

NRCPD-OUAVM Joint Research Report

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1. Principal investigator

Name: Dr. Ruenruetai Udonsom

Position: Scientist Expert Level

Affiliation: Protozoology Department, Faculty of Tropical Medicine, Mahidol University, Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand

2. Project title:

Molecular detection and genotyping of *Toxoplasma gondii*, *Cryptosporidium* spp and *Giardia duodenalis* in domestic animals and wildlife in Thailand

3. Collaborating research group members at NRCPD

Name: Professor Yoshifumi Nishikawa

Position: Professor

4. Research period (in mm/dd/yyyy, and total number of years)

April 1, 2024 to March 31, 2025 (1 year).

5. Purposes and objectives

5.1 This study is to determine the presence and genetic diversity of *T. gondii* infection in cats in Thailand

5.2 To determine *Cryptosporidium* spp and *Giardia duodenalis* infection among domestic animals, including pigs, cats, cattle, and wildlife in Thailand.

6. Outline of research process

Domestic animals such as cattle or pig and several wildlife species are parasitized by *Giardia* and *Cryptosporidium*, and have been considered reservoirs of zoonotic disease. Cats, definitive hosts for *T. gondii*. *T. gondii* oocysts from infected cat can infect human and animals through the environment, including in contaminated foods, water or soil. Therefore, this study the animal stool samples were collected from 61 cattle, 67 pigs, and 175 wildlife specimens from Ayutthaya and Kanchanaburi province and 120 cat stool samples from the refuge at Nakorn Nayok province Thailand for *T. gondii*, *Giardia* and *Cryptosporidium* detection. Ayutthaya province is located in central Thailand which it is a semi-

urban community. Villagers living along the river, namely Chao Phraya River, use the water for agriculture, farming, and transportation. The human activities may produce and discharge waste into water resources, including canals. These characteristics of this area may be suitable for zoonotic risk study. Kanchanaburi is the largest of the western provinces of Thailand. Topographically, it is covered with timber and evergreen forests and has the large numbers of wild animals which can be serve as reservoirs for transmission of zoonotic agents to domestic animals and human. The refuge in Nakhon Nayok province, Thailand is one of the large homes for abandoned of stray cats. Although, cats are potential reservoirs of zoonotic infections to humans and little data exists on the environment and *T. gondii* genotypes in Thailand. **Methods**, *Cryptosporidium* spp and *G. duodenalis* in pigs, cats, cattle and wild-life infection were determined using nested-PCR. The genotype of the pathogens was identified by DNA sequencing of the PCR products. For *T. gondii* detection in cat stool samples were examined using conventional PCR and *T. gondii* isolates were analyzed by multiplex nested PCR-RFLP analysis and DNA sequencing. The results will provide preliminary information of zoonotic protozoan parasites circulating in the study areas which could help to increase our understanding of host-parasite relationships.

7. Outline of research achievements

This research is divided into two objectives as described above.

Firstly, overall, 10.8% (13/120) of the samples tested positive for *T. gondii* infection by conventional PCR using *B1* gene. Of these, eight samples were isolated in Nakhon Nayok province, and five samples were isolated in Kanchanaburi province. All 13 positive samples had 92.94%–100% similarity with *T. gondii* reference sequences in the GenBank database. Using Mn-PCR-RFLP typing, four positive samples were successfully genotyped using at least four genetic markers. For one sample, amplification was successful for eight markers: SAG1, (5' + 3')-SAG2, alt.SAG2, SAG3, GRA6, C22-8, C29-2, and L358. Amplification was achieved for four markers, namely SAG1, (5' + 3')-SAG2, alt.SAG2, and SAG3, for another sample. Amplification for six markers (alt.SAG2, SAG3, GRA6, C22-8, C29-2, and L358) was observed for two samples. Genotyping for *T. gondii* of the positive samples revealed the presence of types I (two samples) and III (one sample) in Kanchanaburi province, whereas one sample from Nakhon Nayok province was isolated as type II. The manuscript of this work is now “under review” process.

Secondly, *Cryptosporidium* spp. was detected in 12 of 67 pig samples and there was no positive in other animals. For *G. duodenalis* was detected in 4 samples of pig, 2 samples of cat and 8 samples of wildlife. DNA sequencing for genotypes identification is in progress. *Cryptosporidium* spp. and *G. duodenalis* detection in animals are summarized in Table 1. **(If the results of DNA sequencing are satisfied, the manuscript will be prepared for publication).**

Pathogens	Infected animals	No. of positive/ No. of samples
<i>Cryptosporidium</i> spp.	Pig	12/67
<i>G. duodenalis</i>	Pig	4/67
	Cat	2/80
	wildlife	8/175

8. Publication of research achievements

Manuscript for the detection and genetic characterization of *T. gondii* infection in cats in Thailand is submitted to Heliyon journal (SJR; Q1) on 12 March 2025 and is now “under review” process (Manuscript Number: HELIYON-D-25-04115).