An Integrated Morphological and Molecular Approach for Detection of Enteric Parasites in Captive Reptiles

Tanapon Sukkankah[1] and Chanakan Chotiphutthikul[1,*]

Abstract

Background: The rising popularity of exotic pet cafés and animal markets has created high-density, mixed-species environments for captive reptiles. These settings can act as epidemiological hotspots for gastrointestinal parasites, increasing the risk of cross-contamination and impacting animal welfare. Due to limited baseline surveillance in these settings, a targeted pilot study is necessary to determine the diversity of existing endoparasites.

Objectives: This pilot study aimed to detect and identify gastrointestinal parasites in captive reptiles from exotic pet cafés and animal markets using an integrated morphological and molecular approach.

Methods: Twelve fecal samples (10 tortoises and 2 iguanas) were submitted to the parasitology laboratory from zoo collections and exotic pet cafés in Chonburi, Saraburi, and Phra Nakhon Si Ayutthaya provinces between January and February 2026. Initial morphological screening was conducted using direct smears and simple flotation to detect ova, cysts, and trophozoites. In addition, modified Ziehl–Neelsen (MZN) staining was performed for specific screening of *Cryptosporidium* spp. For molecular identification, DNA was extracted from fecal samples and subjected to polymerase chain reaction (PCR) amplification targeting protozoa (EukA/B primers targeting 18S rRNA), nematodes (NEM18F/R primers targeting 18S rRNA), and *Cryptosporidium* (nested PCR: SSU2F/R and SSU3F/R targeting SSU rRNA). Positive PCR amplicons were subsequently sequenced for species-level identification. Sequence identification was performed using BLAST against the NCBI database and selecting the result with the highest percent identity.

Results: Microscopic examination detected gastrointestinal nematode eggs (4/12), ciliate protozoan cysts consistent with *Nyctotherus*-like organisms (7/12), and coccidian oocysts (3/12), while *Cryptosporidium* spp. were not detected. PCR amplification using NEM18F/R primers detected nematodes in 6/12 samples, whereas EukA/B primers detected protozoa in 10/12 samples. No amplification of *Cryptosporidium* was observed using nested PCR. All visible PCR bands were excised and sequenced. High-quality sequences were obtained from six samples, based on clear chromatogram peaks without overlapping signals. For nematodes (NEM18F/R primers), two samples yielded identifiable sequences, corresponding to *Thelandros tinerfensis* (97.50% identity) and *Ozolaimus linstowi* (99.41% identity). For protozoa (EukA/B primers), four samples were successfully identified, including *Nyctotherus ovalis* (92% identity) and *Nyctotheroides* spp. (99.12%, 98.35%, and 92.08% identity). Low sequence quality limited identification in some samples. Overall, molecular findings were generally consistent with microscopic observations, particularly for ciliate protozoa. However, PCR detected additional positive samples that were not identified by microscopy.

Conclusion: This study demonstrates that an integrated morphological–molecular approach enhances the detection and identification of gastrointestinal parasites in captive reptiles. While microscopic examination identified broad parasite groups, molecular analysis enabled species-level identification and detected a greater number of positive samples. These findings contribute to improving diagnostic approaches for parasite surveillance in captive environments.

Keywords: Reptiles, Gastrointestinal parasites, Molecular diagnosis, Protozoa, Nematodes

[1] National Institute of Animal Health, Department of Livestock Development, Bangkok, 10900 Thailand
[*] Corresponding author; E-mail: chanakan.can@gmail.com