challenges: identifying target participation rates and optimizing ensemble performance of participating models. To guide this decision-making, we analyzed data from recent US-based collaborative outbreak forecast hubs to identify how the size and composition of an ensemble influences performance.

We analyzed hub forecasts for influenza-like illness (ILI) from 2010–2017 (*5*); for COVID-19 reported cases, hospital admissions, and deaths from 2020–2023 (*6*); and for influenza hospital admissions from 2021–2023 (*7*). For each hub, we identified time periods with maximal model participation that had at least 2 increasing and 2 decreasing epidemiologic phases and obtained forecasts for individual models that produced ≥90% of all possible forecasts (Appendix Table 1, Figure 1, https://wwwnc.cdc.gov/EID/article/30/9/24-0026-App1.pdf). For each ensemble size, $n_D \in \{1, \ldots, N_D\}$, where N_D is the disease-specific total number of models matching our inclusion criteria; we created unweighted ensemble forecasts for every combination of individual models of size n_D. We followed the hub forecast methodologies and made probabilistic forecasts for ILI by using a linear pool methodology (*5*), and we made quantile forecasts for all others by taking the median across all individual forecasts (Figure 1) (*8*). For each hub, we compared the ensemble performance against 2 hub-produced models. The first is a baseline model that produces naive forecasts and serves as a skill reference point; and the second is the published ensemble produced in real-time that is an unweighted ensemble of all submitted forecasts and is the current standard for performance (*3*,*5*). We summarized probabilistic ensemble forecast skill by using the log score for ILI forecasts and the weighted interval score for all others (*9*,*10*). We took the reciprocal of the log score so that lower values would indicate better performance similar to the weighted interval score (Appendix).

Looking across all ensemble sizes and combinations, we found that including more models improved average forecast performance and that all ensembles composed of >3 models outperformed the baseline model (Figure 2). Further increases to the ensemble size slightly improved the average forecast performance, but substantially decreased the variability of performance across ensembles. When we increased the ensemble size of influenza hospital admission forecasts from 4 to 7, the average