COMPARISION BETWEEN KKT, FECT AND FAFA FECES ANALYZER IN DETECTING LIVER FLUKE (*OPISTHORCHIS VIVERRINI*)

ALISA BOONSUYA

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master in Translational Medicine

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การเปรียบเทียบวิธีการตรวจวิเคราะห์ตัวอย่างอุจจาระระหว่างวิธีคาโต้แคท วิธีตกตะกอนด้วยฟอร์มาลิน-อะซีเตต และเครื่องตรวจอุจจาระแบบอัตโนมัติ ในการตรวจหาพยาธิใบไม้ตับออร์พิสทอร์คิสวิเวอร์รินีในมนุษย์

นางสาวอลิษา บุญสุยา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญามหาบัณฑิต
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COMPARISION BETWEEN KKT, FECT AND FAFA FECES ANALYZER IN DETECTING LIVER FLUKE (*OPISTHORCHIS VIVERRINI*)

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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อลิษา บุญสุยา : การเปรียบเทียบวิธีการตรวจวิเคราะห์ตัวอย่างอุจจาระระหว่างวิธีคาโต้แคท วิธีตกตะกอนด้วยฟอร์มาลิน-อะซีเตต และเครื่องตรวจอุจจาระแบบอัตโนมัติ ในการตรวจหาพยาธิใบไม้ตับออร์พิสทอร์คิสวิเวอร์รินีในมนุษย์ (COMPARISION BETWEEN KKT, FECT AND FAFA FECES ANALYZER IN DETECTING LIVER FLUKE (*OPISTHORCHIS VIVERRINI*)) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ แพทย์หญิงชวัลญ์ญา รัตนพิทูลย์, 135 หน้า

คำสำคัญ: พยาธิใบไม้ตับ/ เครื่องตรวจอุจจาระแบบอัตโนมัติ/ วิธีคาโต้แคท/ วิธีตกตะกอนด้วยฟอร์มาลิน-อะซีเตต

การติดเชื้อพยาธิใบไม้ตับออร์พิสทอร์คิสวิเวอร์รินิ เป็นปัญหาสำคัญทางด้านสาธารณสุข มีความสัมพันธ์กับการเกิดโรคมะเร็งท่อน้ำดี ซึ่งพบอุบัติการณ์สูงในประเทศไทย และเป็นสาเหตุการเสียชีวิตอันดับหนึ่งโดยเฉพาะภาคตะวันออกเฉียงเหนือ

การวิจัยนี้มีวัตถุประสงค์เพื่อเปรียบเทียบความแม่นยำในการวินิจฉัยของสามวิธีทางปรสิตวิทยา ได้แก่ วิธีคาโต้แคท (KKT), วิธีการตกตะกอนด้วยฟอร์มาลิน-เอทิล อะซีเตท (FECT), และเครื่องตรวจอุจจาระแบบอัตโนมัติ (FAFA) สำหรับการตรวจหาพยาธิใบไม้ตับออร์พิสทอร์คิสวิเวอร์รินิมีการเก็บตัวอย่างอุจจาระทั้งหมด 455 ตัวอย่างจากพื้นที่ชนบทในห้าจังหวัดภาคตะวันออกเฉียงเหนือของประเทศไทย ตัวอย่างทั้งหมดถูกตรวจแต่ละวิธี โดยการตรวจสอบด้วยกล้องจุลทรรศน์สำหรับ วิธีคาโต้แคทและวิธีการตกตะกอนด้วยฟอร์มาลิน-เอทิล อะซีเตท และโดยใช้เครื่องตรวจอุจจาระแบบอัตโนมัติ การวิเคราะห์ข้อมูลได้ดำเนินการเพื่อประเมินอัตราการติดเชื้อปรสิตและสังเกตความแม่นยำในการวินิจฉัย ผลที่ได้นนำมาวิเคราะห์ค่าความไว ความจำเพาะ ทำนายผลลบ ทำนายการผลบวก ความถูกต้อง และค่าสัมประสิทธิ์แคปปา

ผลการวิจัยพบว่าอัตราการติดเชื้อปรสิตอยู่ที่ 19.34% โดยการติดเชื้อส่วนใหญ่เกิดจากออร์พิสทอร์คิสวิเวอร์รินิ(18.02%) ตามด้วยพยาธิสตรองจิลอยด์ สเตอร์โคราลิส(0.88%) FECT แสดงผลการตรวจพบไข่ออร์พิสทอร์คิสวิเวอร์รินิที่สูงที่สุด (16.48%) ตามด้วย FAFA (10.55%) และ KKT (8.57%) ตามลำดับ การวิเคราะห์ทางสถิติแสดงค่าความไวและความจำเพาะสำหรับการตรวจหาออร์พิสทอร์คิสวิเวอร์รินิโดย KKT (85.15% และ 42.65%), FECT (100% และ 97.73%), และ FAFA (99.74% และ 64.73%) ค่า predictive value เชิงบวก, ค่า predictive value เชิงลบ, และค่า

kappa ได้รับการรายงานสำหรับ FECT (99.54%, 100%, 0.98), FAFA (94.18%, 97.81%, 0.67), และ KKT (97.92%, 100%, 0.56) นอกจากนี้ เวลาที่ใช้ในการเตรียมสำหรับ KKT, FECT, และ FAFA คือ 30, 15, และ 10 นาทีตามลำดับ สรุปผลการวิจัยนี้ชี้ให้เห็นว่า FECT, KKT, และ FAFA มีความไวใกล้เคียงกันในการวินิจฉัยพยาธิใบไม้ตับออร์พิสทอร์คิสวิเวอร์รินิเครื่อง FAFA แสดงศักยภาพในการเป็นเครื่องมือที่มีค่าสำหรับการตรวจหาพยาธิใบไม้ตับออร์พิสทอร์คิสวิเวอร์รินิและการติดเชื้อปรสิตอื่น ๆ ซึ่งมีความเป็นไปได้สำหรับการใช้งานทางคลินิก ผลการวิจัยให้ข้อมูลเชิงลึกที่มีคุณค่าสำหรับการวินิจฉัยและเน้นถึงศักยภาพของ FAFA ในการเพิ่มประสิทธิภาพและความแม่นยำในการประเมินทางปรสิตวิทยา

สาขาเวชศาสตร์ปริวรรต ลายมือชื่อนักศึกษา .....

ปีการศึกษา 2566 ลายมือชื่ออาจารย์ที่ปรึกษา .....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

ALISA BOONSUYA : COMPARISION BETWEEN KKT, FECT AND FAFA FECES ANALYZER IN DETECTING LIVER FLUKE (*OPISTHORCHIS VIVERRINI*) THESIS ADVISOR : THESIS ADVISOR: ASSOC. PROF. SCHAWANYA RATTANAPITOON, MD, 135 PP.

Keyword: *Opisthorchis viverrini*; Fully Automatic Feces Analyzer; Kato-Katz technique; Formalin-ethyl acetate concentration technique.

Liver fluke infection, particularly *Opisthorchis viverrini*, poses a significant public health risk in Thailand, where it is closely associated with cholangiocarcinoma and contributes to substantial mortality in the northeastern region. Diagnosis of this condition employs various parasitological approaches.

This research aims to compare the diagnostic accuracy of three parasitological techniques: the Kato Katz technique (KKT), the formalin-ethyl acetate concentration technique (FECT), and the Fully Automatic Feces Analyzer (FAFA) for *O. viverrini* identification. A total of 455 fecal specimens were collected from rural areas across five provinces in northeastern Thailand. The specimens were processed according to each method and examined through microscopy for KKT and FECT, and by utilizing an artificial intelligence-based machine for FAFA. Data analysis was conducted to assess parasitic infection rates and observe diagnostic accuracy.

The results revealed a parasitic infection rate of 19.34%, with the majority of infections attributed to *O. viverrini* (18.02%), followed by *Strongyloides stercoralis* (0.88%). FECT exhibited the highest positive detection of *O. viverrini* eggs (16.48%), followed by FAFA (10.55%), and KKT (8.57%), respectively. Statistical analysis indicated sensitivity and specificity values for *O. viverrini* detection by KKT (100% and 89.21%), FECT (98.67% and 97.63%), and FAFA (97.92% and 91.15%). The positive predictive value, negative predictive value, and kappa were reported for FECT (89.16%, 99.73%, 0.92), FAFA (56.63%, 99.73%, 0.67), and KKT (45.78%, 100%, 0.58). Additionally, the preparation time for KKT, FECT, and FAFA was 30, 15, and 10 min, respectively. In conclusion, this study highlights FECT, KKT, and FAFA as comparably sensitive in

other parasitic infections, showcasing promise for clinical use. The findings provide valuable insights into the diagnostic landscape and underscore the potential of FAFA in enhancing efficiency and accuracy in parasitological assessments.

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Student's Signature .....
Advisor's Signature .....
Co-advisor's Signature .....

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Alisa Boonsuya

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CHAPTER I

INTRODUCTION

1.1 Rational and Background

Opisthorchis viverrini (*O. viverrini*) infection is a major public health problem in Thailand and Southeast Asian countries (Buathong et al., 2017; Conlan et al., 2011; Chai et al., 2005). The infections are associated with hepatobiliary diseases mainly cholangitis, periductal fibrosis, cholecystitis, cholelithiasis and cholangiocarcinoma (CCA) (Elkins et al., 1990; Pungpak et al., 1989). *O. viverrini* was classified as group 1 carcinogens by World Health Organization (WHO 1994). In Thailand, people are infected with *O. viverrini* about eight million (Sayasone et al., 2017). The highest prevalence of *O. viverrini* infection was found in north and northeastern. The infection occurs after eating raw or undercooked cyprinoid fish containing infective stage metacercariae (Sithithaworn et al., 2012). Therefore, *O. viverrini* infection is still a sporadic problem in some rural areas of Thailand. Active screening is needed requires solving the problem. The microscopic-based stool examination is still useful for detecting various parasitic infection including helminth and protozoa in the community. The Number of parasitological techniques is available for diagnosis. WHO divided types of diagnosis of *O. viverrini* infection including parasitological techniques, immunological techniques, and molecular techniques. Microscopic examination is a gold standard to diagnose *O. viverrini* eggs in stool specimens (Bogoch et al., 2016). Parasitological techniques are used to diagnosis of *O. viverrini* infection by stool examination. The number of parasitological techniques is available for diagnosis. The most common techniques to detect eggs or larva of intestinal helminths in stool samples include direct simple smear technique, Kato-Katz technique (KKT) and the formalin-ethyl acetate concentration technique (FECT) (Bergquist et al., 2009; Qian et al., 2013). However, FECT and Kato-Katz are more labour-intensive with potent danger of acquiring infection and outbreak

of fire because of the use of ether in an “open” system. Moreover, eggs could not be differentiated with those of other human liver flukes (*Clonorchis sinensis* and *Opisthorchis felineus*) and small intestinal flukes (Sripa et al., 2011).

Thus, molecular techniques have been used to improve the specificity of the diagnostic test to determine the true prevalence of *O. viverrini* infection in the study population. Polymerase chain reaction (PCR)–based assays have been developed that could discriminate *O. viverrini* eggs from those of *C. sinensis* and small intestinal flukes using the internal transcribed spacer 1 (ITS1) and ITS2 regions of the ribosomal RNA (rRNA) gene (Sato et al., 2009). In addition, Sato et al (2009) showed that PCR-based assays have been developed that could discriminate *O. viverrini* eggs from those of *C. sinensis* and small intestinal flukes using the ITS1 and ITS2 regions of the rRNA gene. A previous studied by Stensvold et al (2006). They collected stool samples at Vientiane province, Lao PDR. The stool samples determined by using the Kato katz technique, FECT technique and PCR technique. The results were analyzed for the distinction between *O. viverrini* infection and other food-borne trematodes (FBT) eggs, and Kato katz technique was characterized by a sensitivity of 85% when compared with FECT technique. Although, PCR method is useful for confirmed *O. viverrini* like-eggs in the epidemic areas. However, this molecular method is found to be difficult, take time, high cost and unreliable for routine examinations.

Automatic stool processing machine (FA280 Fully Automatic Feces Analyzer; FAFA) is established based on artificial intelligent (AI) technology to accurately distinguish liver fluke, hookworm, roundworm, whipworm, pinworm, tapeworm etc (Orienter, China 2022). FAFA machine is more performance including test speed ≥80 tests / hour, Detection rate ≥95% and accuracy deviation in counting ≤20% (50~100 units/μL simulation samples). FAFA is eased and safety including sample collection tube is fully sealed after sampling, pipeline system is fully sealed when be analyzing and waste samples, test kits and liquid are sealed collected. However, routine laboratory using FAFA for *O. viverrini* examination in Thailand is limited. Therefore, the efficacy of *O. viverrini* detection using FAFA is needed. Particularly, the comparison

between routine parasitological concentration and commercial methods will be performed.

1.2 Research Objective

The study aims to

1.2.1 Detect *O. viverrin* eggs from faecal specimens using routine parasitological concentration and commercial methods.

1.2.2 Identify *O. viverrini eggs* by using molecular technique.

1.2.3 Comparison of efficacies between routine parasitological concentration and commercial methods.

1.3 Research hypotheses

FA 280 Fully Automatic Feces Analyze can be detected liver fluke eggs in stool samples. This method has the effective more than the formalin-ethyl acetate concentration technique (FECT).

1.4 Scope and limitations of the study

This study was contributed to for the operating staff. Because this machine is a tool to perform for shorten the time for large-scale examination, while sensitivity and species identification remain consistent. In terms of safety, the specimen loaded into the container is processed within the machine, there is no hazard to the operating staff. The machine runs in a standardized manner leading to negligible errors when compared against human errors.

1.5 Contribution

This study was contributed to for the operating staff. Because this machine is a tool to perform for shorten the time for large-scale examination, while sensitivity and species identification remain consistent. In terms of safety, the specimen loaded into the container is processed within the machine, there is no hazard to the operating staff. The machine runs in a standardized manner leading to negligible errors when compared against human errors.

1.6 Expected results

This study was revealing the novel information of diagnostic performance of newly developed automatic instrument (FA280 Fully Automatic Feces Analyzer; FAFA) is unknown in a real laboratory use. However, the efficacy of *O. viverrini* detection using FAFA is needed. Particularly, the comparison between routine parasitological concentration and commercial methods was performed.

CHAPTER II

LITERATURE REVIEWS

2.1 Human Liver Flukes

Human liver fluke infections caused by *Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis* remain major public health problems in many parts of the world ranging from eastern and southeastern Asia to Europe (Sripa et al., 2010). Clonorchiasis caused by *C. sinensis* is endemic in northern Vietnam, southern and northeastern China, Korea, and eastern Siberia, whereas opisthorchiasis caused by *O. viverrini* is endemic in Thailand, Lao PDR, Cambodia, and central-southern Vietnam (Doanh and Nawa, 2016; Sripa et al., 2010). *O. felineus* is endemic in the Russian Federation, particularly Western Siberia and in Central-Eastern Europe (Keiser and Utzinger, 2005; Armignacco et al., 2008; Fedorova et al., 2018; Fedorova et al., 2020). It has been estimated up to 14 million people are infected with *C. sinensis* in China alone (Furst et al., 2012; Lai et al., 2017; Brattig et al., 2020). A total of 12.39 million people were recently estimated to be infected with *O. viverrini* in 2018 in four major endemic countries including Thailand (6.71 million), Lao PDR (2.45 million), Vietnam (2.07 million), and Cambodia (1.00 million) (Zhao et al., 2021). For *O. felineus*, although it is not our focus in this review, a recent report revealed more than 60% prevalence in rural districts of Western Siberia (Fedorova et al., 2020). Throughout the world, over 700 million people are at risk of infection with these liver flukes (Keiser and Utzinger, 2005). The infections are associated with hepatobiliary diseases including hepatomegaly, cholangitis, fibrosis of the periportal system, cholecystitis, and gallstones (Fedorova et al., 2020; Qian et al., 2016; Qian et al., 2021; Sripa et al., 2018). Moreover, there are strong evidence from epidemiological, laboratory animal and human studies implicating *O. viverrini* and *C. sinensis* in the development of CCA (Laha

et al., 2007; Sripa et al., 2012; Na et al., 2020). Thus, the International Agency for Research on Cancer, World Health Organization (WHO) classified *O. viverrini* and *C. sinensis* as Group 1 biological carcinogens (Bouvard et al., 2009; IARC, 2011).

2.2 *Opisthorchis viverrini*

2.2.1 History of Opithorchiasis in Thailand

The fish-borne trematode *Opisthorchis viverrini* was first described in Thailand during a post-mortem examination of two prisoners from a jail in Chiengmai, northern Thailand, in 1911 by Leiper, who obtained specimens from Kerr. Kerr reported that 17% of 230 adult male prisoners examined in Chiengmai prison were infected with *O. felineus* (Kerr, 1916). In 1927, Prommas identified the worms found during an autopsy of a 17-year-old male residing in Roi-et, northeast Thailand, as *O. felineus* (Prommas, 1927). However, later studies clarified that the liver fluke infection in Thailand was caused by *O. viverrini*, not *O. felineus* (Sadun, 1955). This was further confirmed by Wykoff et al. in 1965 (Wykoff et al., 1965). Since then, cases of opisthorchiasis have been reported annually. *O. viverrini* remains prevalent and poses a serious health problem in some parts of Thailand. Consequently, ongoing health education and promotion efforts are essential to address this issue.

2.2.2 Morphology and Life cycle of *O. viverrini*

2.2.2.1 Adult

Adult worms of *O. viverrini* are a monoecious dorso-ventrally flattened, lancet shaped, thin and transparent. This parasite was measured of the average size by 7.0 (5.4-10.2) x 1.5 (0.8-1.9) mm and adult worms called monoecious. Its suckers are existed of both oral and ventral sucker with approximately one-fifth of body length at anterior. The deeply lobed and diagonal consisted of two testes located at posterior extremity, (Ash ,2007). The similarity structure form with *O. viverrini* is *C. sinensis* and the mainly different is the size of both parasites. The slightly coiled seminal vesicle is long, and an end bound in the ejaculatory duct, genital pore in front of ventral sucker and cirrus sac and cirrus are absent. Moreover, the multilobate of ovary is situated in

front of the anterior testes and nearby to seminal receptacle and Laurer's canal. The vitellaria comprised a lot of follicles disposed. Additionally, the eggs are yellowish-brown, oval, and have a tubercle-like knob at the opercular end. Therefore, the eggs are containing miracidium average 28 µm by 16µm in size when it laid.

2.2.2.2 Cercariae

The *O. viverrini* cercaria emerge from the infected snails by stimulation of light Then swim freely to find second intermediate host, cyprinid fish. The averages size of bodies is 154 x 75 µm cover with minute spines and the tail is 392 x 26 µm with dorso ventrally fin-fold. *O. viverrini* cercaria are oculate, pleurolophocercus and tobacco pipe-formed shape at resting stage or hanging head down in water. Two eyespots locate laterally between oral sucker and pharynx. Brownish pigments scatter in the body portion. Oral sucker is subterminal anteriorly with several rows of tooth-like structures, ceca is not well developed. Five pair of penetration glands are massed forming behind pharynx. Ventral sucker is underdeveloped, slightly anterior to spherical excretory bladder which is lined with epithelium and flame cell. Genital primordia present by a group of cells at dorsal to ventral sucker. The tail has transversely striated tegument at the proximal third and a fin fold on dorsal through ventral surface of the distal part (Wykoff et al., 1965; Pinlaor, 2003). Cystogenous glands are dorsolateral in both sides of the body (Arunsan et al., 2014)

2.2.2.3 Metacercaria

The metacercaria are formed after penetration of cercariae underneath the skin of cyprinid fish. The average size of the mature encysted metacercaria is 201 x 167 µm. Mostly *O. viverrini* metacercaria are oval shape, and some are occasionally round. Cyst walls consist of 2 layers, the oyster wall is thicker than inner wall. Early stage metacercaria cover with a thin layer inner cyst wall, thick outer cyst wall produces by fish host in later stage metacercaria (Donthaisong et al., 2014).

2.2.2.4 Eggs

The *O. viverrini* eggs are 19-30 µm long by 10-20 µm wide and are often indistinguishable from the eggs of Clonorchis sinensis. The eggs are operculated and possess prominent opercular 'shoulders' and and abopercular knob. The eggs are embryonated when passed in feces. (CDC, 2018)

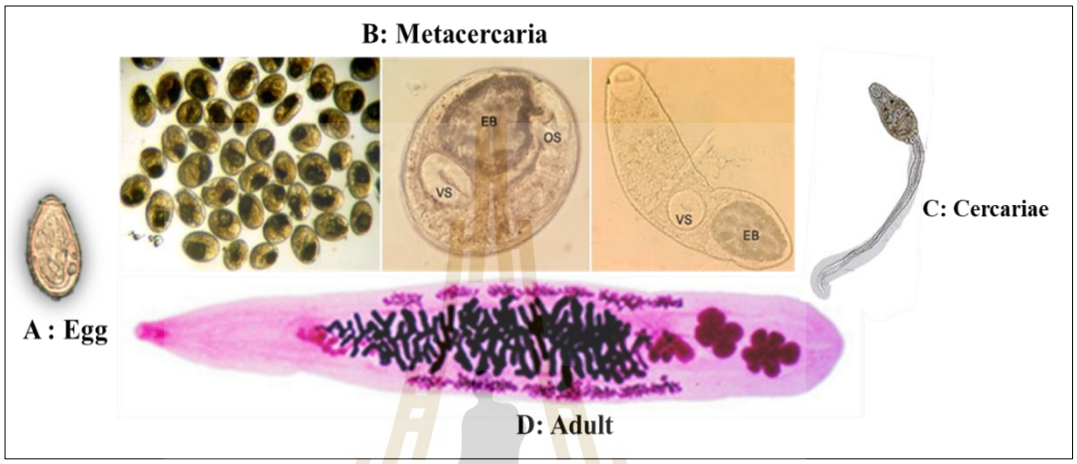


Figure 2.1 The morphological periods of *O.viverrini*

(Modified from CDC, 2018; Charoensuk et al., 2022; Rachprakhon and Purivirojkul, 2021)

2.2.3 Life cycle

The adult worms of *O. viverrini* and *C. sinensis* inhabit the intra- and extrahepatic biliary system. The life cycle of the two liver flukes is shown in Figure 2. Embryonated eggs containing the miracidium release from gravid worms are passed into the bile and eventually the faecal stream. After reaching freshwater, these eggs are ingested by Bithynia snails and hatch. The miracidia then transform to sporocysts, and asexual reproduction occurs giving rise to rediae and, in turn, to cercariae. After escape from the snail, the free-living cercaria attaches to and penetrates the skin of 18 susceptible species of fish in the family Cyprinidae. Once on and within the skin, the cercariae encysts as metacercaria. Metacercaria are infective to final definitive hosts including humans, dogs and cats when they ingest raw or inadequately cooked fish. After ingestion, the metacercaria is digested by gastric and intestinal juices, respectively. Excysted juvenile flukes at the duodenum then migrate through the ampulla of Vater

into the common bile duct and into the intrahepatic bile ducts, where they mature and reproduce. Some flukes can be found in the common bile duct, cystic duct and even in the gallbladder. The adult liver fluke is a hermaphrodite, and can live for several years or even decades.

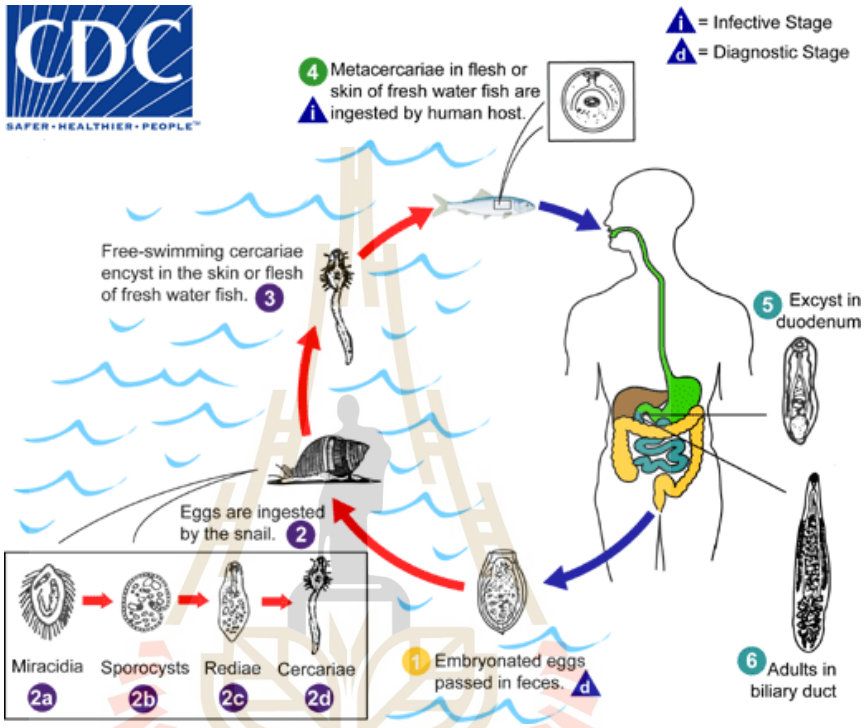


Figure 2.2 Life cycle of *O. viverrini*

(Modified from https://www.cdc.gov/dpdx/opisthorchiasis/index.html)

2.2.4 Risk factors of *O. viverrini* infection

There are several risk factors associated with *O. viverrini* infection such as demographic factors: male, age, younger than 24 years, older than 55 years, low and level of education, ethnicity, laborer, and farmer, (Wongba et al., 2011). Environmental and geographic factors: population located close reservoir: habitation distance nearby less than 1 kilometer from water source and food resource, (Chudthaisong et al., 2015) near the Mekong corridor where households with pet dogs or cats, and no sanitation. Health behavior factors (persistent smoking, alcohol consumer, consumption of raw

freshwater fish, and unsafe disposal of food waste). Moreover, mother infected with *O. viverrini* and parents or guardians ate raw fish. Medical history and Praziquantel treatment factors, (Pengput et al., 2020). Therefore, the important risk factors of severe morbidity are bile duct cancer and coinfection of other parasitic or viral (hepatic) infection and smoking (Forrer A. et al., 2012).

2.2.5 Signs and symptoms

Hepatobiliary tract diseases are induced by liver fluke infection leading to chronic inflammatory diseases that infected with highly of worm burden resulting bile duct cancer (Sithithaworn et al. 2007; Pakharukova and Mordvinov, 2016; Qian et al. 2016). However, most of these clinical signs are mild and asymptomatic, which manifested as jaundice appearing of the cases, but 99% without symptoms (Sripa B. 2021). Moreover, if the flukes present less than 100 worms may not symptom (Armignacco et al. 2008), but the 10 present of the flukes 100-1000 worms whatever might showing with acute clinical sign that caused jaundice, indigestion, epigastric discomfort, general malaise, anorexia, mild fever, and diarrhea (Chai et al., 2005). The chronic infection if not treated might causes liver enlargement, congestion of the spleen, allergic lesions, bile stone development, cholecystitis, and cirrhosis of liver. Nevertheless, the severe condition inducing CCA development, however, benign hepatobiliary disease including cholangitis, periductal fibrosis (PDF), obstructive jaundice hepatomegaly, cholecystitis, and gallbladder stone were shown clinically features. In addition, liver fluke infection mortality and morbidity resulting have worsen complicated (e.g., hepatic lesions, cholangitis, and CCA), which occurred in chronic infection with a lot of worm burden that became a highly impact health status endemicity areas. Recent year evaluation of the clonorchiasis affected on morbidity gained a Disability-adjusted life years (DALY) value of 275,370 cases have associated with high impact of helminthic disease. Finally, highly endemic of *O. felineus* in Western Siberia associated with CCA was detected in 77% of Opisthorchiasis cases and followed by 34.2% cases without Opisthorchiasis (Pakharukova et al., 2016). Therefore, human

liver flukes, *O. viverrini* infection have not shown the signs and symptoms for long times that silent period lasts up to 25-30 years of the parasite's life span if not treated.

2.2.6 Pathophysiology of *O. viverrini* infection

Human infected liver fluke by consumption of other fermented food products, especially from freshwater fish. The principal mechanism of *O. viverrini* infection that leading to massive chronic infection by three pathways: (1) Mechanical damage, (2) Immunopathology, and (3) excretory/secretory (ES) molecules the products of parasite. These main pathways are indicated the mechanism related with *O. viverrini* infection induced to CCA, (Bissell et al., 2001). Mechanical damage, because of parasite feeds in the biliary tract by sucker to hook into biliary epithelial cells that leading to infection of bile duct. However, as the flukes are matured the more lesion enlarged and ulcerated, which induced the nitric oxide and oxygen radicals leading to DAN damage. Immunopathology occurred when the host immune responses to the parasite penetration to damages the biliary epithelial that resulting in inflammation and shown both parasite-specific and nonspecific plasma IL-6 associated with risk of advance periductal fibrosis (APF) in opisthorchiasis leading to hepatobiliary abnormalities including CCA, (Sripa et al., 2009;2012). There were reported the proof of inflammation around infected hamster bile duct that host cell responded to Opisthorchiasis antigens infiltration of inflammation cells in periductal region of infected hamster liver was related with presence of fluke antigens, (Sripa et al., 2000). However, Tolllike receptors (TLRs) recognized and response to different molecules and pathogen associated molecular patterns (PAMPs) merged to activates cellular signaling pathway to induce immune response genes mainly pro-inflammation cytokines, (Venugopal et al., 2009). Moreover, TLR signaling was assessed when ES *O. viverrini* products stimulated the normal immortalized human cholangiocytes cells line (H69) of *O. viverrini* infected people in early stage of 10 immunopathology in biliary tract. Additionally, the ES products effect to express the receptors and proteins such as LTR4 mRNA, I**K**B-degradation and activated NF-**K**B nuclear translocation, (Ninlawan et al., 2010). Thus, IL-6 and IL-8 secreted from cholangiocytes that induced expression by *O. viverrini* ES

products, (Sripa et al. 2009). Liver fluke, ES of *O. viverrini* products the way of parasite to survive in long periods within the host environment by parasite helminths secrete and excrete of soluble proteins and other mediators. These ES products are digestion nutrients, host immune system regulation, and tissue invasion that played typically roles at the host-parasite interface. The long acting of interaction has been changed the homeostasis and involving to malignant transformation, (Pairojkul et al., 1991; Vatanasapt et al., 1998). The cell states altered by the way investigation in fluke infection as an entrance to genomic unsteady that induced by the different of growth (Schwartz et al., 2002). The most common lesions are epithelial dysplasia and metaplasia with goblet cell metaplasia and adenomatous hyperplasia, (Moore et al. 1988). The demonstration of fluke feed on the biliary epithelium and stimulate damage through mechanical harm and inflammatory response shown that the ES proteins from the tegument and excretory pore into bile, or culture *in vitro*, which are highly immunogenic, (Mulvenna et al., 2010). Likewise, the metabolic products might be toxic to or interact with biliary epithelium that resulting from inducing immune response. They had performed co-cultured between murine fibroblasts (NIH-3T3) and *O. viverrini* (but physically separated from the worms in Transwell plates) proliferate compared to cells in media alone, which reported the hyperplasia of opisthorchiasis-associated biliary epithelial cells, (Bhamarapravati et al.,1978; Thuwajit et al., 2004). Therefore, the characterization of transcriptome and ES proteome of *O. viverrini*, the molecular identities of some liver fluke proteins have been revealed, (Daorueang et al., 2012; Smout and Mundt, 2009).

2.3 Source of Thai human infection

Three types of preparations contain uncooked, small and medium-sized, fish: (1) Koi pla, eaten soon after preparation; (Sadun, 1955) Moderately fermented pla som; stored for a few days to weeks; and (2) Pla ra extensively fermented, highly salted fish, stored for at least 2-3 mo (Kaewpitoon et al., 2008). The consumption frequencies of koi pla in some communities every week was approximately 80% (Migasena et al.,

1983). In the northeasterners who have eaten koi pla, studies found the highest prevalence of liver fluke infection (Kurathong et al., 1985). The frequencies of koi pla consumption have declined and are generally confined to special social occasions, while other under-cooked fish preparations like pla som and other moderately preserved fish are generally eaten several times a week (Sithithaworn et al., 2003). Pla ra and jaewbhong, fully preserved fish, is an important staple and consumed daily by 60%-98% of northeasterners and lowland Laotians (Migasena et al., 1983; Upatham et al., 1984; Kurathong et al., 1985; Changbumrung et al., 1988). At present, the patients still show that Koi pla is probably the most infective, followed by fish preserved for < 7 d, then pla ra and jaewbhong, in which viable metacercaria are rare (Sithithaworn et al., 2003).

2.4 Epidemiology of *O. viverrini*

2.4.1 Prevalence

In Thailand, the first report of a high prevalence of *Opisthorchis viverrini* infection, reaching up to 100% in certain villages of northeast Thailand, was documented by Sadun in 1955. Nearly 30 years later, a similarly high prevalence and intensity of *O. viverrini* infection were reported in the Chonnabot district of Khon Kaen Province, confirming Khon Kaen as one of the hot spots for liver fluke infection in Northeast Thailand (Upatham et al., 1982; 1984; 1985). The first nationwide survey in Thailand during 1980-1981 revealed an overall prevalence of *O. viverrini* infection at 14%, with regional prevalence as follows: Northeast (34.6%), Central (6.3%), North (5.6%), and Southern region (0.01%). Due to intensive and continuous control programs and public health service activities, the average national prevalence of infection declined to 9.4% in 2000 (Jongsuksuntigul and Imsomboon, 2003) and further decreased to 8.7% in 2009 (Sithithaworn et al., 2012). In 2009, high prevalence was still observed in the Northeast (16.6%), followed by the North (10.0%), Central (1.3%), and Southern regions (0.01%). This prevalence in the Northeast was similar to that observed in the year 2000 (15.7%). Following this, the Thai Ministry of Public Health (MOPH)

decided to conduct nationwide helminthiasis surveillance every five years. In 2014, the overall national prevalence of *O. viverrini* infection had reduced to 5.1%, with the Northeast at 9.2%, North at 5.2%, Central at 0.9%, and South at 0% (Wongsaroj et al., 2014).

In 2016, the Thai MOPH launched a National Campaign to Eliminate *Opisthorchis viverrini* and Cholangiocarcinoma, targeting 84 endemic districts in the Northeast (20 provinces), North (6 provinces), and one eastern province. This campaign, the largest national effort so far, reinforced intersectoral involvement and utilized integrated control strategies across primary, secondary, and tertiary prevention, aiming to eliminate the liver fluke within the next ten years (by 2025). As a result of this intensive campaign, the prevalence of *O. viverrini* had dramatically decreased to 2.2% nationally by the latest National Helminthiasis Surveillance in 2019 (Wattanawong et al., 2021). The infection pattern remained similar to previous surveys but with lower prevalence, with the Northeast being the highest at 4.98%, followed by the North at 1.79%, Central at 0.87%, and South at 0.01%.

In addition to the national survey data, several cross-sectional studies have been conducted in both non-endemic and endemic areas of Thailand over the past ten years. These studies report a prevalence of *O. viverrini* infection ranging from 0.4% to over 20% in certain high-risk villages (Buathong et al., 2017; Laoraksawong et al., 2018; Pumidonming et al., 2018; Boondit et al., 2020; Mairiang et al., 2021). Based on these findings, *O. viverrini* infection remains prevalent in Thailand, particularly in the Northeast. Therefore, the average national infection prevalence data for the entire country and for specific regions is likely a significant underestimate (Sithithaworn et al., 1991).

2.4.2 Status of Liver fluke infection in Thailand

Opisthorchiasis is a serious public health problem in many parts of the Lower Mekong Basin, including Thailand, Lao PDR, Vietnam, and Cambodia. *Opisthorchis viverrini* has long been known to cause chronic hepatobiliary diseases, including cholangiocarcinoma (CCA), a fatal bile duct cancer. Thailand has reported the highest

incidence of CCA, which overlaps with the highest prevalence of *O. viverrini* infection, particularly in its Northeast region (Sripa et al., 2007; Sripa and Pairojkul, 2008). Based on several national surveys, the overall prevalence of opisthorchiasis in Thailand has gradually decreased from 35% in 1981 (Jongsuksuntigul and Imsomboon, 2003) to 5.3% in 2014 (Wongsaroj et al., 2014). However, the prevalence of liver fluke infection remains high, coinciding with a persistently high incidence of CCA (Khuntikeo et al., 2018). Similarly, the prevalence of soil-transmitted helminths (STH), particularly hookworms, has decreased over the past decades from 11.4% in 2001 (Jongsuksuntigul et al., 2003) to 6.5% in 2009 (Wongsaroj et al., 2014). Several factors may influence the sustained helminth transmission in Thailand, including cultural practices, behavior, occupations, parasite reproduction, environmental conditions, and even government policy (Sripa et al., 2015; Sripa and Echaubard, 2017; Steele et al., 2018). According to a report by Wattanawong in 2021, the prevalence of *O. viverrini* infection was estimated at 2.2% for the whole country. The highest prevalence was in Northeast Thailand at 4.97%, followed by the North (1.79%), Central (0.87%), and South (0.11%) regions. In contrast, the prevalence of hookworms was highest in the South at 9.84%, whereas the Northeast showed the lowest prevalence at 2.43%.

2.5 Diagnosis of *O. viverrini* infection

O. viverrini infection is a major public health problem in Thailand and Southeast Asian countries. The infections are associated with cholangiocarcinoma (CCA) and WHO (1994) are classified as Group 1 carcinogens. In Thailand, people are infected with *O. viverrini* about eight million (Sayasone et al., 2017). The highest prevalence of *O. viverrini* infection was found in north and northeastern. The infection occurs after eating raw or undercooked cyprinoid fish containing infective stage metacercariae (Sithithaworn et al., 2012). The microscopic-based stool examination is still useful for detecting various parasitic infection including helminth and protozoa in the community. The Number of parasitological techniques is available for diagnosis. The World Health Organization (WHO) divided types of diagnosis of *O. viverrini* infection including

parasitological techniques, immunological techniques, and molecular techniques. Microscopic examination is a gold standard to diagnose *O. viverrini* eggs in stool specimens (Bogoch et al., 2016). However, eggs could not be differentiated with those of other human liver flukes (*C. sinensis* and *O. felineus*) and small intestinal flukes (Sripa et al., 2011). Thus, molecular techniques have been used to improve the specificity of the diagnostic test to determine the true prevalence of *O. viverrini* infection in the study population. Polymerase chain reaction (PCR)–based assays have been developed that could discriminate *O. viverrini* eggs from those of C. sinensis and small intestinal flukes using the internal transcribed spacer 1 (ITS1) and ITS2 regions of the ribosomal RNA (rRNA) gene (Sato et al., 2009). Diagnosis of both *O. viverrini* and *O. felineus* is suspected based on the clinical picture, on the anamnestic recall of consuming raw fish, on the detection of eosinophilia, and on typical findings of ultrasound (US), computed tomography (CT) or magnetic resonance imaging (MRI) scans. Confirmation of diagnosis relies on different types of diagnostic techniques.

2.5.1 Parasitological techniques

Parasitological techniques are used to diagnosis of *O. viverrini* infection by stool examination. The number of parasitological techniques is available for diagnosis. The most common techniques to detect eggs or larva of intestinal helminths in stool samples include direct simple smear technique, Kato-Katz technique and the formalin-ethyl acetate concentration technique (FECT) (Bergquist et al., 2009; Qian et al., 2013). The fecal parasite concentrator kits (FPCK) were developed to minimize the complicated process of conventional FECT. This technique can be performed in only four steps, easier and faster to process specimen than FECT technique (Charoensuk et al., 2019). Kaewpitoon et al (2016) studied the border areas of three provinces which are in the northeast part of Thailand. They conducted in the area at Kaeng Sanam Nang district of Nakhon Ratchasima Province, Waeng Noi district of Khon Kaen province, and Khon Sawan district of Chaiyaphum province, Thailand. The stool samples are examined in laboratory by using a modified Kato-Katz Thick smear technique. Charoensuk et al (2019) studied compare the performance of the different

parasitological techniques to detect *O. viverrini* infections. They study at Kanam Chai Khet district in Chachoengsao province and Khao Chakan district in Sa Kaeo province by using formalin- ethyl acetate concentration (FECT) technique, kato- katz technique, direct simple smear and parasite concentrator kit (FPCK) technique for detection *O. viverrini* egg examination in stool. They found that FECT technique provides the highest sensitivity in comparison of Kato-Katz, FPCK and direct simple smear techniques. Again, the FPCK and direct simple smear technique have better sensitivity in EPG ≥ 50 groups compared with EPG < 50 groups. Nevertheless, the FPCK technique has sensitive similar as simple direct smear technique statistically that are in EPG around 50 groups.

2.5.1.1 Direct smear technique

This test is simple demonstration and not complicated detection test of intestinal parasite infected subjects pass through feces. The protozoa (trophozoites or cysts) or helminth eggs has detected by light microscopy (WHO and SOP, 2014). The direct microscopy examination is important to observe the larvae of *Strongyloid stercoralis* (*S. stercoralis*) and detection of high concentration of helminth's egg of *Ascaris lumbricoides* (*A. lumbricoides*) infection. The advantage of this test is faster and cheaper, but it is semi-quantitative and rare for control programs utilized. However, there is more range uses to detect protozoan parasites including trophozoites of *Entamoeba histolytica*, *Giardia lamblia*, and rarely *Balantidium coli* infection. The procedure of this thin smear is involved of small quantity of fresh stool add a drop of saline on microscope glass slide and cover glass on the emulsified stool. Finally, the eggs, larvae, trophozoites of parasites are demonstrated under light microscopy and eosin or iodine utilization is essential to detect the cysts, oocytes of intestinal protozoa (Cheesbrough 2005; World Health Organization 1994). Therefore, accurate examination result is upon the stool specimens, which is commonly used for field survey of parasitic infection in endemicity arears and commonly for transcribing the results from the hospital lab registers to the lab case report

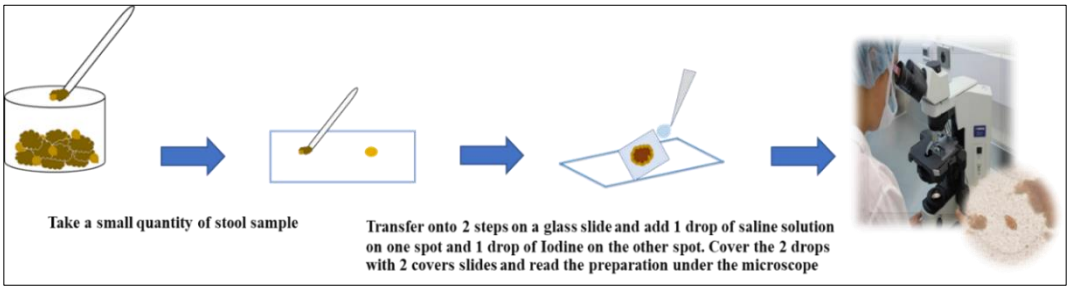


Figure 2.3 Operating steps for the direct smear technique. Adapted from (WHO 1994)

2.5.1.2 Kato's thick smear technique

Kato's thick smear technique is thick glass coverslips are used to detect *Schistosoma mansoni* (*S. mansoni*) infection compares with Kato technique that substituted by cellulose acetate film soaked in glycerine for field use to estimate the sensitivity infection. This technique is widely used to diagnose the helminthic disease that improved various workers (Katz, 1970). This method was evaluated of modified compared with the Bell technique and digestion technique that was indicated of both sensitive and suitable for field use. There were reported that the accuracy and capable comparison with the orthodox Kato & Miura method of thick glass coverslip. Firstly, 1 gram (g) of feces is placed on filter paper (5 cm. square) and pressed through a 100 mesh (150 µm pore size) sieve. The schistosome and other helminth eggs are contained in the sieve stool sample, but the no particles larger than sieve mesh size. The wire surface of the sieve with the edge of a microscope slide and packed into the head of disposable syringe, previously calibrated to deliver 30/g. Next step is place on a slide with faecal smear diameter about 25 mm and then inverted by pressing on the thick glass coverslip firmly. Finally, slide should be examined within 2 hours before it's dried up. The efficacy of modified technique is estimated the numbers of *S. mansoni* eggs that had added into stool of uninfected subject. The observation number of parasite's eggs as a drop of suspension on slide by using microscopy (40X) of magnification were existed by heavy infected human stool sieving, sedimentation, washing, and centrifugation of the emulsified stool. Moreover, this procedure has compared with

cellulose acetate film technique of Martin & Beaver. Therefore, this method requires expertise and commonly used cellophane sheet instead of cover glass for convenience with feces amount 30-60 milligram (mg) that gave accurate results, but helminth eggs may change their shape somewhat (Teesdale and Amin, 1976).

2.5.1.3 Kato-Katz technique

WHO has reveal Kato-Katz technique as the gold standard and essential used to approach the prevalence and infection intensity of soil-transmitted helminths (STHs). The advantages of the Kato-Katz are high sensitivity, egg quantification, cost effectiveness and utilized minimal infrastructure through copro-microscopy methods. This test is used to stratify infection intensity by egg count and cut-off values that straining feces specimen about 41.7 mg, 20 mg, or 50 mg upon the template size place on a glass slide. The glycerol has soaked with piece of the cellophane to cover the preparation and inverted slide with gently press down in a thin smear. Next add glycerol serves for clearing fecal material (fat) within surround the eggs. However, it takes within 1 to 24h before observing under microscopy for other species except hookworm eggs require approximately 30 minutes and then eggs were counted under microscopy per gram of feces (WHO. 2002; Katz et al., 1972). Therefore, this technique has used for general lab in hospital.

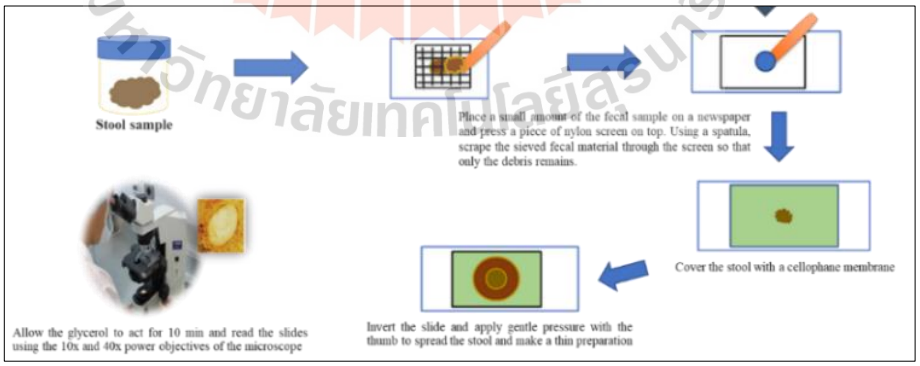


Figure 2.4 Operating steps of the Kato-Katz technique

2.5.1.4 Formalin ethyl-acetate concentration technique (FECT)

The common diagnostic testing of liver fluke's eggs by using light microscopy examination available and cheaper for detection in stool specimens. Even though it is capable diagnostic tool, but the lower flukes and lower sensitivity of parasitological technique, which is the burden of illness affecting of accuracy examination. However, light microscopy detection based FECT is commonly used to investigate parasite eggs for opisthorchis diagnosis. The FECT technique has limited of diagnosis in light *O. viverrini* infection for sensitivity and specificity that required an expert parasitologist to confirm the differentiation between *O. viverrini* with minute intestinal flukes' eggs (MIFs) in faecal specimens. Therefore, FECT is a gold standard for measuring the *O. viverrini* eggs (Worasith et al. 2015).

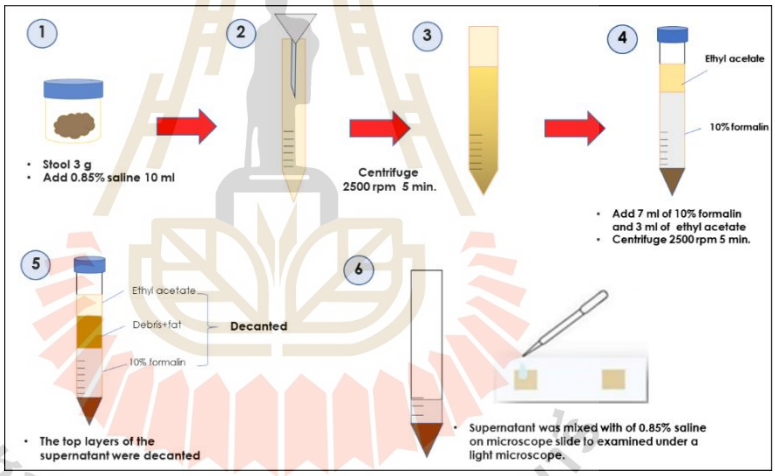


Figure 2.5 Operating steps of the Kato-Katz technique

2.5.2 Molecular techniques

Molecular techniques have been used to improve the specificity of the diagnostic test to determine the true prevalence of *O. viverrini* infection in the study population (Boondit et al., 2020). Sato et al (2009) showed that polymerase chain reaction (PCR)-based assays have been developed that could discriminate *O. viverrini* eggs from those of *C. sinensis* and small intestinal flukes using the internal transcribed spacer1 (ITS1) and ITS2 regions of the ribosomal RNA (rRNA) gene. A previous studied

by Stensvold et al (2006). They collected stool samples at Vientiane province, Lao PDR. The stool samples determined by using the Kato-Katz technique, formalin ethyl acetate concentration technique and PCR technique. The results were analyzed for the distinction between *O. viverrini* infection and other food-borne trematodes (FBT) eggs, and Kato-Katz technique was characterized by a sensitivity of 85% when compared with FECT technique. Demonstrating a PCR technique sensitivity of approximately 50% in samples with faecal egg counts > 1000, the previously studies was not supported. Aung et al (2017) studied *O. viverrini* infection in the lower Myanmar. The stool samples determined by using modified formalin-ether concentration technique and polymerase chain reaction (PCR) technique. The total positive sample is 34 participants of *O. viverrini* infection. The DNA sequences was accomplished 18 positive samples and confirmed *O. viverrini* like eggs by using molecular technique. The sequences show 99.7% identity with *O. viverrini* mitochondrial *cox1*. Buathong et al (2020) studies genetic differentiation of *Opisthorchis*-like Eggs in Northern Thailand by using stool specimens. The results showed prevalence of *Opisthorchis*-like eggs in studies conducted in a rural community of Sanamchaikaet district, Chachoengsao Province, central Thailand, was 17.4–21.3%. From PCR assays, all liver flukes were *O. viverrini* and all Minute intestinal flukes were *H. taichui.* ITS2 nucleotide sequencing analysis showed a single variant of *O. viverrini* with no variation and two variants of *H. taichui*

2.5.2.1 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) has invented by Mullis in 1983 that patented in 1985. The polymerase has used *in vitro* replication of specific DNA sequences. This technique is able generated tens of billions of copies from a particle fragment of DNA extract (DNA template). PCR is competent based on the accurate amount of matrix DNA and purification or cloning. The discrimination between liver flukes and others small intestinal flukes' infection and their eggs were difficulties to detect under microscopy examination (Sithithaworn et al., 1991; Lee et al. 2012). PCR based method is competence to detect DNA template for diagnosis from 28.6% to 76.6% incase microscopy examination negative and classify between *opisthorchis*-like eggs and others small intestinal flukes' sensitivities up to 93.7% of *Opisthorchis* genetic

from faecal specimen (Umesha et al., 2008; Lovis et al., 2009; Won et al., 2016). Therefore, DNA extraction protocol using cetyltrimethylammonium bromide to remove inhibitors have been applied for higher sensitivity diagnosis (Umesha et al. 2008). This technique highly sensitive specimens have contained more than 200 eggs per gram of feces according to *O. viverrini* infection intensity (Wongratanacheewin et al., 2002). However, DNA template analysis using PCR-based ITS2 of the rRNA genes have been used as markers to differentiate *O. viverrini*, *C. sinensis*, and small intestinal flukes. Additionally, mitochondrial genes have also been used *COX*1 and *NAD*1 to discriminate between *O. viverrini*, *C. sinensis*, and *H. taichui* (Traub et al., 2009; Kaewkong et al., 2013; Lamaningao et al., 2017). Thus, the validation potential of ITS1 region is classified of related sequencing species targeting rDNA, whereas *COX*1 and *NAD*1 are identified of opisthorchis species and its complementary in mtDNA (Yao et al., 2010).

2.5.2.2 The principle of PCR

The possible of PCR amplification a large amount from fragment DNA sample into new strand that replicated from double-stranded DNA template. which is broke into three phases including denaturation, annealing, and extension step. These steps are comprised of buffer, 10x taq buffer, dNTP, primers, MgCl2, Taq polymerase, and DNA extract in access the cycles with volume from 10µl to 100 µl according to each target DNA template (PCR; Belkum et al., 2008). The denaturation is the step to separate the double-strands DNA that temperature is obtained at 94°C of the first period, that is not allowed the hydrogen bonds to maintain at a temperature over than 80°C and the double-strands DNA is broke as a single stranded DNA to replicate the new strand. The second step is annealing that carried the temperature between 40 and 70°C low down temperature to permit the hydrogen bonds to reform and complementary strands to hybridize, which is better than longer strand matrix DNA for hybridization and more specific. The final step is extension that carried out at temperature 72°C to syntheses the complementary strand when the Taq polymerase binds to prime single-stranded DNA and catalyzes replication by deoxyribonucleoside triphosphate. Thus, DNA synthesis an analysis amount is take 20-40 cycles when the

sequencing of interest greater than 1 kilobase at a rates of 2 minutes per kilobase. Finally, PCR reaction was extremely rapid that lasts a few hours for 30 cycles.

2.5.2.3 Primers

The primer design to achieve selective amplification nucleotide sequences of DNA template by using PCR with one pair of oligonucleotides, which is use primer to replicate and synthesis chemical. The best amplifies of complementary are composed of both ends interest sequence fragment 5′-3′ strand DNA, which recognized of the same fragment DNA. As the sequence of interest was allowed to replicate of primers single-strand DNA, which is usually between 10 and 30 nucleotides in general guarantee of sufficiently specific hybridization (Tools, 2015).

2.5.2.4 Detection and analysis of the PCR products

Agarose gel electrophoresis (or acrylamide), the products are viability to analyze the PCR products that composed of one or more DNA fragments (the sequence of interest). Gel electrophoresis is used to separate the size of DNA by electrolysis DNA charged. The movement of DNA molecules is migrated from the negative to the positive pole that depended on the size and shape of the DNA. Interestingly, the smaller DNA moves rapids than larger DNA and supercoiled DNA moves rapids than linear double-strand DNA and circular DNA respectively (Supaporn Pumpa. 2019). Ethidium bromide staining is dyeing the DNA for visualization and ultraviolet transillumination (280-320 nm). If there are formed diffuse band and the small PCR products will be visible depend on the reaction and condition of nonspecific DNA fragment amplified greater or lesser forming net band. This principal of capillary electrophoresis (automated system) is detected by laser diode for analysis primers fragment of possible PCR products (Belkum et al., 2008).

2.5.2.5 Target genes: Internal transcribed spacer 2 (ITS 2)

In all living organisms have existed protein synthesis and gene expression where these main roles maintained by ribosomes, intracellular, and molecular machines. The phylogenetic information in prokaryotes and eukaryotes have

extended by ribosomal RNA (rRNA), whereas ribosomal DNA (rDNA) has collected related with composed of coding regions (18S, 5.8S and 28S) and non-coding region of internal transcribed spacers 2 (ITS2), (Wei et al., 2006). Molecular markers of ITS regions have extended used to analyze for taxonomy and phylogenetic, which preferred over non-coding region including multiple copies of rRNA genes, suitable for PCR amplification with typical universal primers for diversity organisms, average sequence length for sequencing, and high degree of diversity at the generic and species level suitable to frequent nucleotide polymorphism in sequences (Poczai and Hyvonen, 2010). ITS region was emphasized depended on both high degree and low of inter-specific genetic diversity of population in the different hosts and geographical areas of species level. However, the gene region a single copy portion of chloroplast genome and nuclear rDNA has been used to construct for phylogeographic inference in bryophytes.

2.6 Treatment

Praziquantel is the current drug of choice for the treatment of opisthorchiasis patients infected has been recommended as follows. Praziquantel 25 mg/kg three times daily for 2-3 days (Sithithaworn et al., 2007). Another chemotherapy with praziquantel 40 mg/kg in a single dose has been recommended by WHO (1995), but the sustainability control of this approach is unclear (Chai et al., 2005; Clausen et al., 2015). Moreover, human *O. felineus* infection treatment with praziquantel (25 mg/kg orally 3x/day for 1-2 days). The standard dose of praziquantel is 75mg/kg orally three times for 1 day which results in an egg-decreasing rate of 98% to 99% for both infected by *O. viverrini* and *C. sinensis* (Keiser and Utzinger, 2010). Therefore, the adverse effects of praziquantel treatment such as dizziness, headache, and nausea are uncommon. Additionally, Tribendimidine is one of the agents for treating liver fluke has been reported in several novels. Indeed, tribendimidine is an amidantel derivative which forced against not only liver fluke but some intestinal roundworms including hookworms, ascariasis, and enterobiasis (Xu, 2014). Tribendimidine treatment against both *O. viverrini* and *C. sinensis* to reduce egg rate of 400 mg single dose with higher effect of 98% to 99% compared to praziquantel with fewer side effects. The

most common side effects of tribendimidine such as dizziness, vertigo, headache, nausea, and fatigue (Sayasone et al., 2016). Even though the treatment of liver fluke is high effectiveness can be defined based on eggs in feces samples but very few eggs are produced in case of not efficacy treatment.

2.7 Prevention and control

Human liver fluke infection must be start with effective education on prevention and control to encouragement raw freshwater fish consumers understand the risk factors the source of problem. Whenever, the evidence is significant transmission from fish cultured that important source of human liver fluke infections. The implementation in aquaculture systems is intervention to control fish-borne liver and intestinal flukes. However, interventions, developed in extensive field trial was designed to eliminating egg contamination by control fish infections in the ponds and reducing snail population, (Clausen et al., 2015; Khambooraung et al., 1997). The program of farmer education and improvements require a firm commitment to manipulation practices and ponds infrastructure. The farmers should achieve from basic training of the biology and epidemiology of fishbone zoonotic trematodes that benefits to their families for participating in prevention and control interventions. There is initiation involve from the house members aware of not eating raw fish as well as prevention of raw or dead fish by farm animals such as dogs, cats, and pigs. Prevention egg and host fecal contamination of the pond environment are setting the embankments to prevent surface water run-off from entering the pond using cement barrier at least 10-15 cm above the bank top. The reservoir hosts including cats and dogs used installation of fencing to protection of entering the pond and do not discharge the waste from latrines and livestock pens. Intervention to prevent and control snails in the fishpond require the following: the pond has drained and dried completely before restocking the ponds after harvest at least 5 days, remove of top bottom mud 3-5 cm and the site not adjacent to the pond. Moreover, all vegetation in ponds should be removed and aquatic vegetation must be removed at least 3 meters from the water portal (inlet pond water replenishment), all incoming water filtered through a 5 mm mesh screen before entering the pond. The important

intervention is public health actions for endemic community's education households including the risk from inadequately fish dish preparation; avoid contaminating water bodies with human and animal waste to the extent possible; seek the medical treatment when suspected signs and symptoms appeared due to liver fluke infection. Additionally, the laboratory condition has trained parasitologists are needed to make some information on survival of *C. sinensis* eggs survival in isotonic solution at 2 °C-4 °C for up to 3 months, 26 °C for up to 1 month. In fresh night soil, the survival time was 2 days at 25 °C; an observed survival of *O. felineus* eggs for 160 days in river water held at 0 °C-5 °C, (Drozdov et al., 1962). The egg persistence in soil and water under natural environmental condition is needed further field research. Therefore, similarly studies survival of eggs in sewage, sludge, surface water, wastewater and irrigation water are insufficient to draw any conclusion.

Education the community people do not eat raw or uncooked freshwater fish especially lightly salted, smoked or pickled fish that contained the parasites. However, FDA recommended for fish dish serve or storage to prevent of parasites as following: cooking fish properly requiring internal temperature at least 145°F (~63°C). Freezing (fish): at -4°F (-20°C) or below for at least 7 days or -31°F (-35°C) or below for at least 15 hours; or -31°F (-35°C) or below until solid and storing at -4°F (-20°C) or below for at least 24 hours, (Prevention, 2020)

2.8 Differences Between the Eggs of *O. viverrini* and the Eggs of MIF

The eggs of the liver fluke, *O. viverrini* are ovoid in shape and brownish yellow in color, which is due to bile pigmentation. These eggs possess an operculum, a distinct shoulder that is clearly visible, and a knob at the posterior end. When stained with potassium permanganate, the rough surface of the eggshell becomes clearly observable. In contrast, the eggs of MIF resemble those of *O. viverrini* but differ in that the shoulder of the egg is less prominent. These eggs also have a posterior knob which contains a structure resembling a droplet. When stained with potassium permanganate, the eggshell surface appears smooth. This distinction is Illustrated in Figure 2.6

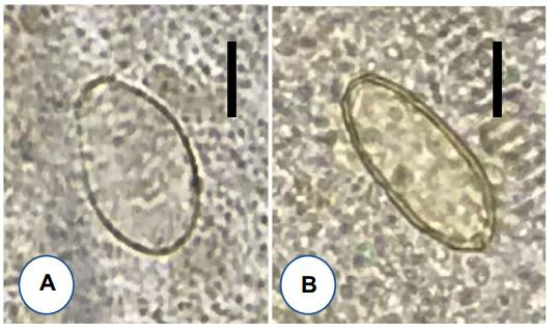


Figure 2.6 Morphology of eggs (A) Presumed to be an egg of *O. viverrini.* (B) Presumed to be an egg of *H. taichui*. Scale bars (A, B) = 10 μm. (Jung et al., 2023)

2.9 Automatic stool processing machine

Automatic stool processing machine (FA280 Fully Automatic Feces Analyzer; FAFA) is established based on artificial intelligent (AI) technology to accurately distinguish liver fluke, hookworm, roundworm, whipworm, pinworm, tapeworm etc (Orienter, China 2022). FAFA machine is more performance including test speed ≥80 tests / hour, Detection rate ≥95% and accuracy deviation in counting ≤20% (50~100 units/μL simulation samples). FAFA is eased and safety including sample collection tube is fully sealed after sampling, pipeline system is fully sealed when be analyzing and waste samples, test kits and liquid are sealed collected. However, routine laboratory using FAFA for *O. viverrini* examination in Thailand is limited. Therefore, the efficacy of *O. viverrini* detection using FAFA is needed. Particularly, the comparison between routine parasitological concentration and commercial methods will be performed.

Figure 2.7 Machine composition of FA280 Fully Automatic Feces Analyzer (Orienter, China 2022)

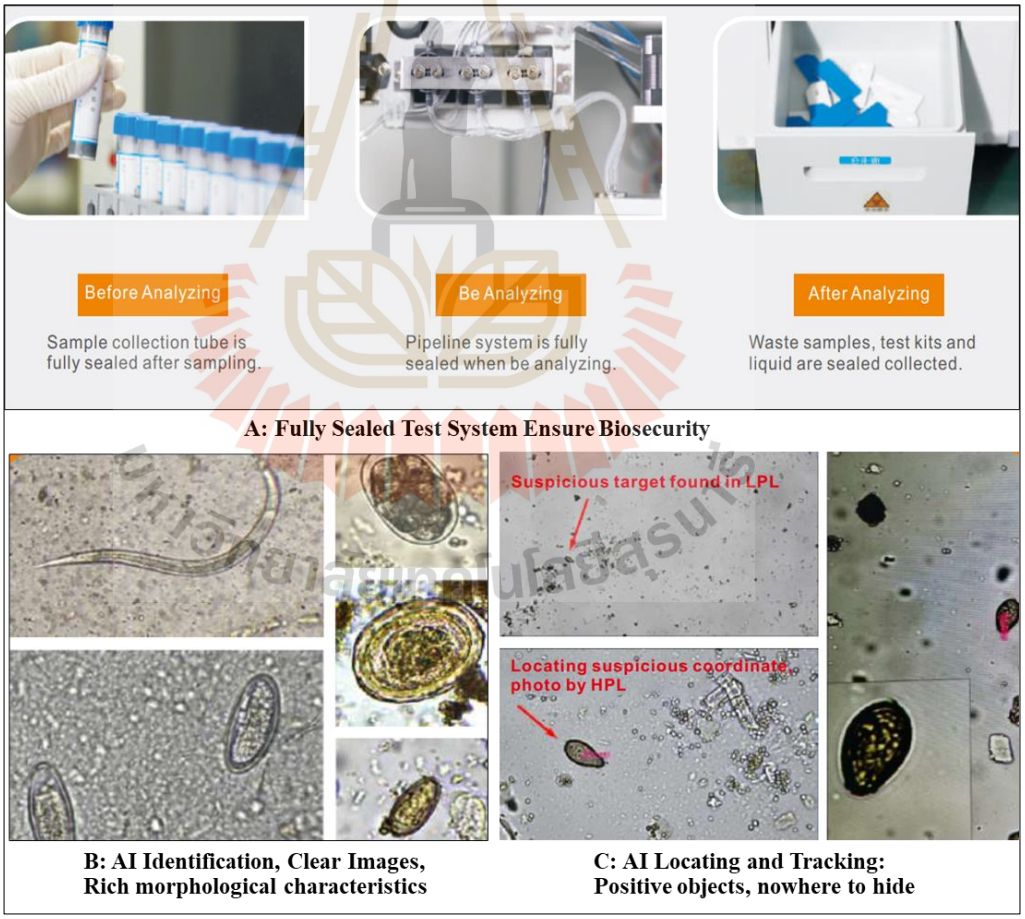


Figure 2.8 A feature on Automatic stool processing machine (A-C)

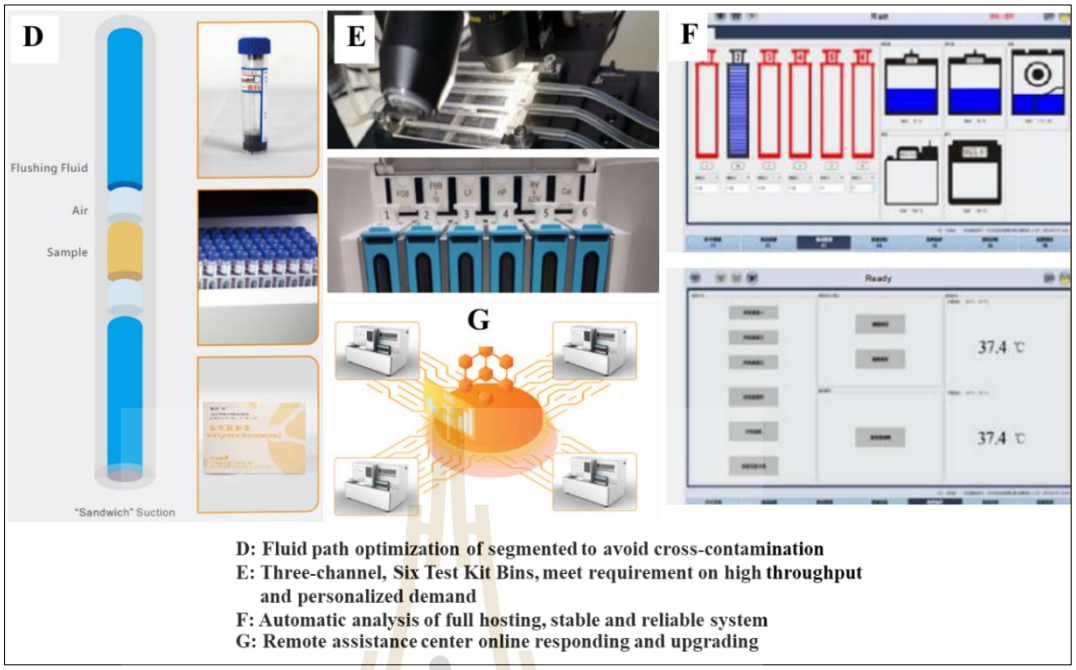


Figure 2.9 A feature on Automatic stool processing machine (D-G)

2.10 Related research

Diagnosis of *O. viverrini* infection has traditionally relied on parasitological techniques, such as microscopic examination of stool samples to detect eggs. The Kato-Katz technique, formalin-ethyl acetate concentration technique (FECT), and direct simple smear are commonly used (Bergquist et al., 2009; Qian et al., 2013). However, these methods have limitations in sensitivity and specificity. Eggs of *O. viverrini* are morphologically similar to those of other liver flukes and intestinal flukes, necessitating confirmatory testing by expert parasitologists (Sripa et al., 2011).

Screening of individuals with opisthorchiasis for surveillance and control in endemic areas has traditionally relied on conventional parasitological methods, i.e., Kato-thick smear (Jongsuksuntigul, 2003), formalin-ethyl acetate concentration technique (FECT), Kato-Katz (KK) method and simple smear method (Petney et al., 2018). These methods are known to have several drawbacks, including limited analytical sensitivity, i.e., low-intensity infections can go undetected and may require repeated fecal examination over several days. In addition, the KK method has been

shown to have limited analytical specificity, with *O. viverrini* eggs often confused with the eggs from minute intestinal flukes (MIF) such as *Phaneropsolus bonnei* and *Prosthodendrium molenkampi*, and accurate distinction requires experienced microscopists (Kaewkes, 2003; Lovis et al., 2009; Sayasone et al., 2009; Chai et al., 2015).A previous study suggested that FECT is superior to the KK method for screening of *O. viverrini*, but FECT is logistically complicated, i.e., samples require centrifugation, and has practical disadvantages compared with the KK method (Charoensuk et al., 2019). To ease the complications of performing FECT, stool concentrator kits such as the Mini Parasep® Kit have been developed to minimize specimen handling within a disposable enclosed system (Saez et al., 2011). In addition, after specimen preparation, the samples can be kept for later examination, which eases time demands when screening

Molecular techniques, including polymerase chain reaction (PCR)-based assays, have been developed to improve diagnostic specificity. PCR can differentiate *O. viverrini* eggs from those of Clonorchis sinensis and other small intestinal flukes by targeting the internal transcribed spacer regions of ribosomal RNA genes (Sato et al., 2009). These advanced techniques have enhanced the accuracy of prevalence estimates and are crucial for epidemiological studies.

CHAPTER III

RESERCH METHODOLOGY

3.1 Study design

The experimental study was conducted over four months from August to December 2022, to demonstrate the examination of gastroenterological parasitic infections among 455 samples available left in the PDRC, Institute of Medicine, Suranaree University of Technology by using FECT, KKT, Automatic concentration feces analyzer, which is required to perform under light microscope.

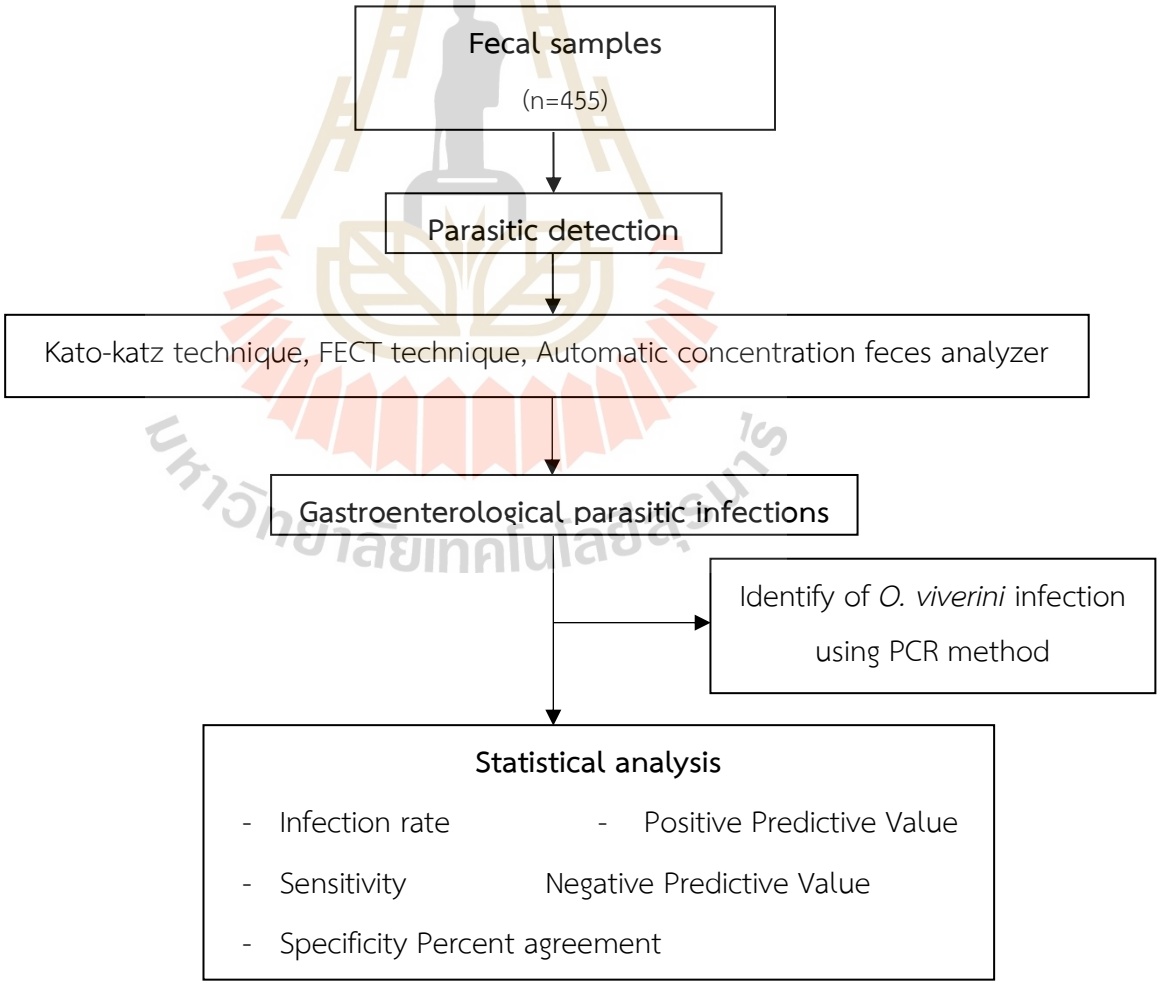


Figure 3.1 Flowchart of study design

3.2 Location of research

The research was conducted at the Parasitic Disease Research Center (PDRC), Institute of Medicine, Suranaree University of Technology, located at 111 Maha Witthayalai Rd, Suranaree, Mueang Nakhon Ratchasima District, Nakhon Ratchasima 30000. The experiments were conducted between August 2022 and December 2023

3.3 Population and sample size

Population (faecal specimens) was collected from parasitic disease research center (PDRC), Institute of Medicine, Suranaree University of Technology. Samples size will be calculated according to the formula for comparing the sensitivity (or specificity) of two diagnostic tests followed the formula (Buderer, 1996). That reported by Hajian-Tilaki K, in 2014 as following:

$$\boldsymbol{n} = \frac{\left[Z_{\frac{\alpha}{2}}\sqrt{2\,x\,\overline{P}(1-\overline{P})} + Z_{\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right]^2}{(P_1-P_2)^2}$$

$\boldsymbol{n}$ = sample size,

$Z_{\frac{\alpha}{2}}$ = statistic for a level of confidence (= 1.96)

Z_{β} = statistic for a level of confidence (= 0.84)

$\overline{P}$ = the average of P_1 and P_2

P_1, P_2 = the sensitivity (or specificity) of two diagnostic tests

$$\boldsymbol{n} = \frac{\left[1.96\,x\,\sqrt{2\,x\,(0.95\,x\,0)} + 0.84\,x\,\sqrt{0.91\,x\,(1-0.95)+0.91\,(1-0.91)}\right]^2}{(1-0.91)^2}$$

$$\boldsymbol{n} = 455\ case$$

The required sample size for this study comprised 455 individuals. A cross-sectional design was employed to gather fecal specimens, the study population mostly

comprised a high-risk group or area where parasitic infections have been reported, and a simple random sampling technique was utilized to select the 455 participants from the population in Nakhon Ratchasima (n=146), Chaiyaphum (n=204), Kalasin (n=54), Roi-Et (n=25), and Nong Khai (n=26) in northeastern Thailand during 2021 to 2023 (Figure 1). Male and female aged 15 years or older were recruited. After the participants informed consent, they were registered for demographic information and fecal specimens' collection, respectively.

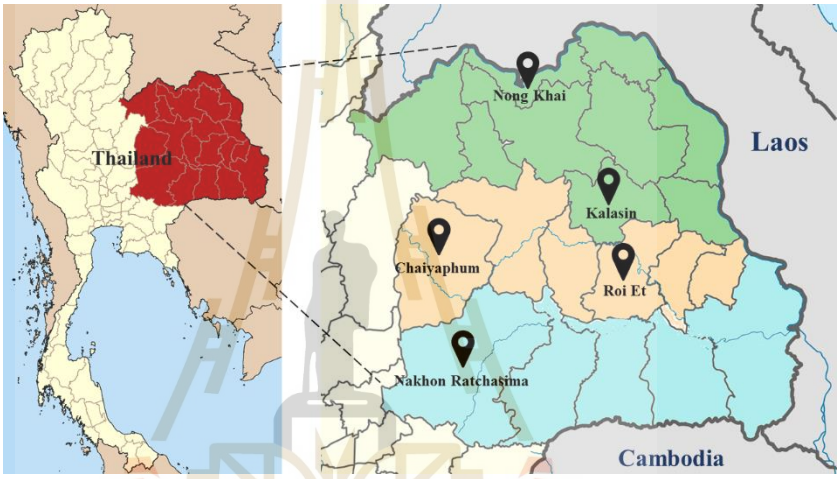


Figure 3.2 Map of fecal specimens collection areas. The representation of five provinces (Nong Khai, Kalasin, Chaiyaphum, Roi Et, and Nakhon Ratchasima) was marked with a black dropped pin (Modified from Wikimedia Commons, 2010).

3.4 Ethical statement

The procedure was approved by the Human Ethics Committee of the health office in Nakhon Ratchasima Province (Reference number NRPH013), and the ethical aspects of fecal examination were approved by the Bioethics Committee of Suranaree University of Technology, Thailand (Reference number SUT-IBC-019/2022). (Figure 1E)

3.5 Materials

The parasitological and molecular techniques was used for examined the gastroenterological parasitic infections in stool samples including Kato-Katz technique, Formalin-ethyl acetate concentration technique, Automatic concentration feces analyzer and Molecular technique. Materials and methods for each technique in this study including reagents and equipment as below.

3.5.1 Kato-Katz technique

One gram of feces was pressed through a mesh screen to remove large particles, and then a portion of the sieved sample was transferred to the hole of a template on a slide. After filling the hole, the template was removed, and the remaining sample was covered with a piece of cellophane pre-soaked in a glycerol-malachite green solution. The number of eggs was counted under a light microscope and multiplied by 24 to calculate the eggs per gram (EPG) (Kato and Miura, 1954; Katz et al., 1972).

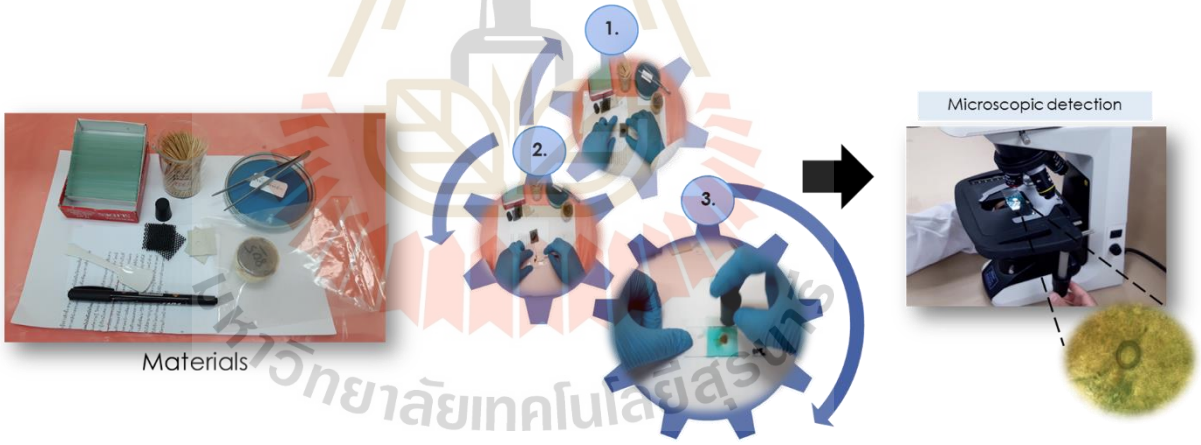
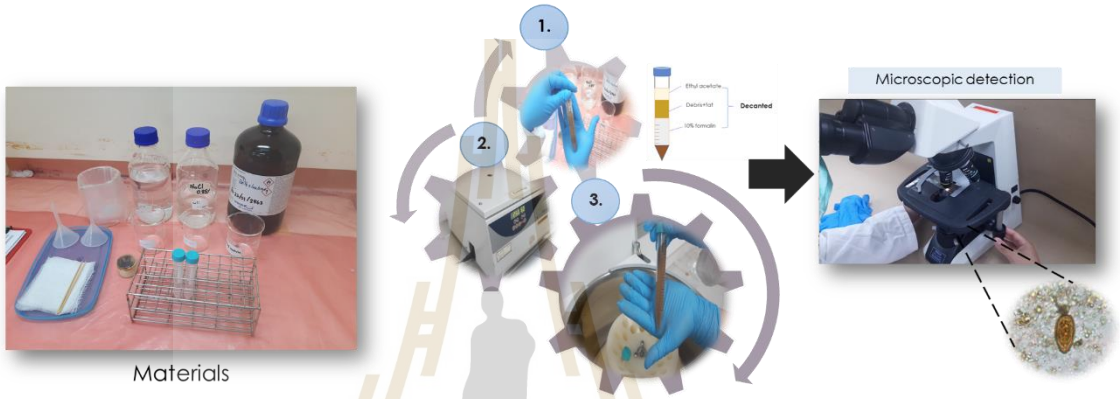


Figure 3.3 The process of Kato-Katz technique

3.5.2 Formalin-ethyl acetate concentration technique

Three grams of stool were dissolved in 10 ml of 0.85% saline, and the debris was strained on gauze to bring the volume in the centrifuge tube to 15 ml and then centrifuged at 1,500 rpm for 5 min. Thereafter, 7 ml of 10% formalin and 3 ml of ethyl acetate were added to the sediment and mixed thoroughly. The sample was

centrifuged at 1,500 rpm for 5 min and then the top layers of the supernatant were decanted. A cotton-tipped applicator was used to remove the debris from the sides of the centrifuge tube. The resulting sediment was fixed with 1 ml of 10% formalin. The final fecal suspension was examined with two drops of 40 µl per sample by the same microscopist using a compound microscope at 100× and 400× magnifications with the results combined and multiplied by the number of drops in the suspension and divided



by the mass of stool in grams to calculate the number of EPG (Truant et al., 1981; Charoensuk et al., 2019; Kopolrat et al., 2022).

Figure 3.4 The process of FECT technique

3.5.3 Automatic Feces Analyzer

The techniques employed by the FA280 Fully Automatic Feces Analyzer entail the utilization of artificial intelligence (AI)-based machine. The collection tube was added 0.5 g of fecal specimen. The machine operates with the following steps of liquidation. Briefly, sample dilution was added into the sample container for liquidation. After mixing, the liquidized samples were passed to the concentrated specimen. The microscope of the machine was located and identified the terminator of parasite detection and then the results reporting showed the images and other parameters such as color and character of feces.

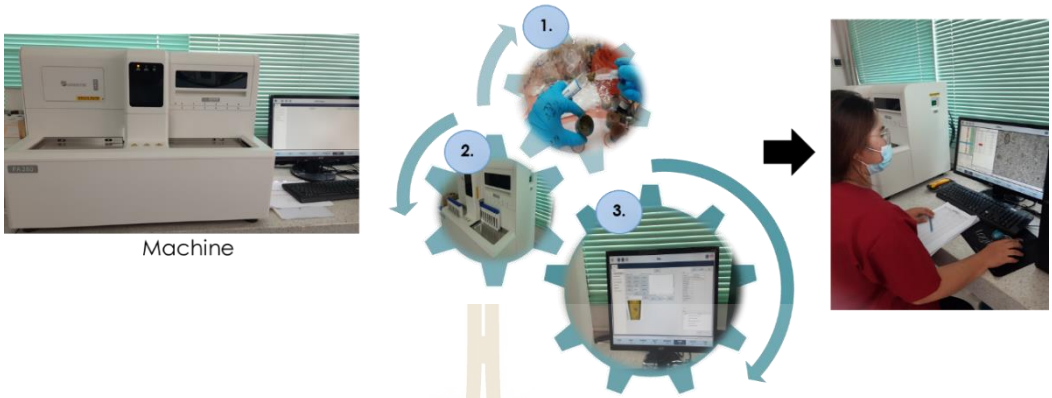


Figure 3.5 The process of machine FA280 Fully Automatic Feces Analyzer.

3.5.4 Polymerase chain reaction (PCR) technique

3.5.4.1 Genomic DNA extraction

DNA was extracted from faecal specimens using a feces QIAamp1 DNA mini kit (Qiagen, Hilden, Germany) and following manufacturer's instructions. In concise, 180-220 mg of stool in 2 mL was added by 1mL of inhibit EX buffer in microcentrifuge tube to each specimen respectively. In each following step is vortex 1 min or until stool sample has homogenized, heat suspension temperature at 95 ℃ for 5 minutes. And then centrifuge with 14,000 rpm for 1 minute to pellet stool particles. Add 200 µL of supernatant into 15 µL of proteinase K tube and add 200 µL buffer AL and vortex with 15 sec. The next is incubate at 70 ℃ for 10 min with Ethanol 95% (200µL), and vortex 15 sec. The supernatant 600µL will be transferred to a new spin column and centrifuge for 1 min then place the QIAamp spin column into 2 ml collection tube. Add 500µL buffer AW1 to centrifuge for 1 min then discard the tube containing the filtrate. The buffer AW2 will be added into spin column with 500 µL then centrifuge for 3 minutes and discard collection tube containing filtrate. The spin column place into the new collection tube and centrifuge for 3 minutes. The QIAamp was transferred into a new

labeled 1.5 ml microcentrifuge tube and then add 25 µL buffer ATE into spin column and incubate for 1 min at the room temperature then centrifuge for 1 min to elute DNA. Finally, DNA extraction sample stored at –20 ºC until use.

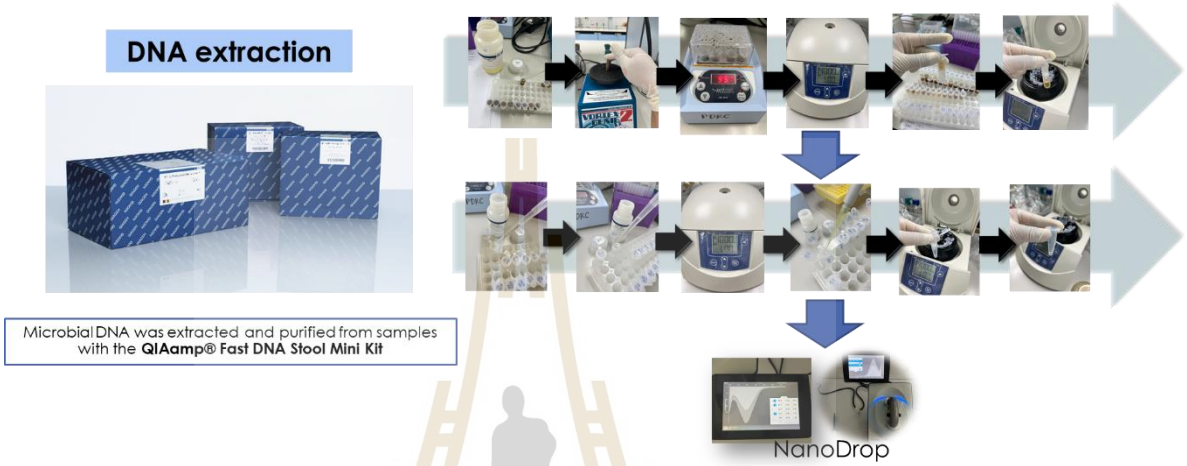


Figure 3.6 The process of DNA extraction

3.5.4.2 Primer design

Polymerase chain reaction (PCR) is most important in molecular biology with specific primer based on rDNA in the nucleotide database of NCBI. Primers for amplification, discrimination, and sequencing of the mitochondrial protein-coding and nuclear ribosomal genes used in this study. The ITS2-PCR sequence-Ov-6F 5´-CTG AAT CTC TCG TTT GTT CA-3´ as a forward primer and Sequence-Ov-6R 5´-GTT CCA GGT GAG TCT CTC TA-3´ as a reverse primer that produced an amplicon was generated of fragment 330 bp from nuclear ribosomal genes (Wongratanacheewin et al, 2003)

3.5.4.3 Polymerase chain reaction (PCR) using ITS2

The ITS2-PCR was amplified using OV-6F 5´-CTG AAT CTC TCG TTT GTT CA-3´ as a forward primer and OV-6R 5´-GTT CCA GGT GAG TCT CTC TA-3´ of 330 bp. The ITS2-PCR reaction with total volume 25µL and containing of PCR buffer (20 mM Tris-HCl), 50 mM KCl, 1.5 mM of $MgCl_2$, 200 µM of dNTP, and 1 µM of Taq DNA polymerase (Duenngai

et al. 2008). The PCR products was amplified in the G-STORMTM GS482 thermal cycler (G-STORM) (Figure 3.6). There are three steps of PCR as following denaturation, annealing, and extension, which is initial denaturation temperature at 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and extension at 72°C for 45 sec, which is accompanying by 30 cycles (Duenngai et al. 2008). Finally holding temperature at 12°C. However, the higher specificities of PCR, it fails to be negative if there is a PCR inhibitor in stool. DNA extraction protocol using cetyltrimethylammonium bromide to remove inhibitors had been applying for the better sensitivities of diagnosis (Duenngai et al. 2008). PCR-based method test is highly sensitive if the specimens contained more than 200 eggs per gram of feces according to *O. viverrini* infection intensity (Wongratanacheewin et al. 2002).

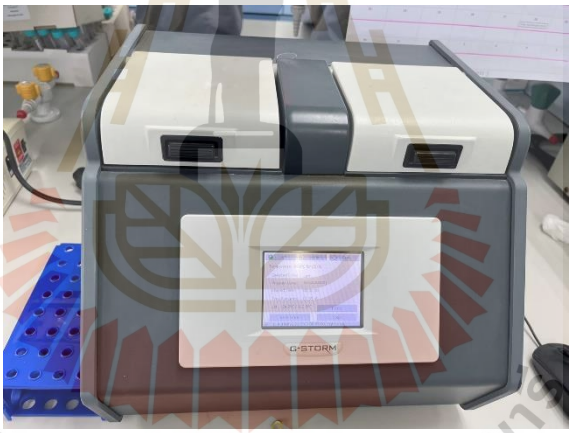


Figure 3.7 The PCR machine in this study.

3.5.4.4 Agarose gel electrophoresis

The PCR product was confirmed by electrophoresis under 1.5% of agarose powder for detection PCR products by using TAE buffer, 6x DNA loading, DNA ladder, Maestrosafe nucleic acid stain (MNAS), and DNA extracted. Before the gel electrophoresis run the solution has diluted comprising of 1µL MNAS, 1 µL of 6x DNA loading dye, and 3-5 µL of DNA template depending on the gel well number that selected for gel electrophoresis that sorted, respectively. However, the 1 µL of DNA

ladder dye mixed with MNAS only for ladder band. The PCR products were electrophoresed by 100 V at room temperature approximately 30-40 minutes. The final step, the agarose gel was transferred to visualize under molecular image® Gel Doc TMXR + Imaging System (Bio-Rad).

3.6 Statistical analysis

Statistical analysis was performed using SPSS version 26.0 for Windows (SPSS Inc., Chicago, USA). The gold standard was defined as the combined method. Positive results referred to the presence of parasite eggs or larvae in the examined fecal specimen of three methods: KKT, FECT, and FAFA. The positive result regardless of the technique was considered a true positive. Therefore, specificity would be judged 100% from this setting. *O. viverrini* eggs were identified and counted to report the prevalence of infection with 95% confidence interval (CI). Each examination technique was evaluated for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and percent agreement was reported as Cohen's kappa (0.00-0.19; none, 0.20-0.39; minimal, 0.40-0.59; weak, 0.60-0.79; moderate, 0.80-0.89; strong, 0.90-1.00; almost perfect). Data was reported as a percentage with 95% CI. The statistical significance level was predetermined at P-value < 0.05.

3.7 Ethical statement

The procedure was approved by the ethical aspects of fecal examination were approved by the Bioethics Committee of Suranaree University of Technology, Thailand (Reference number SUT-IBC-019/2022).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Detection of *O. viverrini* eggs from fecal specimens using routine parasitological concentration and commercial methods.

4.1.1 Prevalence of Intestinal parasites infections (IPIs)

The population in the study area includes five provinces from which cases of parasitic infections were reported, namely Nakhon Ratchasima, Chaiyaphum, Kalasin, Roi Et, and Nong Khai. These provinces were located in the northeastern region of Thailand (Figure 4.1). The results of screening individuals for new infections using the Kato Katz method were based on a sample of 455 case, including 22.42% (101/455) males and 77.58% (353/455) females, with a mean age of 54.53 years (S.D.=10.80).

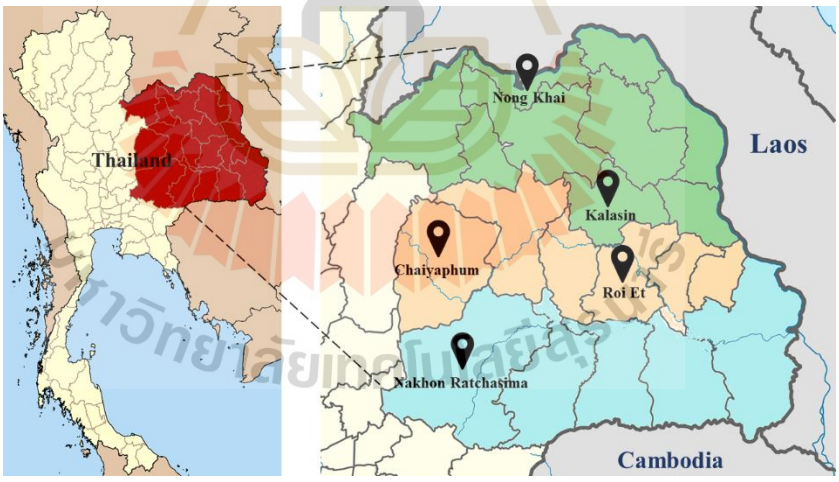


Figure 4.1 Map of fecal specimens collection areas. The representation of five provinces (Nong Khai, Kalasin, Chaiyaphum, Roi Et, and Nakhon Ratchasima) was marked with a black dropped pin (Modified from Wikimedia Commons, 2010).

The examination of fecal specimens from a total of 455 samples were revealed an overall prevalence of parasitic infection at 19.34% (88/455). The most prevalent

infection was *O. viverrini* at 18.02% (82/455), followed by *S. stercoralis* at 0.88% (4/455), *Taenia* spp. and *Enterobius vermicularis* at 0.22% each (1/455). Parasite eggs and larvae were identified using KKT, FECT, and the FAFA machine, as illustrated in Figure 4.2. Upon provincial analysis, Kalasin province was exhibited the highest infection rate at 55.55% (30/54), followed by Chaiyaphum at 22.55% (46/204), and Roi Et at 16.0% (4/25) , respectively, (Table 4.1).

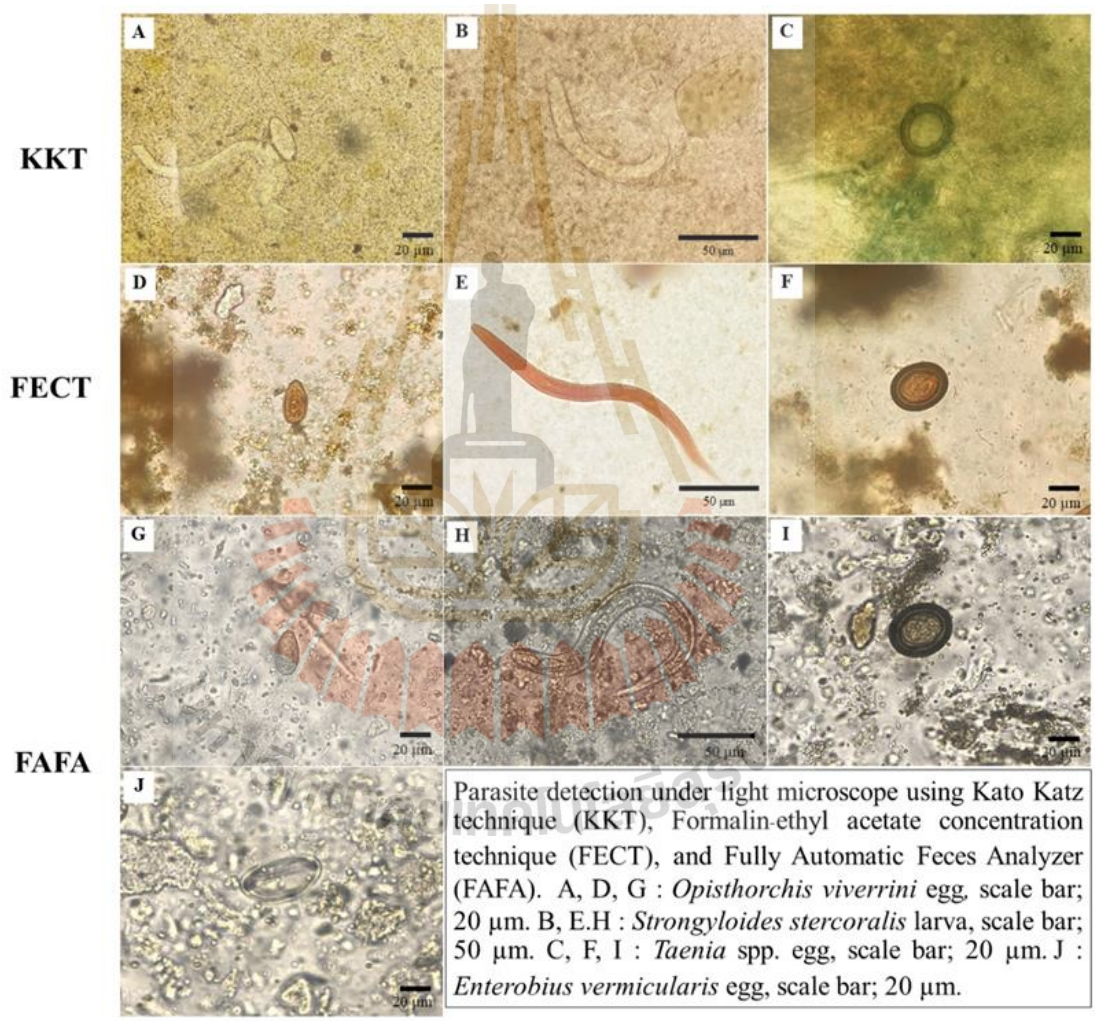


Figure 4.2 Intestinal helminth eggs and larva in fecal specimens under light microscopy.

Table 4.1 Parasitic infections among the population in five provinces through the examination of fecal specimens using KKT, FECT, and FAFA (*n*=455).

Parasite	Province					
	NR (*n*=146) (%)	CP (*n*=204) (%)	KL (*n*=54) (%)	RE (*n*=25) (%)	NK (*n*=26) (%)	Total (%)
O. viverrini	3 (2.05)	46 (22.55)	30 (55.55)	4 (16.00)	0	83 (18.02)
S. stercoralis	3 (2.05)	0	1 (1.85)	0	0	4 (0.88)
Taenia spp.	0	0	0	0	1 (3.85)	1 (0.22)
E. vermicularis	1 (0.68)	0	0	0	0	1 (0.22)
Total	**7 (4.79)**	**46 (22.55)**	**31 (57.41)**	**4 (16.00)**	**1 (3.85)**	**88 (19.34)**

NR= Nakhon Ratchasima, CP= Chaiyaphum, KL=Kalasin, RE= Roi-Et and NK= Nong Khai

4.1.2 Identification of *O. viverrini* eggs

The identification of *O. viverrini* eggs through the FECT predominantly was yielded a detection rate of 16.48% (75/455). Subsequently, the FAFA machine was demonstrated a rate of 10.55% (48/455), while the KKT exhibited a rate of 8.57% (39/455). Moreover, *S. stercoralis* larvae were primarily identified at 0.88% (4/455) using FECT, 0.66% (3/455) using FAFA, and 0.44% (2/455) using KKT. *Taenia* spp. eggs were detected at 0.22% (1/455) using FECT, FAFA, and KKT. *E. vermicularis* was identified at 0.22% (1/455) using FAFA. The same specimens, total of 23 cases were positively detected the helminths by FAFA, KKT, and FECT (22 cases of *O. viverrini* eggs and 1 case of *S. stercolaris* larvae). Additionally, double infection (*O. viverrini* eggs and *S. stercoralis* larvae) was observed at 0.22% (1/455) using both FECT and FAFA.

Table 4.2 Identification of parasitic infections through the examination of fecal specimens using KKT, FECT, and FAFA (*n*=455).

Parasites	KKT		FECT		FAFA		*P*-value
	n(%)	95%CI	*n*(%)	95%CI	*n*(%)	95%CI	
O. viverrini	39 (8.57)	0.54-0.72	75 (16.48)	0.88-0.97	51 (10.55)	0.62-0.79	0.000
S. stercoralis	2 (0.44)	0.45-1.00	4 (0.88)	1.00-1.00	3 (0.66)	0.58-1.00	0.000
Taenia spp.	1 (0.22)	1.00-1.00	1 (0.22)	1.00-1.00	1 (0.22)	1.00-1.00	0.002
E. vermicularis	-	-	-	-	1 (0.22)	1.00-1.00	0.002
Double infection	-	-	1 (0.22)	1.00-1.00	1 (0.22)	1.00-1.00	0.002

4.2 Results of Identify *O. viverrini* eggs by using molecular technique.

4.2.1 Confirm PCR product by gel electrophoresis

A total of 83 *O. viverrini* positive fecal samples were available for DNA extraction and PCR method. To identify species of *O. viverrini*, the PCR method using ITS2 primers was 91.57% (76/83) positive in specimens with *O. viverrini* positive from three methods of stool examination. As shown in Table 4.3, The number of *O. viverrini* positive by using PCR methods for confirm was 95.83% (46/48) from FAFA, 94.67 (71/75) from FECT and 92.31% (36/39) from KKT. The PCR products showed the fragments of 330 bp for *O. viverrini* positive (Figure 4.3).

Table 4.3 The results of PCR methods for confirm *O. viverrini* infection.

Method	Number of *O. viverrini* eggs positive	confirm *O. viverrini* infection by PCR method	
		Positive (%)	Negative (%)
KKT	39	36 (92.31)	3 (7.69)
FECT	75	71 (94.67)	4 (5.33)
FAFA	48	46 (95.83)	2 (4.17)

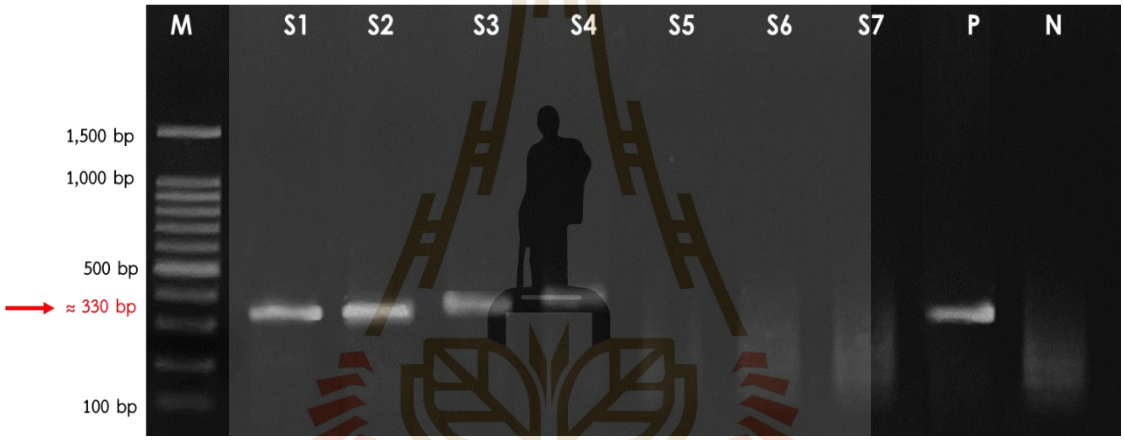


Figure 4.3 PCR products with *O. viverrini* positive using ITS2 primer. Lanes S1-S4 was *O. viverrini* positive, Lanes S5-S7 was Sample negative, P was Positive control and N was negative control.

4.3 Results of comparison of efficacies between routine parasitological concentration and commercial methods.

4.3.1 Comparison of efficacies between routine parasitological concentration and commercial methods.

The sensitivity for *O. viverrini* egg detection was found to be 88.15%,100.00%, and 99.74% for KKT, FECT, FAFA machine, and PCR, respectively. In terms of specificity,

KKT, FECT, and FAFA demonstrated values of 42.65%, 97.06%, and 64.73% , respectively. The KKT exhibited the highest positive predictive value (PPV) for detecting *O. viverrini* eggs at 100.00%, followed by FECT at 99.54%, while FAFA displayed the lowest PPV at 94.18 %. On the other hand, KKT and FECT demonstrated the highest negative predictive value (NPV) at 100.00%, followed by FAFA machine, both with a NPV of 97.81%. Substantial agreement was observed between the total positive results and the three methods: FECT (kappa=0.98; almost perfect), FAFA (kappa=0.75; moderate), and KKT (kappa=0.67; weak) (Table 4.5).

Table 4.5 Diagnostic accuracy of KKT, FECT, and FAFA for *O. viverrini* examination in fecal specimens.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa (95%CI)
KKT	85.15	42.65	100.00	100.00	0.56 (0.41-0.67)
FECT	100.00	97.06	99.54	100.00	0.98 (0.95-1.00)
FAFA	99.74	64.73	94.18	97.81	0.75 (0.54-0.839)

The preparation times for KKT, FECT, and FAFA were 30, 15, and 10 min, respectively. Notably, FECT involved exposure to formalin and ethyl acetate, whereas both KKT and FAFA remained chemical-free in their processing methods. The cost of sample examinations associated with each technique was outlined in Table 4.4 It is important to note that the price of the FAFA machine is not included in the cost analysis.

Table 4.4 Considerations of preparation time, cost implications associated with individual techniques, and the extent of chemical exposure during experimentation.

	Methods			
	KKT	FECT	FAFA	PCR
Preparation time (min/case)	30	15	10	120
Cost per sample (Thai Baht/case)	60	250	160	
Chemical exposure				
Ethyl acetate	-	+	-	-
Formalin	-	+	-	-

4.4 Discussion

This study conducted a comparative analysis of three parasitological methodologies-namely KKT, FECT, and FAFA-aimed at diagnosing *O. viverrini* and parasitic infections within the fecal samples of a community population in northeastern Thailand. The results of the parasitic infection examination using all three methods revealed the presence of parasitic infections in all provinces, with a higher prevalence observed in Kalasin province, followed by Chaiyaphum province. These findings align with the study conducted by Wattanawong et al. (2019) which reported a higher frequency of infections in the northeastern region, particularly with represent to *O. viverrini*, *S. stercoralis*, *Taenia* spp., and E. vermicularis. From the findings of this study, it is evident that the prevalence of *O. viverrini* infection is higher than that reported in the national survey of 2019. This highlights the ongoing presence of infections at various community levels, emphasizing the critical necessity for continuous surveillance by relevant authorities. In assessing the qualitative and quantitative diagnosis of *O. viverrini*, the three methodologies demonstrated comparable performance, as assessed through sensitivity, specificity, PPV, and NPV. The calculated kappa coefficient also underscored substantial agreement among the three

tests. In addition, when diagnosing various parasitic helminth infections, the FECT demonstrated significantly elevated diagnostic parameters in comparison to the FAFA and KKT. In the context of population screening for *O. viverrini*, FECT exhibited noteworthy performance in helminthiasis diagnosis, particularly in the case of opisthorchiasis. Although the FAFA yields lower diagnostic results than the FECT method, it is noteworthy that, when compared to the KKT method, FAFA demonstrates superior diagnostic outcomes. Nevertheless, our findings are consistent with previous research, underscoring a limitation shared by both FECT and KKT—namely, their incapacity to differentiate between minute intestinal fluke (MIF) eggs and *O. viverrini* eggs (Chai et al., 2005; Buathong et al., 2017; Lamaningao et al., 2017). Prior investigations in the northeastern region of Thailand have predominantly focused on *O. viverrini* infection, with a low prevalence of MIF reported (Ramsay et al., 1989; Elkins et al., 1990; Boonjaraspinyo et al., 2013). In contrast, the central region of the Lao People's Democratic Republic (Lao PDR) identified *Haplorchis* spp. as more abundant than *O. viverrini* (Chai et al., 2013). Furthermore, a study in northern Thailand employing molecular diagnostics revealed the occurrence of MIF to be 3.8 times higher than that of *O. viverrini* (Buathong et al., 2020). Epidemiological surveys of parasitic helminths in Southeast Asia, including Thailand, frequently indicate coinfections with multiple species (Boonjaraspinyo et al., 2013; Chai et al., 2013; Sayasone et al., 2015). Given this scenario, there is a preference for diagnostic methods that can reliably classify helminth eggs from different species. This underscores the importance of employing methodologies with the capacity to discriminate among various parasitic infections, facilitating a more nuanced understanding of the complex epidemiological landscape in the region. While the examination and differentiation of both *O. viverrini* and MIF using the FAFA have not been extensively studied before, this research represents the inaugural investigation assessing both types of flukes, demonstrating effective discrimination in comparison to the KKT.

In this study, the PCR method targeting the ITS2 region was employed to confirm *O. viverrini* egg infections. Some samples yielded negative PCR results, potentially due

to the presence of strong PCR inhibitors in fecal samples and the unsuccessful disruption of the eggs. Therefore, improvements in DNA extraction and PCR assays for detecting Opisthorchis-like eggs in fecal samples are needed, especially for cases with light infections. Discrimination between human liver fluke eggs and those of small intestinal flukes (MIF) was introduced using PCR assays of the ITS2 region. A related study demonstrated that the ITS2-PCR assay had a sensitivity of 71.0%, with detection limits as low as 0.6–3 pg. of Opisthorchis-like eggs (Traub et al., 2009). Currently, the diagnosis of *O. viverrini* and MIF infections is primarily performed via microscopy-based stool examinations. Techniques such as KKT or FECT can increase the sensitivity of these tests (Tungtrongchitr et al., 2007; Qian et al., 2013; Tesana et al., 1991; Glinz et al., 2010). Screening for *O. viverrini* infection is conducted through direct stool examination using the KKT, where stool samples are examined for *O. viverrini* eggs under a microscope. This method provides good accuracy and remains practical for local settings with limited resources (Bergquist et al., 2009; Tungtrongchitr et al., 2007; Qian et al., 2013). However, *O. viverrini* eggs are morphologically similar to MIF eggs, making differentiation challenging in routine practice (Buathong et al., 2017; Tesana et al., 1991). Consequently, diagnosis relies on identifying eggs under a light microscope. Studies of *Opisthorchis*-like eggs conducted by Radomyos et al. (Radomyos et al., 1998; 1994) and Wijit et al. (Wijit et al., 2013) in the upper northern provinces revealed that most adult flukes collected from participants were MIFs, which are not risk factors for CCA. In central and northeastern Thailand, *Opisthorchis*-like eggs were mostly identified as *O. viverrini* infections (Sithithaworn et al., 2012; Boondit et al., 2020), which are significantly related to CCA occurrence (Poomphakwaen et al., 2009; Kamsa-ard et al., 2018). Several studies have utilized PCR to differentiate between *O. viverrini* and MIFs by targeting the rDNA ITS1 and ITS2 regions (Sato et al., 2009; 2010). PCR has shown better performance than concentration techniques such as FECT, especially in areas with low infection intensity. Studies in northern provinces of Thailand revealed that MIFs, particularly *H. taichui*, were dominant fish-borne trematodes in these regions. A study conducted from 2015 to 2016 in upper northern provinces using purgative

magnesium sulfate found that 70% of *Opisthorchis*-like eggs were positive for *H. taichui*, with *O. viverrini* undetected by PCR (Wijit et al., 2013). In this study, the PCR assay based on the rDNA ITS2 region was used to detect *Opisthorchis*-like eggs from stool specimens. The ITS2 primer demonstrated high sensitivity, detecting *Opisthorchis*-like eggs with a sensitivity of 95.2%, whereas the RTFluke primer showed only 71.0% sensitivity (Traub et al., 2009). The ITS2 primer revealed a higher proportion of *H. taichui* infections and co-infections of *H. taichui* and *O. viverrini* compared to single *O. viverrini* infections (Buathong et al., 2020).

The FAFA machine employs the sedimentation concentration technique to process fecal specimens, resembling the FECT. This method demonstrates reliability in automatically concentrating stool samples to detect parasites commonly endemic in Thailand. Both preserved and fresh specimens are compatible with the FAFA machine, and our findings align with the established reliability demonstrated in other published studies on commercial kits (Sawangkla et al., 2022). This machine proves particularly suitable for laboratory use, offering an advantageous solution for regions lacking specialized personnel. In terms of safety, the FAFA machine ensures the processing of specimens within a sealed container, mitigating potential hazards for operating staff. The standardized operation of the machine contributes to minimal errors compared to human-induced errors. Furthermore, the turnaround time for specimens processed by the FAFA machine is notably shorter than conventional methods, enhancing efficiency in diagnostic procedures. However, a notable drawback pertains to the associated costs, encompassing the specimen container and the requisite training for machine operation. Several factors may contribute to the observed variance among distinct tests. Firstly, the FAFA machine processes a greater amount of fecal specimen (0.5 g) compared to FECT (3 g) and KKT (1 g). Secondly, procedural stages within FECT involving the filtration of fecal debris and fat play a pivotal role in enhancing the isolation of eggs, thereby amplifying the likelihood of detecting parasite eggs or larvae (Kopolrat et al., 2022). Moreover, the long-standing use of the KKT in screening and control programs in Thailand (Jongsuksuntigul and Imsomboon, 2003; Wattanawong et al., 2019) may be

associated with underreporting in current epidemiological settings, given the prevalence of light infections of *O. viverrini*. While FECT requires specific laboratory equipment and proves impractical in resource-poor settings, the FAFA machine's design as an all-in-one tube with a fixative and built-in filtration apparatus removes fecal debris akin to the FECT procedure. Importantly, the FAFA allows for sample storage after preparation, providing a distinct advantage over KKT, which necessitates immediate reading by technicians within 30 min of slide preparation. Our findings indicate that the FAFA performs equally well compared to FECT and outperforms KKT. This suggests that the FAFA may emerge as the method of choice for future field applications in parasite surveys, particularly in the northeastern region of Thailand (Charoensuk et al., 2019). Advancements in science and technology have led to the development of commercial kits with accuracy comparable to conventional techniques (Soares, 2020). Numerous studies evaluating the clinical use of such kits, including Feconomics®, Mini Parasep®, and Sciendox 50®, have demonstrated promising results when compared with FECT (Kurt et al., 2012; Sanprasert et al., 2016; Sawangkla et al., 2021). Additionally, the turnaround time for specimens processed by the FAFA machine was comparatively shorter than the conventional method mentioned above.These developments underscore the evolving landscape of diagnostic tools and the potential integration of innovative technologies for improved parasite detection in various settings.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In summary, a noteworthy prevalence of *O. viverrini* infection is evident in northeastern Thailand. The outcomes of this study reveal that the FAFA, FECT, and KKT methodologies demonstrate comparable diagnostic sensitivity for *O. viverrini.* Specifically, the FAFA machine emerges as a potentially valuable tool for the detection of *O. viverrini* and other parasitic infections. Its aptness for clinical application as a concentration technique device is underscored by heightened safety for laboratory technicians and efficient processing times. To further enhance our comprehension, prospective studies should systematically evaluate the reliability of the FAFA machine in comparison to alternative stool concentration techniques, especially considering that our investigation primarily juxtaposed it with FECT and KKT. Furthermore, assigning precedence to the utilization of fresh specimens in clinical assessments can reinforce the validation of this machine's reliability.

5.2 Recommendation

To further enhance our comprehension, prospective studies should systematically evaluate the reliability of the FAFA machine in comparison to alternative stool concentration techniques, especially considering that our investigation primarily juxtaposed it with FECT and KKT. Furthermore, assigning precedence to the utilization of fresh specimens in clinical assessments can reinforce the validation of this machine's reliability.

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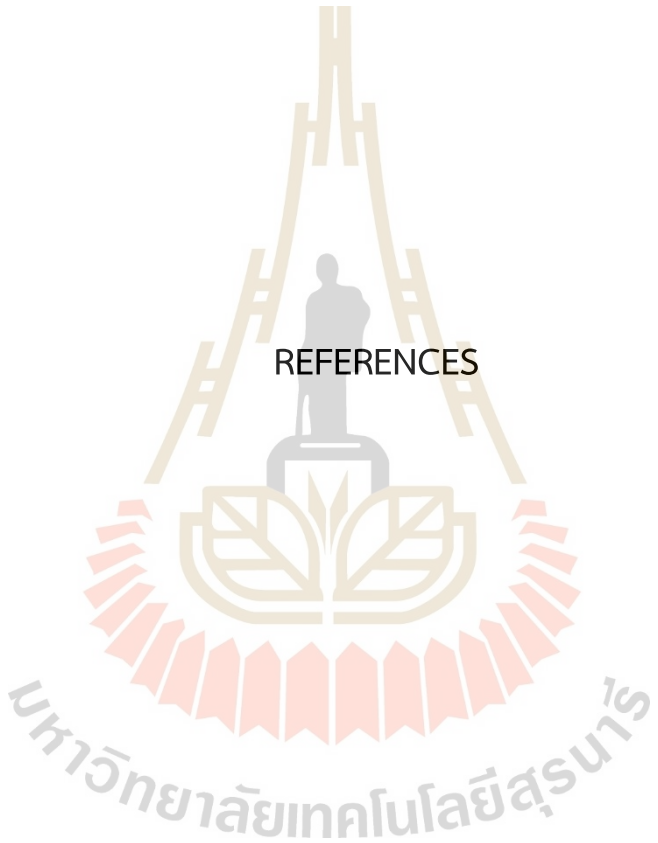

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APPENDICES

APPENDIX A

THE PREPARATIONS OF REAGENTS

Appendix A1 Reagent of glycerol-malachite green

3% aqueous malachite green	1 mL
Glycerol	100 ml
Distilled water	100 ml

Appendix A2 Reagent of PCR Reactions

Distilled water (molecular grade)	16.8 ul
10x Taq Buffer	2.5 ul
dNTP	1 ul
Ov-6F (Forward Primers)	1 ul
Ov-6R (Reverse Primers)	1 ul
Tag DNA Pol.	0.2 ul
$MaCl_2$	1.5 ul
DNA template	1 ul

Appendix A3 Reagent of 1x TAE solution from 50x TAE buffer

1x TAE	980 ml
Distilled water	20 ml

Appendix A4 Reagent of 1.5% Agarose gel

1x TAE	30 ml
Agarose gel	0.45 gram

APPENDIX B

RAW DATA

TABLE 1B Raw data of specimen from specimen

No.	PID	Gender	Age	Location (province)
1	1404	Female	50	Chaiyaphum
2	1405	Male	70	Chaiyaphum
3	1406	Female	56	Chaiyaphum
4	1414	Female	61	Chaiyaphum
5	1415	Female	53	Chaiyaphum
6	1416	Female	51	Chaiyaphum
7	1417	Female	53	Chaiyaphum
8	1418	Female	52	Chaiyaphum
9	1419	Female	59	Chaiyaphum
10	1420	Female	53	Chaiyaphum
11	1422	Female	52	Chaiyaphum
12	1423	Female	58	Chaiyaphum
13	1424	Female	48	Chaiyaphum
14	1425	Male	52	Chaiyaphum
15	1426	Female	58	Chaiyaphum
16	1427	Female	49	Chaiyaphum
17	1428	Female	46	Chaiyaphum
18	1429	Male	56	Chaiyaphum
19	1430	Female	47	Chaiyaphum
20	1431	Male	61	Chaiyaphum
21	1432	Male	56	Chaiyaphum
22	1433	Female	59	Chaiyaphum

No.	PID	Gender	Age	Location (province)
23	1434	Male	65	Chaiyaphum
24	1435	Male	57	Chaiyaphum
25	1436	Female	57	Chaiyaphum
26	1437	Male	42	Chaiyaphum
27	1438	Female	53	Chaiyaphum
28	1439	Female	51	Chaiyaphum
29	1440	Female	56	Chaiyaphum
30	1441	Male	38	Chaiyaphum
31	1447	Male	47	Chaiyaphum
32	1448	Female	68	Chaiyaphum
33	1449	Female	25	Chaiyaphum
34	1489	Male	49	Nakhon Ratchasima
35	1490	Female	47	Chaiyaphum
36	1491	Female	51	Chaiyaphum
37	1492	Female	55	Chaiyaphum
38	1493	Female	60	Chaiyaphum
39	1494	Female	70	Chaiyaphum
40	1495	Female	45	Chaiyaphum
41	1496	Female	58	Chaiyaphum
42	1497	Female	53	Chaiyaphum
43	1498	Female	32	Chaiyaphum
44	1499	Female	56	Chaiyaphum
45	1500	Female	46	Chaiyaphum

No.	PID	Gender	Age	Location (province)
46	1501	Female	47	Chaiyaphum
47	1502	Female	34	Chaiyaphum
48	1503	Female	52	Chaiyaphum
49	1504	Female	53	Chaiyaphum
50	1505	Female	59	Chaiyaphum
51	1506	Female	47	Chaiyaphum
52	1507	Female	49	Chaiyaphum
53	1508	Female	55	Chaiyaphum
54	1509	Female	59	Chaiyaphum
55	1510	Female	52	Chaiyaphum
56	1511	Female	47	Chaiyaphum
57	1512	Female	54	Chaiyaphum
58	1513	Female	52	Chaiyaphum
59	1514	Female	47	Chaiyaphum
60	1515	Female	61	Chaiyaphum
61	1516	Female	30	Chaiyaphum
62	1517	Female	49	Chaiyaphum
63	1518	Female	61	Chaiyaphum
64	1519	Female	44	Chaiyaphum
65	1520	Female	55	Chaiyaphum
66	1521	Female	54	Chaiyaphum
67	1522	Female	49	Chaiyaphum
68	1523	Female	63	Chaiyaphum

No.	PID	Gender	Age	Location (province)
69	1524	Female	75	Chaiyaphum
70	1525	Female	49	Chaiyaphum
71	1526	Female	49	Chaiyaphum
72	1527	Female	46	Chaiyaphum
73	1528	Female	63	Chaiyaphum
74	1529	Female	38	Chaiyaphum
75	1530	Female	55	Chaiyaphum
76	1531	Male	62	Chaiyaphum
77	1532	Male	45	Chaiyaphum
78	1533	Female	62	Chaiyaphum
79	1534	Female	59	Chaiyaphum
80	1535	Female	54	Chaiyaphum
81	1536	Female	60	Chaiyaphum
82	1537	Female	48	Chaiyaphum
83	1538	Female	50	Chaiyaphum
84	1539	Female	62	Chaiyaphum
85	1540	Female	60	Chaiyaphum
86	1541	Female	55	Chaiyaphum
87	1542	Male	65	Chaiyaphum
88	1543	Female	55	Chaiyaphum
89	1544	Male	61	Chaiyaphum
90	1545	Male	69	Chaiyaphum
91	1546	Female	67	Chaiyaphum

No.	PID	Gender	Age	Location (province)
92	1547	Male	37	Chaiyaphum
93	1548	Female	48	Chaiyaphum
94	1549	Female	52	Chaiyaphum
95	1550	Male	73	Chaiyaphum
96	1551	Female	55	Chaiyaphum
97	1552	Male	49	Chaiyaphum
98	1553	Female	51	Chaiyaphum
99	1554	Female	50	Chaiyaphum
100	1555	Male	69	Chaiyaphum
101	1556	Female	43	Chaiyaphum
102	1557	Female	52	Chaiyaphum
103	1558	Female	63	Chaiyaphum
104	1559	Male	64	Chaiyaphum
105	1560	Female	72	Chaiyaphum
106	1561	Female	55	Chaiyaphum
107	1562	Female	50	Chaiyaphum
108	1563	Male	60	Chaiyaphum
109	1564	Female	61	Chaiyaphum
110	1565	Female	58	Chaiyaphum
111	1566	Female	48	Chaiyaphum
112	1567	Female	55	Chaiyaphum
113	1568	Female	55	Chaiyaphum
114	1569	Female	47	Chaiyaphum

No.	PID	Gender	Age	Location (province)
115	1570	Female	59	Chaiyaphum
116	1571	Female	54	Chaiyaphum
117	1572	Female	43	Chaiyaphum
118	1573	Female	50	Chaiyaphum
119	1574	Female	71	Chaiyaphum
120	1575	Female	52	Chaiyaphum
121	1576	Female	50	Chaiyaphum
122	1577	Female	50	Chaiyaphum
123	1578	Female	52	Chaiyaphum
124	1579	Female	75	Chaiyaphum
125	1580	Female	54	Chaiyaphum
126	1581	Female	63	Chaiyaphum
127	1582	Female	77	Chaiyaphum
128	1583	Female	51	Chaiyaphum
129	1584	Female	72	Chaiyaphum
130	1585	Female	54	Chaiyaphum
131	1586	Male	67	Chaiyaphum
132	1587	Male	64	Chaiyaphum
133	1588	Female	62	Chaiyaphum
134	1589	Male	60	Chaiyaphum
135	1590	Female	52	Chaiyaphum
136	1591	Female	41	Chaiyaphum
137	1592	Female	45	Chaiyaphum

No.	PID	Gender	Age	Location (province)
138	1593	Male	63	Chaiyaphum
139	1594	Male	61	Chaiyaphum
140	1595	Male	78	Chaiyaphum
141	1596	Male	57	Chaiyaphum
142	1597	Female	60	Chaiyaphum
143	1598	Female	62	Chaiyaphum
144	1599	Female	55	Chaiyaphum
145	1600	Female	47	Chaiyaphum
146	1601	Male	78	Chaiyaphum
147	1602	Female	61	Chaiyaphum
148	1603	Female	50	Chaiyaphum
149	1604	Female	52	Chaiyaphum
150	1605	Female	70	Chaiyaphum
151	1606	Female	52	Chaiyaphum
152	1607	Female	43	Chaiyaphum
153	1608	Male	54	Chaiyaphum
154	1609	Female	53	Chaiyaphum
155	1610	Female	54	Chaiyaphum
156	1611	Female	40	Chaiyaphum
157	1612	Female	59	Chaiyaphum
158	1616	Female	41	Nong Khai
159	1617	Female	50	Nong Khai
160	1618	Female	61	Nong Khai

No.	PID	Gender	Age	Location (province)
161	1619	Female	55	Kalasin
162	1620	Female	71	Kalasin
163	1621	Female	41	Kalasin
164	1622	Female	44	Kalasin
165	1623	Female	70	Kalasin
166	1624	Female	55	Kalasin
167	1625	Female	48	Kalasin
168	1626	Female	49	Kalasin
169	1627	Male	50	Kalasin
170	1628	Female	50	Kalasin
171	1629	Male	51	Kalasin
172	1630	Female	39	Kalasin
173	1631	Male	40	Kalasin
174	1632	Female	69	Kalasin
175	1633	Male	64	Kalasin
176	1634	Female	48	Kalasin
177	1635	Female	61	Kalasin
178	1636	Male	64	Kalasin
179	1637	Male	36	Kalasin
180	1638	Male	38	Kalasin
181	1642	Female	67	Kalasin
182	1643	Male	64	Nakhon Ratchasima
183	1644	Female	62	Nong Khai

No.	PID	Gender	Age	Location (province)
184	1645	Male	48	Nakhon Ratchasima
185	1646	Female	40	Nakhon Ratchasima
186	1647	Female	65	Nakhon Ratchasima
187	1648	Male	39	Nakhon Ratchasima
188	1649	Female	58	Nakhon Ratchasima
189	1650	Female	45	Nakhon Ratchasima
190	1651	Female	52	Nakhon Ratchasima
191	1652	Female	56	Nakhon Ratchasima
192	1653	Female	65	Nakhon Ratchasima
193	1654	Female	66	Nakhon Ratchasima
194	1655	Female	50	Nakhon Ratchasima
195	1656	Female	53	Nakhon Ratchasima
196	1657	Female	61	Nakhon Ratchasima
197	1658	Female	31	Nakhon Ratchasima
198	1659	Female	57	Nakhon Ratchasima
199	1660	Female	65	Nakhon Ratchasima
200	1661	Male	71	Nakhon Ratchasima
201	1662	Female	15	Nakhon Ratchasima
202	1663	Female	58	Nakhon Ratchasima
203	1664	Male	34	Nakhon Ratchasima
204	1665	Female	54	Nakhon Ratchasima
205	1666	Male	15	Nakhon Ratchasima
206	1667	Male	55	Nakhon Ratchasima

No.	PID	Gender	Age	Location (province)
207	1668	Female	55	Nakhon Ratchasima
208	1669	Male	65	Nakhon Ratchasima
209	1670	Female	71	Nakhon Ratchasima
210	1671	Female	45	Nakhon Ratchasima
211	1672	Female	59	Nakhon Ratchasima
212	1673	Female	46	Nakhon Ratchasima
213	1674	Female	58	Nakhon Ratchasima
214	1675	Female	45	Nakhon Ratchasima
215	1676	Male	63	Nakhon Ratchasima
216	1677	Female	59	Nakhon Ratchasima
217	1678	Female	40	Nakhon Ratchasima
218	1679	Female	52	Nakhon Ratchasima
219	1680	Female	57	Nakhon Ratchasima
220	1681	Female	49	Nakhon Ratchasima
221	1682	Female	51	Nakhon Ratchasima
222	1683	Male	68	Nakhon Ratchasima
223	1684	Female	49	Nakhon Ratchasima
224	1685	Female	68	Nakhon Ratchasima
225	1686	Female	58	Nakhon Ratchasima
226	1687	Female	54	Nakhon Ratchasima
227	1688	Female	55	Nakhon Ratchasima
228	1689	Female	61	Nakhon Ratchasima
229	1690	Female	42	Nong Khai

No.	PID	Gender	Age	Location (province)
230	1691	Male	67	Nong Khai
231	1692	Female	49	Nong Khai
232	1693	Female	48	Nong Khai
233	1694	Female	47	Nong Khai
234	1695	Male	61	Nong Khai
235	1696	Male	61	Nong Khai
236	1697	Male	69	Kalasin
237	1698	Male	52	Kalasin
238	1699	Female	38	Nong Khai
239	1700	Female	58	Nong Khai
240	1701	Female	53	Nong Khai
241	1702	Female	66	Nong Khai
242	1703	Male	51	Nong Khai
243	1704	Female	18	Nong Khai
244	1705	Female	62	Nong Khai
245	1706	Male	54	Nong Khai
246	1707	Male	67	Nong Khai
247	1708	Female	48	Nong Khai
248	1709	Female	50	Nong Khai
249	1710	Male	53	Nong Khai
250	1711	Female	62	Nong Khai
251	1712	Female	42	Nong Khai
252	1713	Female	50	Roi Et

No.	PID	Gender	Age	Location (province)
253	1714	Male	62	Roi Et
254	1715	Female	60	Roi Et
255	1716	Female	54	Roi Et
256	1717	Male	69	Roi Et
257	1718	Female	67	Roi Et
258	1719	Male	47	Roi Et
259	1731	Female	69	Kalasin
260	1732	Female	48	Kalasin
261	1733	Male	67	Kalasin
262	1734	Female	36	Kalasin
263	1735	Female	68	Kalasin
264	1736	Male	55	Kalasin
265	1737	Male	62	Kalasin
266	1738	Male	65	Kalasin
267	1739	Male	65	Kalasin
268	1740	Male	62	Kalasin
269	1741	Female	54	Kalasin
270	1742	Female	80	Kalasin
271	1743	Male	66	Kalasin
272	1744	Male	56	Kalasin
273	1745	Male	45	Kalasin
274	1746	Female	59	Kalasin
275	1747	Male	64	Kalasin

No.	PID	Gender	Age	Location (province)
276	1748	Male	61	Kalasin
277	1749	Female	57	Kalasin
278	1750	Male	47	Kalasin
279	1751	Female	48	Kalasin
280	1752	Male	58	Kalasin
281	1753	Female	48	Kalasin
282	1754	Female	45	Kalasin
283	1755	Male	65	Kalasin
284	1756	Male	67	Nong Khai
285	1757	Female	53	Kalasin
286	1758	Female	46	Kalasin
287	1759	Female	50	Kalasin
288	1760	Male	51	Kalasin
289	1761	Female	49	Kalasin
290	1762	Male	53	Kalasin
291	1763	Male	28	Roi Et
292	1764	Male	72	Roi Et
293	1765	Male	67	Roi Et
294	1766	Male	60	Roi Et
295	1767	Female	67	Roi Et
296	1768	Female	70	Roi Et
297	1769	Female	63	Roi Et
298	1770	Male	55	Roi Et

No.	PID	Gender	Age	Location (province)
299	1771	Male	72	Roi Et
300	1772	Female	56	Roi Et
301	1773	Female	48	Roi Et
302	1774	Male	18	Roi Et
303	1775	Male	69	Roi Et
304	1776	Female	64	Roi Et
305	1777	Male	67	Roi Et
306	1778	Female	51	Roi Et
307	1779	Female	56	Roi Et
308	1780	Female	38	Roi Et
309	1781	Female	64	Nakhon Ratchasima
310	1782	Female	44	Nakhon Ratchasima
311	1783	Female	59	Nakhon Ratchasima
312	1784	Female	71	Nakhon Ratchasima
313	1785	Female	58	Nakhon Ratchasima
314	1786	Male	55	Nakhon Ratchasima
315	1787	Male	62	Nakhon Ratchasima
316	1788	Female	51	Nakhon Ratchasima
317	1789	Female	55	Nakhon Ratchasima
318	1790	Female	58	Nakhon Ratchasima
319	1791	Female	56	Nakhon Ratchasima
320	1792	Female	35	Nakhon Ratchasima
321	1793	Female	58	Nakhon Ratchasima

No.	PID	Gender	Age	Location (province)
322	1794	Female	67	Nakhon Ratchasima
323	1795	Female	61	Nakhon Ratchasima
324	1796	Female	52	Nakhon Ratchasima
325	1797	Female	67	Nakhon Ratchasima
326	1798	Female	57	Nakhon Ratchasima
327	1799	Female	45	Nakhon Ratchasima
328	1800	Female	60	Nakhon Ratchasima
329	1801	Female	52	Nakhon Ratchasima
330	1802	Female	37	Nakhon Ratchasima
331	1803	Female	54	Nakhon Ratchasima
332	1804	Female	38	Nakhon Ratchasima
333	1805	Female	53	Nakhon Ratchasima
334	1806	Female	70	Nakhon Ratchasima
335	1807	Male	80	Nakhon Ratchasima
336	1808	Female	55	Nakhon Ratchasima
337	1809	Female	63	Nakhon Ratchasima
338	1810	Female	69	Nakhon Ratchasima
339	1811	Female	61	Nakhon Ratchasima
340	1812	Female	60	Nakhon Ratchasima
341	1813	Female	31	Nakhon Ratchasima
342	1814	Female	28	Nakhon Ratchasima
343	1815	Female	41	Nakhon Ratchasima
344	1816	Female	36	Nakhon Ratchasima

No.	PID	Gender	Age	Location (province)
345	1817	Female	48	Nakhon Ratchasima
346	1818	Female	62	Nakhon Ratchasima
347	1819	Female	36	Nakhon Ratchasima
348	1820	Female	56	Nakhon Ratchasima
349	1821	Female	54	Nakhon Ratchasima
350	1822	Female	63	Nakhon Ratchasima
351	1823	Female	54	Nakhon Ratchasima
352	1824	Female	62	Nakhon Ratchasima
353	1825	Female	48	Nakhon Ratchasima
354	1826	Male	55	Nakhon Ratchasima
355	1827	Female	61	Nakhon Ratchasima
356	1828	Female	71	Nakhon Ratchasima
357	1829	Male	55	Nakhon Ratchasima
358	1830	Female	51	Nakhon Ratchasima
359	1831	Female	58	Nakhon Ratchasima
360	1832	Female	70	Nakhon Ratchasima
361	1833	Female	19	Nakhon Ratchasima
362	1834	Female	39	Nakhon Ratchasima
363	1835	Female	34	Nakhon Ratchasima
364	1836	Female	54	Nakhon Ratchasima
365	1837	Female	51	Nakhon Ratchasima
366	1838	Female	41	Nakhon Ratchasima
367	1842	Male	43	Nakhon Ratchasima

No.	PID	Gender	Age	Location (province)
368	1843	Female	31	Nakhon Ratchasima
369	1844	Female	44	Nakhon Ratchasima
370	1845	Female	34	Nakhon Ratchasima
371	1846	Female	18	Nakhon Ratchasima
372	1847	Female	49	Nakhon Ratchasima
373	1848	Male	66	Nakhon Ratchasima
374	1849	Female	62	Nakhon Ratchasima
375	1850	Female	43	Nakhon Ratchasima
376	1851	Female	41	Nakhon Ratchasima
377	1852	Female	45	Nakhon Ratchasima
378	1853	Female	40	Nakhon Ratchasima
379	1854	Female	51	Nakhon Ratchasima
380	1855	Female	68	Nakhon Ratchasima
381	1856	Female	61	Nakhon Ratchasima
382	1857	Female	53	Nakhon Ratchasima
383	1858	Female	47	Nakhon Ratchasima
384	1859	Female	50	Nakhon Ratchasima
385	1860	Female	44	Nakhon Ratchasima
386	1861	Female	50	Nakhon Ratchasima
387	1862	Female	65	Nakhon Ratchasima
388	1863	Female	70	Nakhon Ratchasima
389	1864	Female	62	Nakhon Ratchasima
390	1865	Female	57	Nakhon Ratchasima

No.	PID	Gender	Age	Location (province)
391	1866	Female	57	Nakhon Ratchasima
392	1867	Female	65	Nakhon Ratchasima
393	1868	Female	65	Nakhon Ratchasima
394	1869	Female	40	Nakhon Ratchasima
395	1870	Female	58	Nakhon Ratchasima
396	1871	Female	66	Nakhon Ratchasima
397	1872	Female	52	Nakhon Ratchasima
398	1873	Female	72	Nakhon Ratchasima
399	1874	Female	55	Nakhon Ratchasima
400	1875	Female	59	Nakhon Ratchasima
401	1876	Female	28	Nakhon Ratchasima
402	1877	Female	60	Nakhon Ratchasima
403	1878	Female	48	Nakhon Ratchasima
404	1879	Female	61	Nakhon Ratchasima
405	1880	Female	47	Nakhon Ratchasima
406	1881	Female	51	Nakhon Ratchasima
407	1882	Female	46	Nakhon Ratchasima
408	1883	Female	77	Chaiyaphum
409	1931	Female	60	Chaiyaphum
410	1932	Female	58	Chaiyaphum
411	1933	Female	50	Chaiyaphum
412	1934	Female	53	Chaiyaphum
413	1935	Female	47	Chaiyaphum

No.	PID	Gender	Age	Location (province)
414	1936	Female	52	Chaiyaphum
415	1937	Female	71	Chaiyaphum
416	1938	Female	56	Chaiyaphum
417	1939	Female	46	Chaiyaphum
418	1940	Female	61	Chaiyaphum
419	1941	Male	25	Chaiyaphum
420	1942	Male	55	Chaiyaphum
421	1943	Female	46	Chaiyaphum
422	1944	Male	77	Chaiyaphum
423	1945	Male	63	Chaiyaphum
424	1946	Male	67	Chaiyaphum
425	1947	Female	64	Chaiyaphum
426	1948	Female	65	Chaiyaphum
427	1949	Female	59	Chaiyaphum
428	1950	Female	61	Chaiyaphum
429	1951	Female	58	Chaiyaphum
430	1952	Female	54	Chaiyaphum
431	1953	Female	67	Chaiyaphum
432	1954	Female	58	Chaiyaphum
433	1955	Female	72	Chaiyaphum
434	1956	Female	48	Chaiyaphum
435	1957	Female	43	Chaiyaphum
436	1958	Female	58	Chaiyaphum

No.	PID	Gender	Age	Location (province)
437	1959	Female	43	Chaiyaphum
438	1960	Female	51	Chaiyaphum
439	1961	Female	71	Chaiyaphum
440	1962	Male	47	Chaiyaphum
441	1963	Female	64	Chaiyaphum
442	1964	Female	62	Chaiyaphum
443	1965	Female	60	Chaiyaphum
444	1966	Female	58	Chaiyaphum
445	1967	Female	52	Chaiyaphum
446	1968	Female	45	Chaiyaphum
447	1969	Female	67	Chaiyaphum
448	1970	Female	60	Chaiyaphum
449	1971	Female	65	Chaiyaphum
450	1972	Female	58	Chaiyaphum
451	1973	Female	61	Chaiyaphum
452	1974	Female	55	Chaiyaphum
453	1975	Female	52	Chaiyaphum
454	1976	Female	51	Chaiyaphum
455	1977	Female	58	Chaiyaphum

Table 2B Results of positive parasite infections.

No.	IPD	FAFA	FECT	KKT	PCR
1	1404	Positive	Positive	Positive	Positive
2	1405	Positive	Positive	Positive	Positive
3	1406	Positive	Positive	Positive	Positive
4	1414	Positive	Positive	Positive	Positive
5	1415	Positive	Positive	Negative	Positive
6	1416	Positive	Positive	Negative	Positive
7	1425	Positive	Positive	Negative	Positive
8	1426	Negative	Positive	Negative	Positive
9	1437	Positive	Positive	Negative	Positive
10	1489	Positive	Positive	Positive	-
11	1496	Positive	Positive	Negative	Positive
12	1499	Positive	Positive	Negative	Positive
13	1501	Negative	Positive	Negative	Positive
14	1517	Positive	Positive	Negative	Positive
15	1518	Positive	Positive	Negative	Positive
16	1530	Positive	Positive	Negative	Positive
17	1531	Negative	Positive	Negative	Positive
18	1539	Positive	Positive	Positive	Positive
19	1541	Negative	Positive	Positive	Positive
20	1544	Negative	Positive	Negative	Positive
21	1548	Positive	Positive	Negative	Positive
22	1549	Positive	Positive	Negative	Positive
23	1556	Negative	Positive	Negative	Positive
24	1557	Negative	Negative	Positive	Positive

25	1560	Negative	Positive	Negative	Positive
26	1561	Negative	Positive	Negative	Positive
27	1565	Positive	Positive	Negative	Positive
28	1566	Positive	Positive	Negative	Positive
29	1567	Negative	Positive	Negative	Positive
30	1568	Positive	Positive	Positive	Positive
31	1573	Negative	Positive	Negative	Positive
32	1580	Negative	Positive	Negative	Positive
33	1582	Negative	Positive	Negative	Positive
34	1589	Positive	Positive	Negative	Positive
35	1593	Positive	Positive	Negative	Positive
36	1595	Negative	Positive	Negative	Positive
37	1619	Negative	Positive	Negative	Positive
38	1620	Positive	Positive	Negative	Positive
39	1621	Positive	Positive	Negative	Positive
40	1624	Negative	Positive	Positive	Positive
41	1626	Negative	Positive	Negative	Positive
42	1627	Positive	Positive	Negative	Positive
43	1633	Negative	Positive	Positive	Positive
44	1635	Negative	Positive	Positive	Positive
45	1636	Positive	Positive	Positive	Positive
46	1637	Negative	Negative	Positive	Positive
47	1638	Negative	Negative	Positive	Positive
48	1642	Positive	Positive	Negative	Positive
49	1643	Negative	Positive	Positive	-
50	1682	Negative	Negative	Positive	Positive

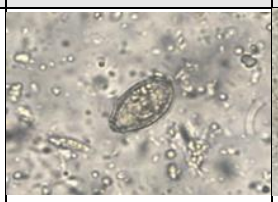
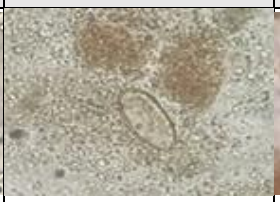
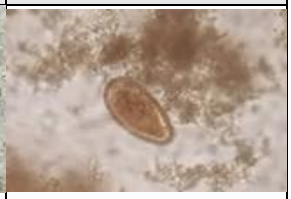
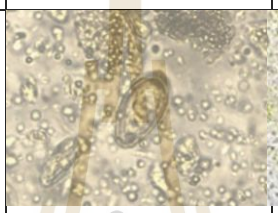
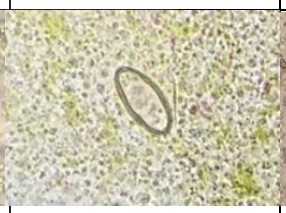
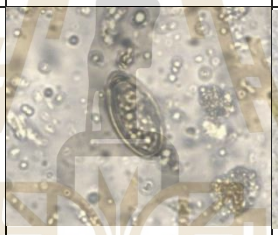
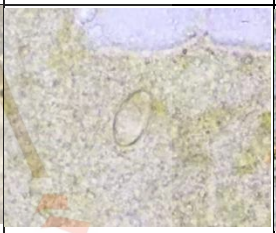
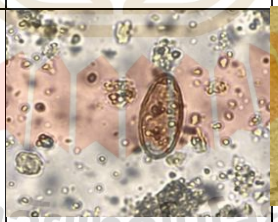
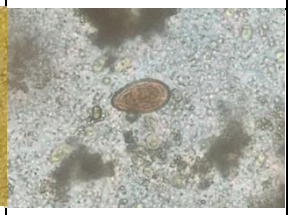
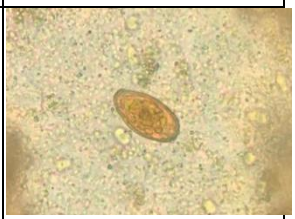
51	1689	Negative	Negative	Positive	Positive
52	1731	Positive	Positive	Negative	Positive
53	1732	Negative	Negative	Positive	Positive
54	1733	Positive	Positive	Positive	Positive
55	1734	Negative	Negative	Positive	Positive
56	1735	Positive	Positive	Positive	Positive
57	1737	Positive	Positive	Positive	Positive
58	1738	Positive	Positive	Positive	Positive
59	1740	Negative	Positive	Positive	Positive
60	1741	Negative	Positive	Positive	Positive
61	1744	Positive	Positive	Positive	Positive
62	1745	Positive	Positive	Positive	Positive
63	1751	Negative	Positive	Negative	Positive
64	1752	Positive	Positive	Negative	Positive
65	1753	Negative	Positive	Negative	Positive
66	1754	Positive	Negative	Negative	Positive
67	1755	Negative	Positive	Negative	Positive
68	1756	Positive	Positive	Positive	-
69	1759	Negative	Positive	Negative	Positive
70	1760	Negative	Positive	Negative	Positive
71	1761	Negative	Positive	Negative	Positive
72	1764	Positive	Positive	Positive	Positive
73	1765	Positive	Positive	Positive	Positive
74	1766	Positive	Positive	Positive	Positive
75	1777	Positive	Positive	Positive	Positive
76	1857	Positive	Negative	Negative	-

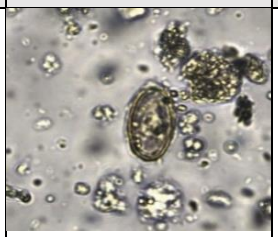
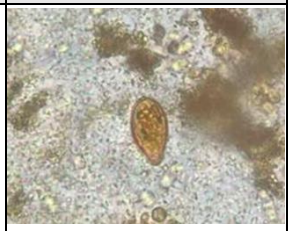
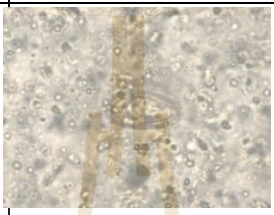
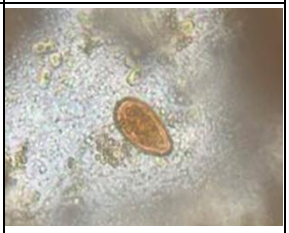
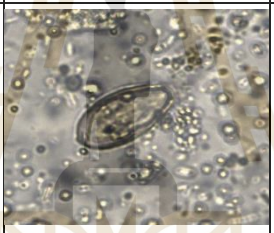
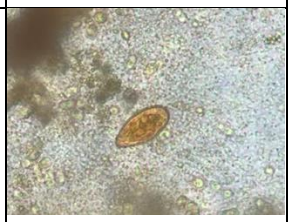
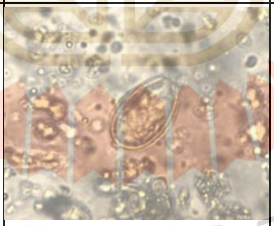
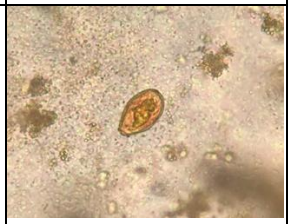
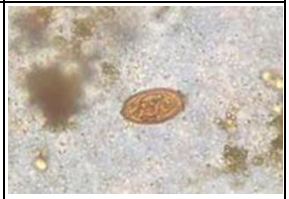
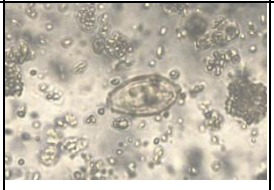
77	1871	Positive	Positive	Negative	-
78	1945	Positive	Positive	Negative	Positive
79	1949	Positive	Positive	Negative	Positive
80	1960	Positive	Positive	Negative	Positive
81	1969	Positive	Positive	Positive	Positive
82	1971	Positive	Positive	Positive	Positive
83	1972	Positive	Positive	Positive	Positive
84	1973	Negative	Negative	Positive	Positive
85	1974	Positive	Positive	Positive	Positive
86	1975	Positive	Positive	Positive	Positive
87	1976	Negative	Positive	Positive	Positive
88	1977	Negative	Positive	Positive	Positive

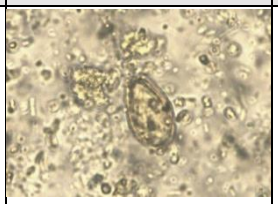
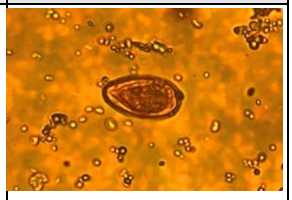
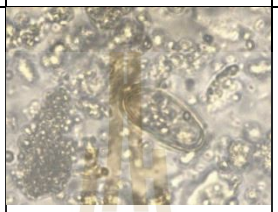
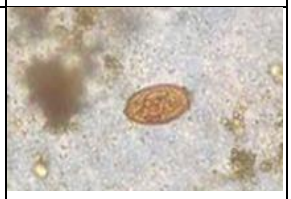
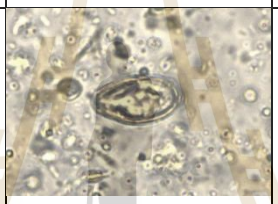
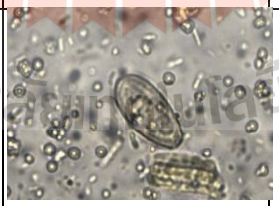
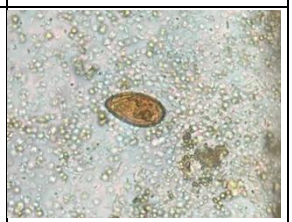
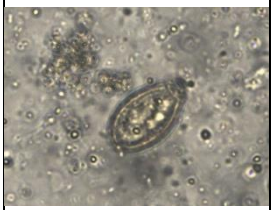
APPENDIX C

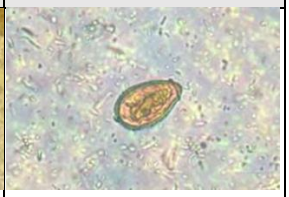
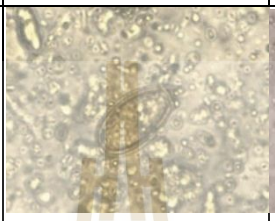
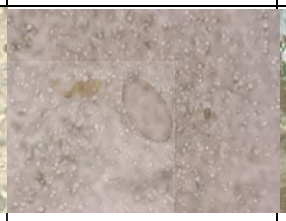
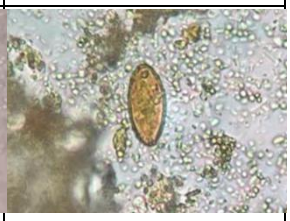
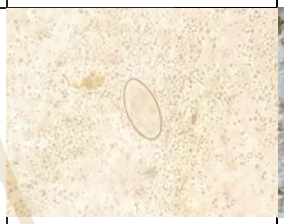
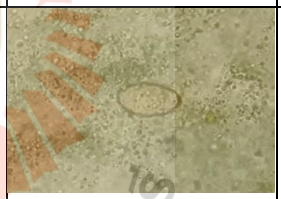
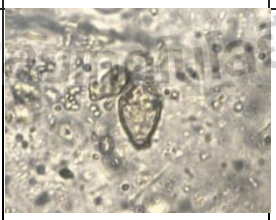
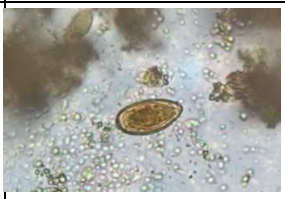
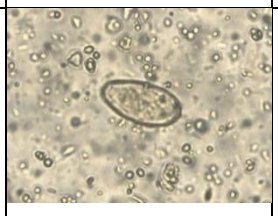
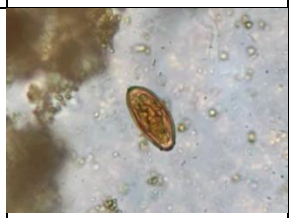
PICTURE OF THE RESULTS OF THREE METHODS

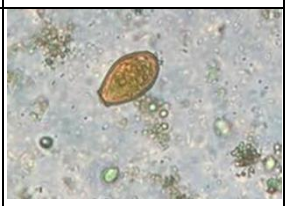
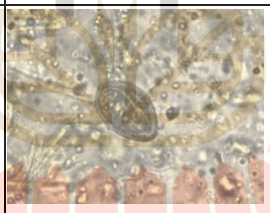
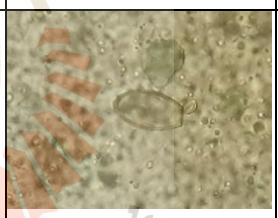
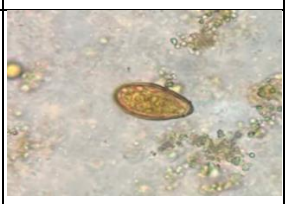
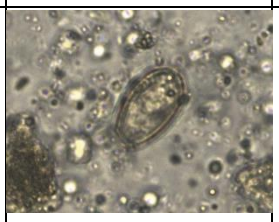
TABLE 1C Three techniques of identifying parasites under a microscope

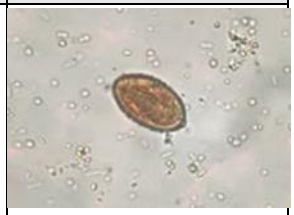
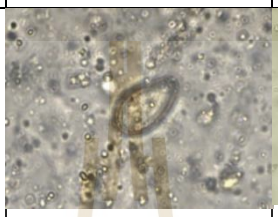
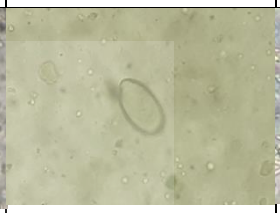
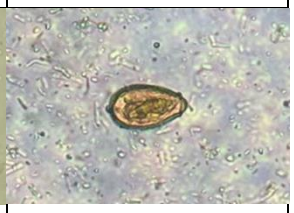
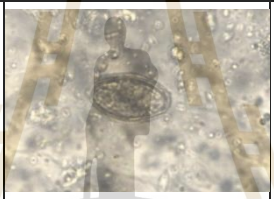
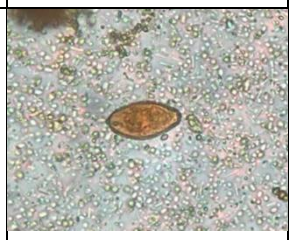
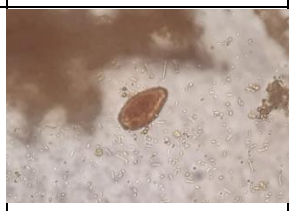
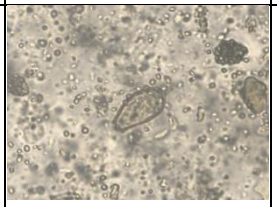
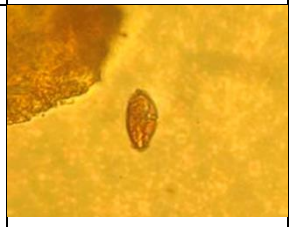
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
1.	1404	*O. viverrini*	 1404 H-6-2	 40x	 40x
2.	1405	*O. viverrine*	 1405 H-12-2	 40x	 40x
3.	1406	*O. viverrini*	 1406 H-3-1	 40x	 40x
4.	1414	*O. viverrini*	 1406 H-3-1	 40x	 40x
5.	1415	*O. viverrini*	 1415 H-3-1	Not found	 40x

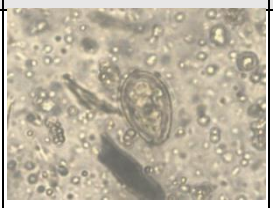
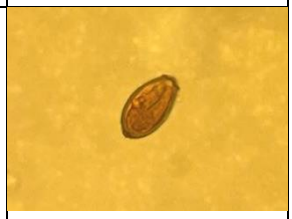
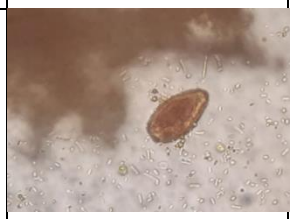
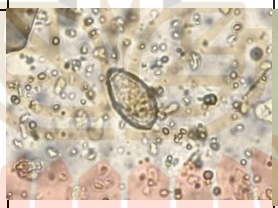
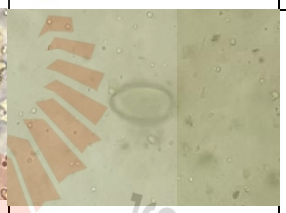
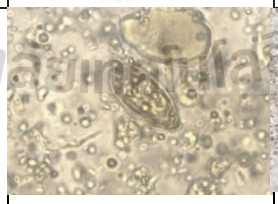
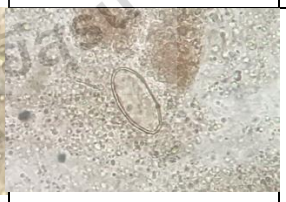
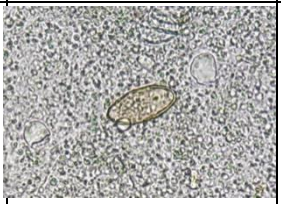
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
6.	1416	*O. viverrini*	 1416 H-5-2	Not found	 40x
7.	1425	*O. viverrini*	 1425 H-5-2	Not found	 40x
8.	1426	*O. viverrini*	 1426 H-5-1	Not found	 40x
9.	1437	*O. viverrini*	 1437 H-5-2	Not found	 40x
10.	1489	*S. stercoralis*	 1489 H-6-1	Not found	 40x
11.	1496	*O. viverrini*	 1496 H-4-2	Not found	 40x

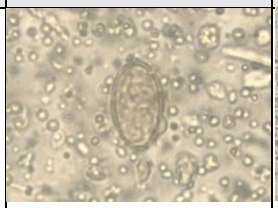
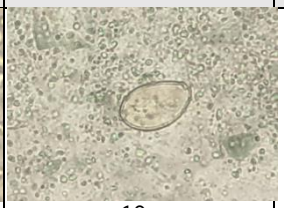
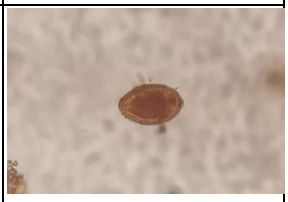
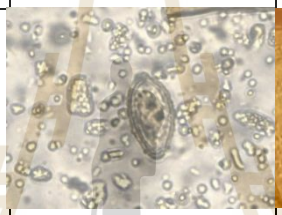
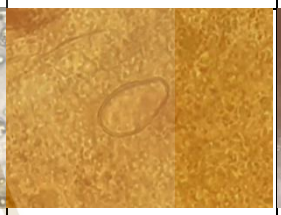
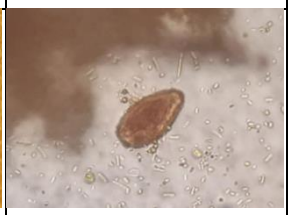
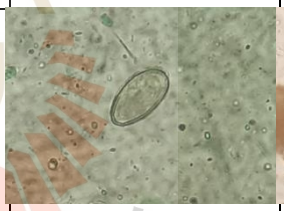
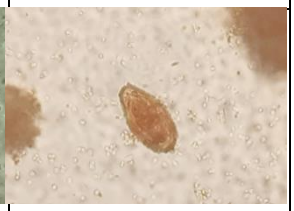
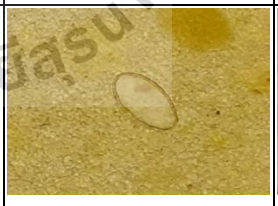
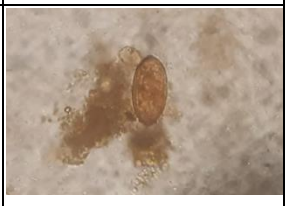
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
12.	1499	*O. viverrini*	 1499 H-3-1	Not found	 40x
13.	1501	*O. viverrini*	 1501 H-3-2	Not found	 40x
14.	1517	*O. viverrini*	 1517 H-3-3	Not found	 40x
15.	1518	*O. viverrini*	 1518- H-7-1	Not found	Not found
16.	1524	*O. viverrini*	 24 H-3-3	Not found	 40x
17.	1530	*O. viverrini*	 1530 H-3-1	Not found	 40x

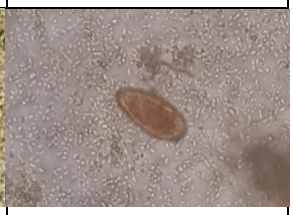
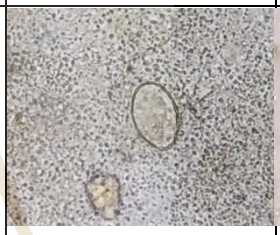
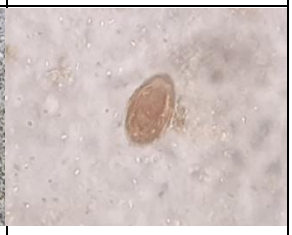
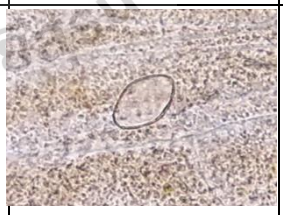
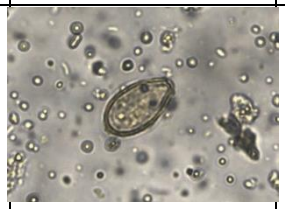
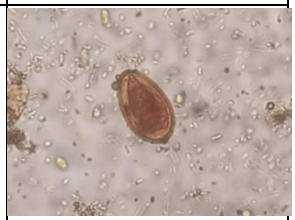
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
18.	1531	*O. viverrini*	Not found	 40X	 40x
19.	1539	*O. viverrini*	 1539 H-8-1	 40x	 40x
20.	1541	*O. viverrini*	Not found	 40x	 40x
21.	1544	*O. viverrini*	Not found	 40x	Not found
22.	1548	*O. viverrini*	 1548 H-4-3	Not found	 40x
23.	1549	*O. viverrini*	 1549 H-3-1	Not found	 40x

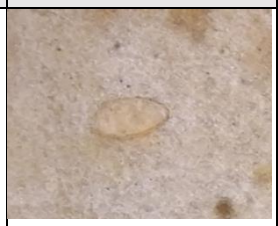
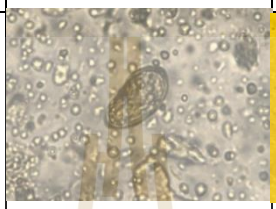
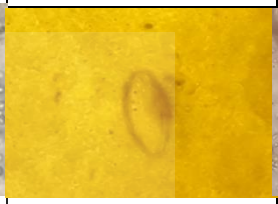
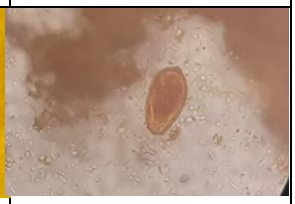
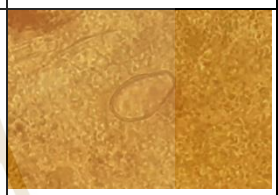
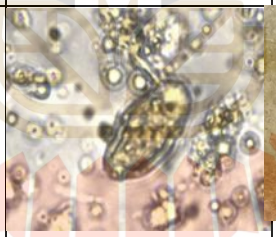
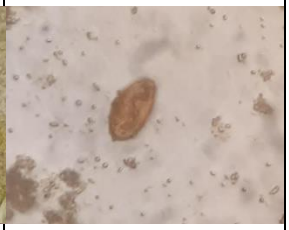
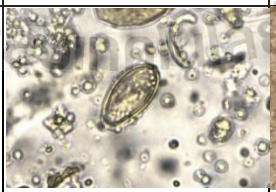
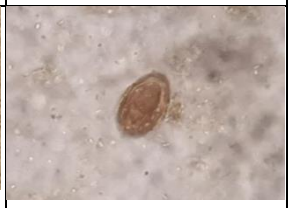
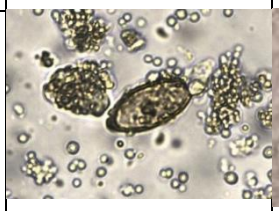
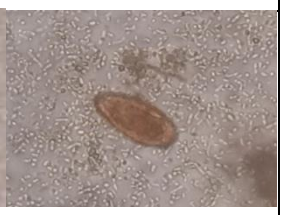
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
24.	1556	*O. viverrini*	Not found	 40X	Not found
25.	1557	*O. viverrini*	Not found	Not found	 40X
26.	1560	*O. viverrini*	Not found	 40x	Not found
27.	1561	*O. viverrini*	 1561 H-5-1	 40x	Not found
28.	1565	*O. viverrini*	 1565 H-3-1	Not found	 40x
29.	1566	*O. viverrini*	 1566 H-6-1	Not found	 40x

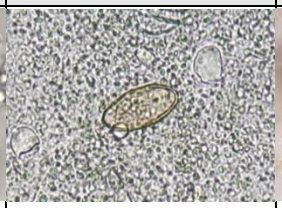
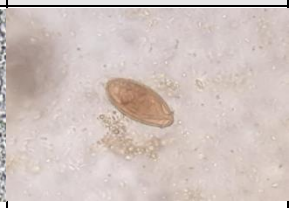
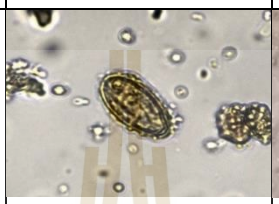
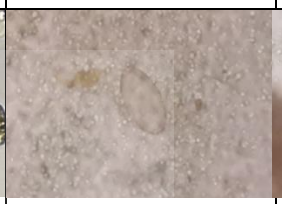
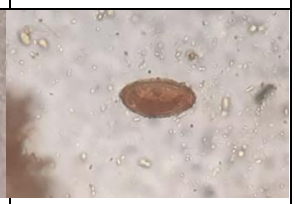
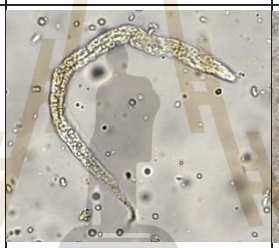
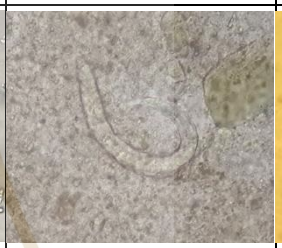
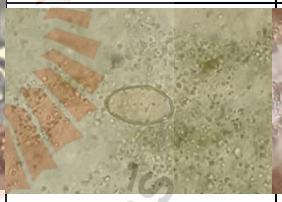
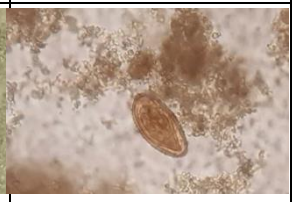
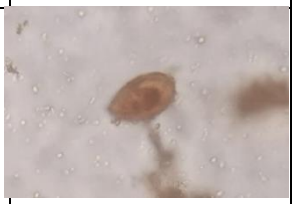
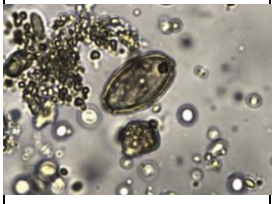
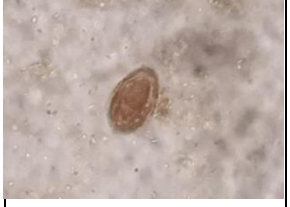
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
30.	1567	*O. viverrini*	Not found	Not found	 40x
31.	1568	*O. viverrini*	 1568 H-4-1	 40x	 40x
32.	1573	*O. viverrini*	 1573 H-7-1	Not found	 40x
33.	1580	*O. viverrini*	Not found	Not found	 40x
34.	1582	*O. viverrini*	Not found	Not found	 40x
35.	1589	*O. viverrini*	 1589 H-4-1	Not found	 40x

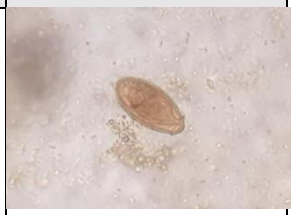
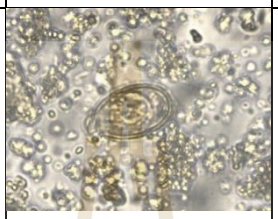
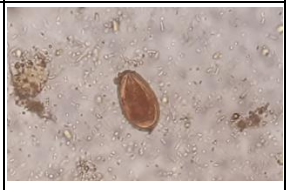
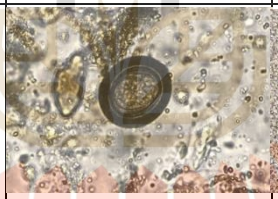
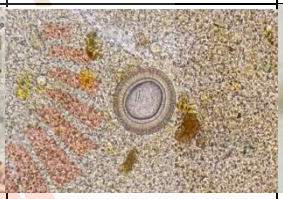
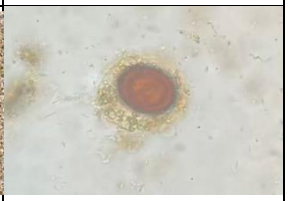
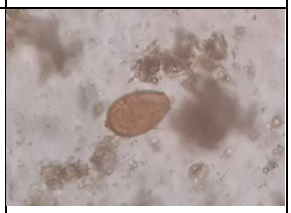
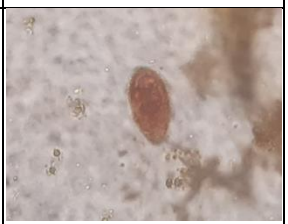
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
36.	1593	*O. viverrini*	 1593 H-5-1	Not found	 40x
37.	1595	*O. viverrini*	Not found	Not found	 40x
38.	1619	*O. viverrini*	Not found	Not found	 40x
39.	1620	*O. viverrini*	 1620 H-3-2	 40x	Not found
40.	1621	*O. viverrini*	 1621 H-3-2	 40x	Not found
41.	1624	*O. viverrini*	Not found	 40x	Not found

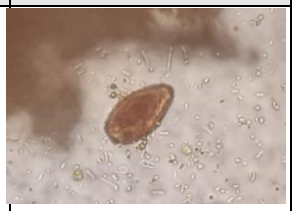
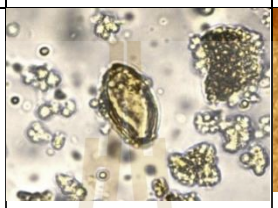
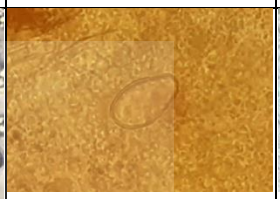
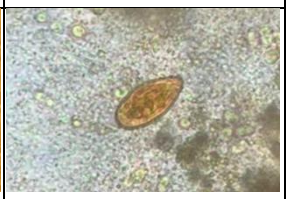
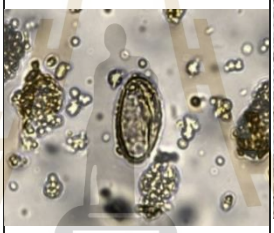
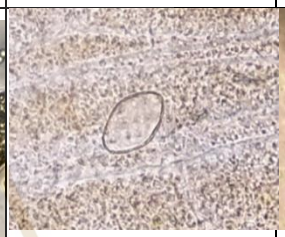
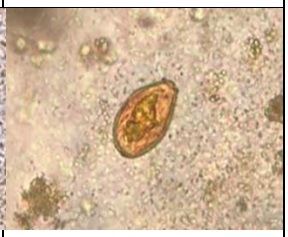
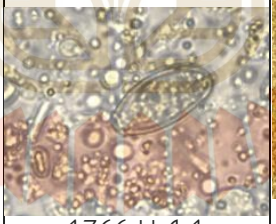
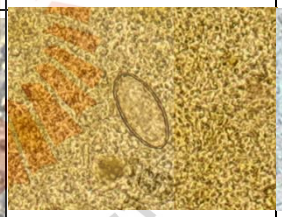
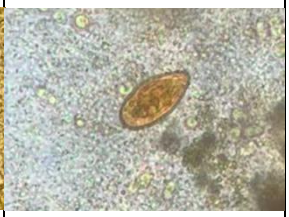
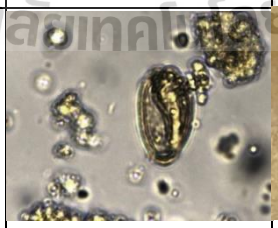
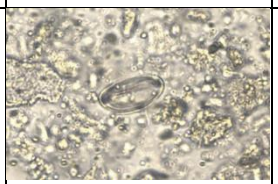
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
42.	1626	*O. viverrini*	 1626 H-4-2	 40x	Not found
43.	1627	*O. viverrini*	Not found	Not found	 40x
44.	1633	*O. viverrini*	 1627 H-3-3	 40x	 40x
45.	1635	*O. viverrini*	Not found	 40x	 40x
46.	1636	*O. viverrini*	Not found	 40x	 40x
47.	1637	*O. viverrini*	Not found	 40x	Not found

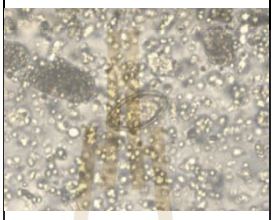
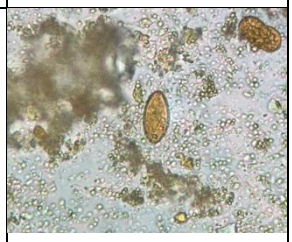
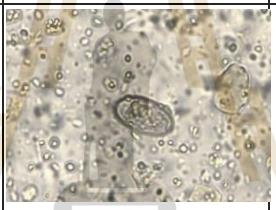
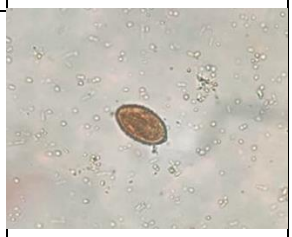
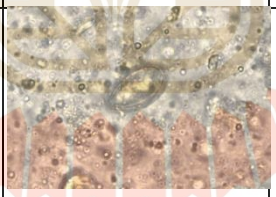
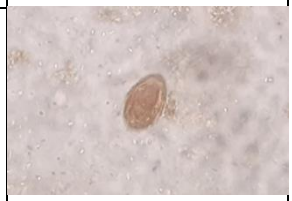
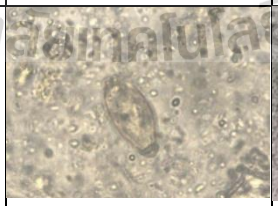
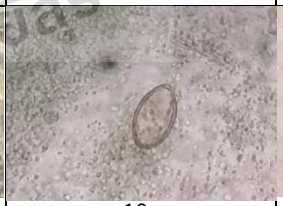
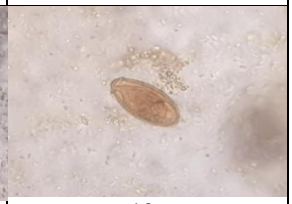
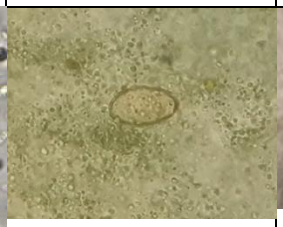
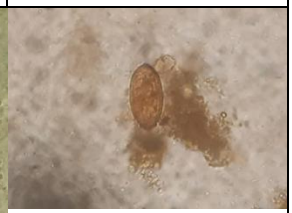
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
48.	1638	*O. viverrini*	Not found	 40x	Not found
49.	1642	*O. viverrini*	Not found	 40x	 40x
50.	1643	*O. viverrini*	Not found	 40x	 40x
51.	1682	*O. viverrini*	Not found	 40x	Not found
52.	1689	*O. viverrini*	Not found	 40x	Not found
53.	1731	*O. viverrini*	 1731 H-5-1	Not found	 40x

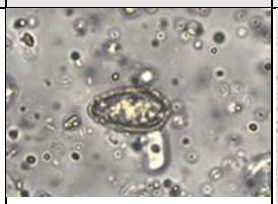
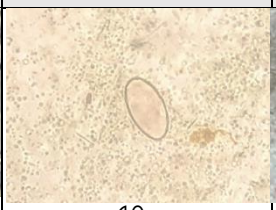
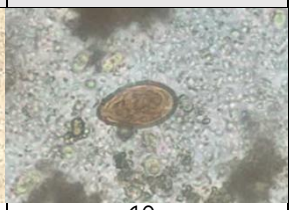
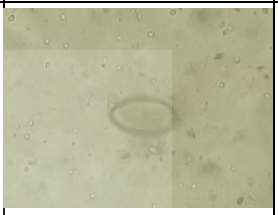
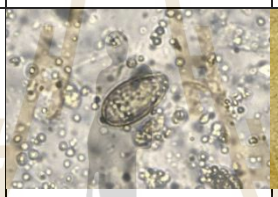
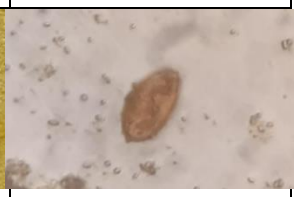
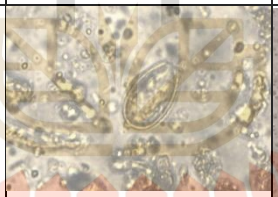
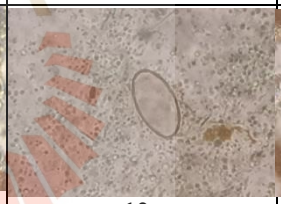
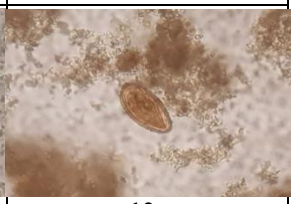
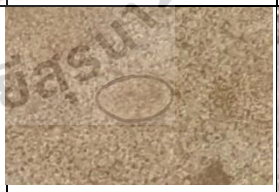
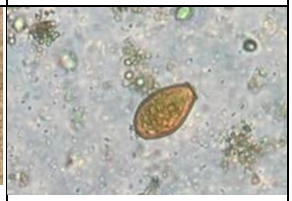
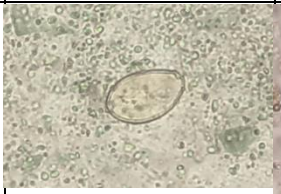
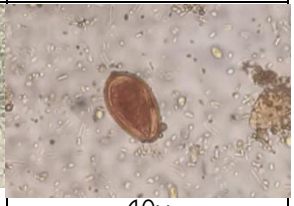
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
54.	1732	*O. viverrini*	Not found	 40x	Not found
55.	1733	*O. viverrini*	 3 H-6-2	 40x	 40x
56.	1734	*O. viverrini*	Not found	 40x	Not found
57.	1735	*O. viverrini*	 1735 H-3-3	 40x	 40x
58.	1737	*O. viverrini*	 1737 H-3-4	 40x	 40x
59.	1738	*O. viverrini*	 1738 H-6-3	 40x	 40x

No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
60.	1740	*O. viverrini*	 1740 H-3-4	 40x	 40x
61.	1744	*O. viverrini*	 1744 H-5-2	 40x	 40x
		SS	 1744 H-4-1	 40x	 40x
62.	1745	*O. viverrini*	 1745 H-4-1	 40x	 40x
63.	1751	*O. viverrini*	Not found	Not found	 40x
64.	1752	*O. viverrini*	 1752 H-3-3	Not found	 40x

No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
65.	1753	*O. viverrini*	Not found	Not found	 40x
66.	1754	*O. viverrini*	 1754 H-3-3	Not found	Not found
67.	1755	*O. viverrini*	Not found	Not found	 40x
68.	1756	*Taenia spp.*	 1756 H-7-3	 40x	 40x
69.	1759	*O. viverrini*	Not found	Not found	 40x
70.	1760	*O. viverrini*	Not found	Not found	 40x

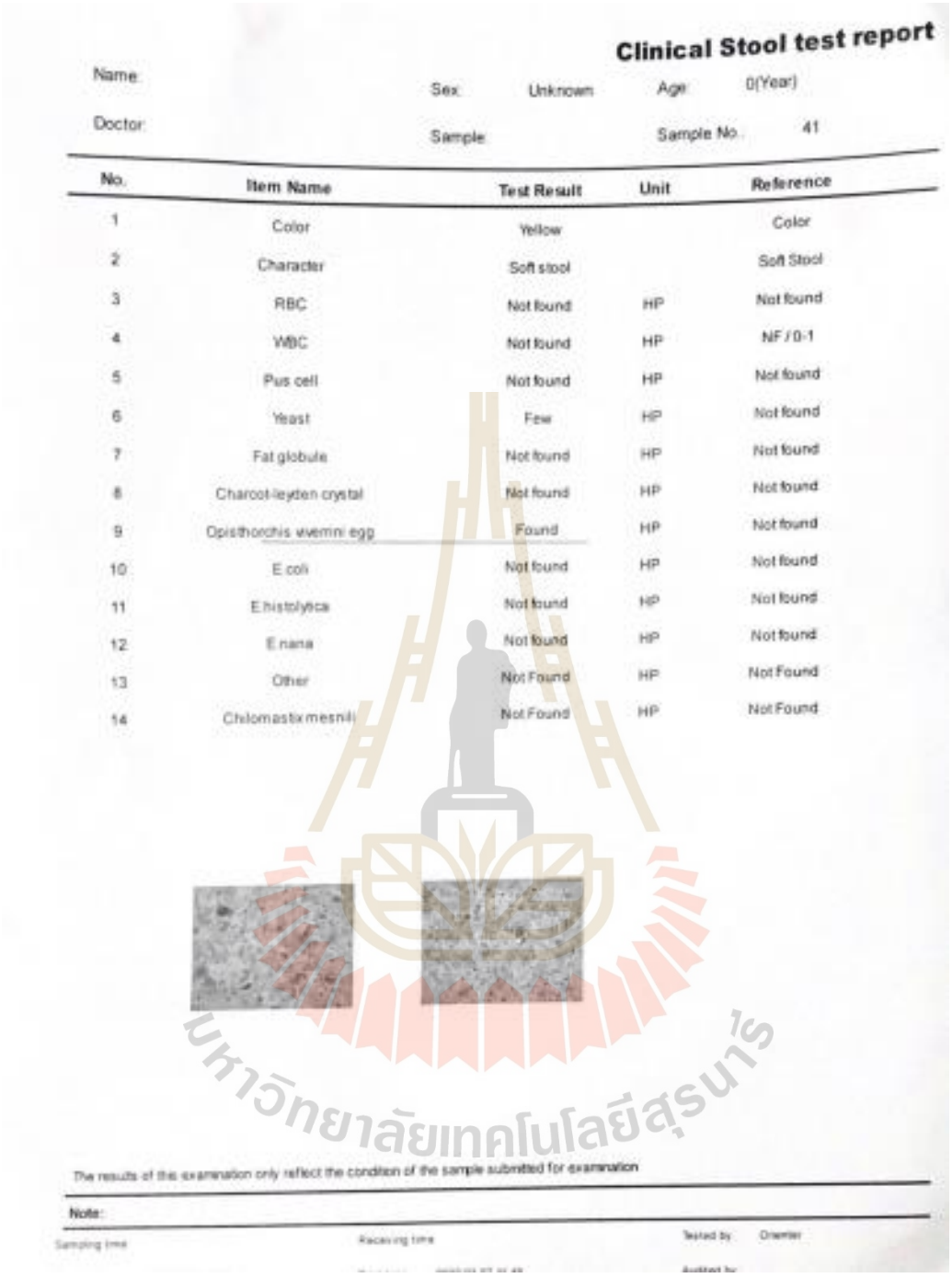
No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
71.	1761	*O. viverrini*	Not found	Not found	 40x
72.	1764	*O. viverrini*	 1764 H-3-1	 40x	 40x
73.	1765	*O. viverrini*	 1765 H-3-3	 40x	 40x
74.	1766	*O. viverrini*	 1766 H-4-1	 40x	 40x
75.	1777	*O. viverrini*	 1777 H-4-4	 40x	 40x
76.	1857	*E. vermicularis*	 1857 H-3-1	Not found	Not found

No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
77.	1871	*S. stercoralis*	 1871 H-7-4	 40x	 40x
78.	1945	*O. viverrini*	 1945 H-4-4-1	Not found	 40x
79.	1949	*O. viverrini*	 1949 H-3-3	Not found	 40x
80.	1960	*O. viverrini*	 1960 H-5-1	Not found	 40x
81.	1969	*O. viverrini*	 1969 H-6-1	 40x	 40x
82.	1971	*O. viverrini*	 1971 H-3-2	 40x	 40x

No.	Code	Parasites	Microscope identification		
			FAFA	KKT	FECT
83.	1972	*O. viverrini*	 1972 H-3-2	 40x	 40x
84.	1973	*O. viverrini*	Not found	 40x	Not found
85.	1974	*O. viverrini*	 1974 H-4-2	 40x	 40x
86.	1975	*O. viverrini*	 1975 H-4-2	 40x	 40x
87.	1976	*O. viverrini*	Not found	 40x	 40x
88.	1977	*O. viverrini*	Not found	 40x	 40x

APPENDIX D

CLINICAL STOOL TEST REPORT



Clinical Stool test report

Name: Sex: Unknown Age: 0(Year)

Doctor: Sample: Sample No.: 41

No.	Item Name	Test Result	Unit	Reference
1	Color	Yellow		Color
2	Character	Soft stool		Soft Stool
3	RBC	Not found	HP	Not found
4	WBC	Not found	HP	NF / 0-1
5	Pus cell	Not found	HP	Not found
6	Yeast	Few	HP	Not found
7	Fat globule	Not found	HP	Not found
8	Charcot-leyden crystal	Not found	HP	Not found
9	Opisthorchis viverrini egg	Found	HP	Not found
10	E.coli	Not found	HP	Not found
11	E.histolytica	Not found	HP	Not found
12	E.nana	Not found	HP	Not found
13	Other	Not Found	HP	Not Found
14	Chlomastix mesnili	Not Found	HP	Not Found

The results of this examination only reflect the condition of the sample submitted for examination

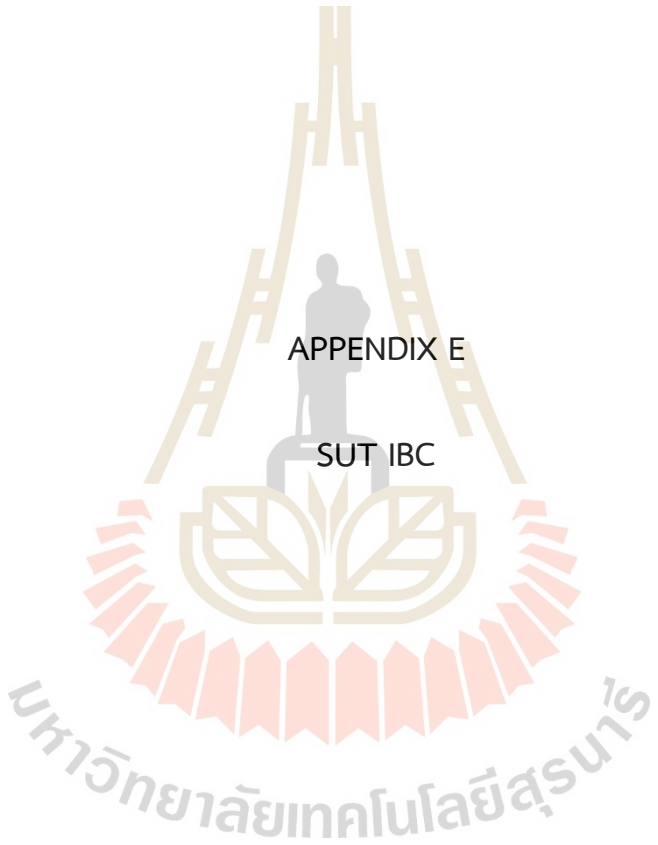
Note:

Sampling time: Receiving time: Tested by: Checker:

Figure 1D The results of clinical stool test report by FAFA machine

APPENDIX E

SUT IBC



เลขที่รับรอง SUT-IBC-019/2022

ใบรับรองการดำเนินงานด้านความปลอดภัยทางชีวภาพ
มหาวิทยาลัยเทคโนโลยีสุรนารี

ชื่อโครงการ	: การเปรียบเทียบวิธีการวิเคราะห์ตัวอย่างอุจจาระระหว่างวิธีคาโต้แคท (KKT), วิธีตกตะกอนด้วยฟอร์มาลิน-อะซีเตต (FECT) และเครื่องตรวจอุจจาระแบบอัตโนมัติ (FAFA) ในการตรวจหาพยาธิใบไม้ตับออร์ทิสทอร์คิสวิเวอร์รินีในมนุษย์
รหัสโครงการ	: IBC-65-24
หัวหน้าโครงการ	: นางสาวอลิษา บุญสุญา
อาจารย์ที่ปรึกษา	: รองศาสตราจารย์ แพทย์หญิงขวัญญ์ญา รัตนพิทูลย์
สังกัด	: สำนักวิชาแพทยศาสตร์
ประเภทงานวิจัย	: งานวิจัยประเภทที่ 1
ระดับห้องปฏิบัติการ	: Biosafety Level 1 (BSL 1)
รายงานความก้าวหน้า	: ส่งรายงานความก้าวหน้าอย่างน้อย 1 ครั้ง/ปี

ข้อเสนอโครงการวิจัยและเอกสารประกอบของข้อเสนอโครงการวิจัยนี้ ได้รับการพิจารณาจากคณะกรรมการควบคุมความปลอดภัยทางชีวภาพ มหาวิทยาลัยเทคโนโลยีสุรนารีแล้ว คณะกรรมการฯ ลงความเห็นว่า ข้อเสนอโครงการวิจัยที่จะดำเนินการมีความสอดคล้องกับแนวทางปฏิบัติเพื่อความปลอดภัยทางชีวภาพ และพระราชบัญญัติเชื้อโรคและพิษจากสัตว์ พ.ศ. 2558 จึงเห็นสมควรให้ดำเนินการวิจัยตามข้อเสนอการวิจัยนี้ได้

กรณีที่มีการปฏิบัติอย่างหนึ่งอย่างใดนอกเหนือจากที่กรอกไว้ในข้อมูลและที่เสนอไว้ในโครงการ คณะกรรมการฯ จะดำเนินการงดใบรับรองนี้ และแจ้งมายังคณะกรรมการควบคุมความปลอดภัยทางชีวภาพ มทส.หรือหน่วยงานที่เกี่ยวข้องทราบ

ลงชื่อ.....
(รองศาสตราจารย์ ดร.ระพี อูทเคอ)
ประธานคณะกรรมการควบคุมความปลอดภัยทางชีวภาพ
มหาวิทยาลัยเทคโนโลยีสุรนารี

วันที่ออกใบรับรอง 5 มกราคม 2566
วันที่ใบรับรองหมดอายุ 4 มกราคม 2567

Figure 1E The Bio Ethics have been approved by Committee of the institute of research and development, Suranaree University of Technology, Thailand

สถาบันวิจัยและพัฒนา
มหาวิทยาลัยเทคโนโลยีสุรนารี

ขอมอบประกาศนียบัตรฉบับนี้ไว้เพื่อแสดงว่า

อลิษา บุญสุยา

ได้สอบผ่านการฝึกอบรมหลักสูตร

"ความปลอดภัยทางชีวภาพ (Biosafety)
และการรักษาความปลอดภัยทางชีวภาพ (Biosecurity)"

(เลขทะเบียนรับรองหลักสูตรจากกรมวิทยาศาสตร์การแพทย์ที่ สธ 0621.06/140)

ให้ไว้ ณ วันที่ 24 พฤษภาคม 2565

หมดอายุ วันที่ 23 พฤษภาคม 2568

รองศาสตราจารย์ ดร.ระพี ฤทเคอ
ผู้อำนวยการสถาบันวิจัยและพัฒนา
มหาวิทยาลัยเทคโนโลยีสุรนารี

Figure 2C Certificate of completion for biosafety and biosecurity course.

APPENDIX F

RESEARCH DISTIBUTIONS

Research Distributions

1. Submitting your manuscript in titled “Detection of intestinal parasitic infections in the population in northeastern Thailand using the Kato-Katz Technique (proceedings) at The 3rd International Conference on Parasitology 2023 (ICP2023). Organized by Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand on 9th -10th November 2023

2. Poster presentation in titled Efficacy of High sensitively diagnosis of Gastrointestinal parasitic infections by Fully Automatic Feces Analyzer at The 59th Annual Scientific Conference of the Malaysian Society of Parasitology & Tropical Medicine. (MSPTM 2023) Organized by Malaysian Society of Parasitology and Tropical Medicine on 15-16 March 2023 at the Wembley Hotel, Penang, Malaysia.

Figure 1F Certificate of appreciation for poster presentation titled Detection of intestinal parasitic infections in the population in northeastern Thailand using the Kato-Katz Technique (proceedings) at The 3rd International Conference on Parasitology 2023 (ICP2023). Organized by Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand on 9th -10th November 2023.

Figure 2F Certificate of appreciation for attending The 3rd International Conference on Parasitology 2023 (ICP2023). Oganiaed by Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand on 9th -10th November 2023.

Figure 3F Certificate of appreciation for poster presentation titled Efficacy of High sensitively diagnosis of Gastrointestinal parasitic infections by Fully Automatic Feces Analyzer at The 59th Annual Scientific Conference of the Malaysian Society of Parasitology & Tropical Medicine. (MSPTM 2023) Organized by Malaysian Society of Parasitology and Tropical Medicine on 15-16 March 2023 at the Wembley Hotel, Penang, Malaysia.

APPENDIX G

RESEARCH ARTICLE ACHIEVEMENT

APPENDIX 1G Publications

1. **Boonsuya, A.**, Arunsan, P., Pechdee, P., La, N., Thanchonnang, C., Rattanapitoon, N.K., & Rattanapitoon, S.K. (2024). Diagnosis of the carcinogenic liver fluke, *Opisthorchis viverrini*: comparison of two coprological methods versus the automatic feces analyzer. *Tropical Biomedicine*, **approved for publication.**
2. **Boonsuya**, A., Pechdee P., Arunsan P., Thanchonnang, C. , Phinsiri, C., Rattanapitoon, N. K., & Rattanapitoon, S. K. (2024). Papaya Seeds Extract Combined Lauryl Glucoside Against Human Parasites Contaminated in Fresh Vegetables. *The Natural Products Journal,* **approved for publication.**
3. Pechdee, P., **Boonsuya, A.**, Arunsan, P., Thanchonnang, C., La, N., Rattanapitoon, N. K.,Laha, T., Nuansing, W., & Rattanapitoon, S. K. (2024). Anthelmintic activity and pathophysiological effect of Allium sativum crude extract against carcinogenic liver fluke, *Opisthorchis viverrini*. *Tropical Biomedicine*, **approved for publication.**
4. Sangkam,W., Arunsan, P., Pechdee, P., **Boonsuya, A.**, Thanchonnang, C., Rattanapitoon, N.K., & Rattanapitoon, S.K. (2024). Anthelmintic activity and pathophysiological effect of anthelmintic drugs against carcinogenic liver fluke, *Opisthorchis viverrini*. Tropical Biomedicine, **approved for publication.**
5. **Boonsuya, A.**, Chitpitaklert, P., Pechdee, P., Srithongklang, W., Thanchonnang, C., La, N., Gordon, C. N., Rattanapitoon, N. K., Arunsan, P., & Rattanapitoon, S. K. (2023). Oral parasitic protozoan *Entamoeba gingivalis* in periodontal disease patients, northeastern Thailand. *Trop Biomed*, *40*(4), 471-477. https://doi.org/10.47665/tb.40.4.013
6. Chitpitaklert, P., **Boonsuya, A.**, Pechdee, P., Thanchonnang, C., La, N., Rattanapitoon, N. K., Arunsan, P., & Rattanapitoon, S. K. (2023). Molecular detection of oral Tri*chomonas tenax* in periodontal disease patients by polymerase chain reaction -based 18S rRNA gene. *Trop Biomed*, *40*(3), 307-312. https://doi.org/10.47665/tb.40.3.00612

7. La, N., Leng, M., Arunsan, P., Pechdee, P., **Boonsuya, A.**, Thanchonnang, C., Rattanapitoon, N. K., & Rattanapitoon, S. K. (2023). Molecular identification of *Opisthorchis viverrini* among the northeastern Cambodian population by internal transcribed spacer 2 based polymerase chain reaction. Trop Biomed, 40(4), 383-391. https://doi.org/10.47665/tb.40.4.002
8. La, N., Leng, M., Rattanapitoon, K., Pechdee, P., **Boonsuya, A.**, Arunsan, P., & Rattanapitoon, S. K. (2022). Intestinal parasitic infections and risk factors among the population in Cambodia. *Trop Biomed, 39*(4), 539–546. https://doi.org/10.47665/tb.39.4.009

4/9/24, 3:41 PM Mail - Schawanya Rattanapitoon - Outlook

Tropical Biomedicine JTB2-23-0543 - Final Decision on Revised Manuscript (Approved)

Opussoft <notification@opussoft.net>
ถึง 9/4/2024 15:32
ถึง:Schawanya Rattanapitoon <schawanya.ratt@sut.ac.th>

Tropical Biomedicine - Final Decision on Revised Manuscript (Approved)

Ref: JTB2-23-0543

Dear Assoc Prof Rattanapitoon, Schawanya,

This is to notify you that your revised manuscript of "**Diagnosis of the carcinogenic liver fluke, Opisthorchis viverrini: comparison of two coprological methods versus the automatic feces analyzer**" is approved for final publication.

A publication fee of USD$300.00 will be levied. However, **the publication fee is waived IF** the first author or corresponding author is a member of Malaysian Society of Parasitology and Tropical Medicine (MSPTM) of at least three (3) years' standing.

The MSPTM Treasurer will issue an electronic invoice to the corresponding author via email sent from the payment gateway system within 3 working days after this acceptance letter is being issued by the Editor. The payment instructions are listed below. Kindly make the payment within three weeks from this notification.

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Swift Code : **SCBLMYKXXXX**
Bank name : **Standard Chartered Bank Malaysia Berhad**
Bank address : **Lot 4 & 5, Level G2, Publika Shopping Gallery, Solaris Dutamas, No.1 Jalan Dutamas1, 50480 Kuala Lumpur, Malaysia.**

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Figure 1G Acceptance email from the Tropical Biomedicine Journal for the manuscript titled "Diagnosis of the carcinogenic liver fluke, *Opisthorchis viverrini*: comparison of two coprological methods versus the automatic feces analyzer" for publication.

Gmail Schawanya Rattanapitoon <schawanya.ratt@g.sut.ac.th>

Preliminary Acceptance | BMS-NPJ-2024-156

The Natural Products Journal <admin@bentham.manuscriptpoint.com> ๙. 8 ก.ค. 08:13
ตอบกลับ: The Natural Products Journal <npj@benthamscience.net>
ถึง: <schawanya.ratt@g.sut.ac.th>
สำเนา: <ambreenirshad@benthamscience.net>

Reference#: BMS-NPJ-2024-156

Submission Title: Papaya Seeds Extract Combined Lauryl Glucoside Against Human Parasites Contaminated in Fresh Vegetables

Dear Dr. Schawanya Rattanapitoon,

On behalf of Bentham Science, I am pleased to inform you that your article titled **"Papaya Seeds Extract Combined Lauryl Glucoside Against Human Parasites Contaminated in Fresh Vegetables"**, with Reference No. "BMS-NPJ-2024-156" has been found generally acceptable for publication, following an independent peer review.

Certain aspects of your submission relating to the quality of language, plagiarism/ similarity, figures and use of AI tools (if any) may require additional scrutiny by our Quality Assurance Department before final acceptance and publication.

Attached is your digitally signed copyright letter for your records.

We appreciate your valuable contribution to "**The Natural Products Journal**" and look forward to successful collaboration in the future.

With warm regards,

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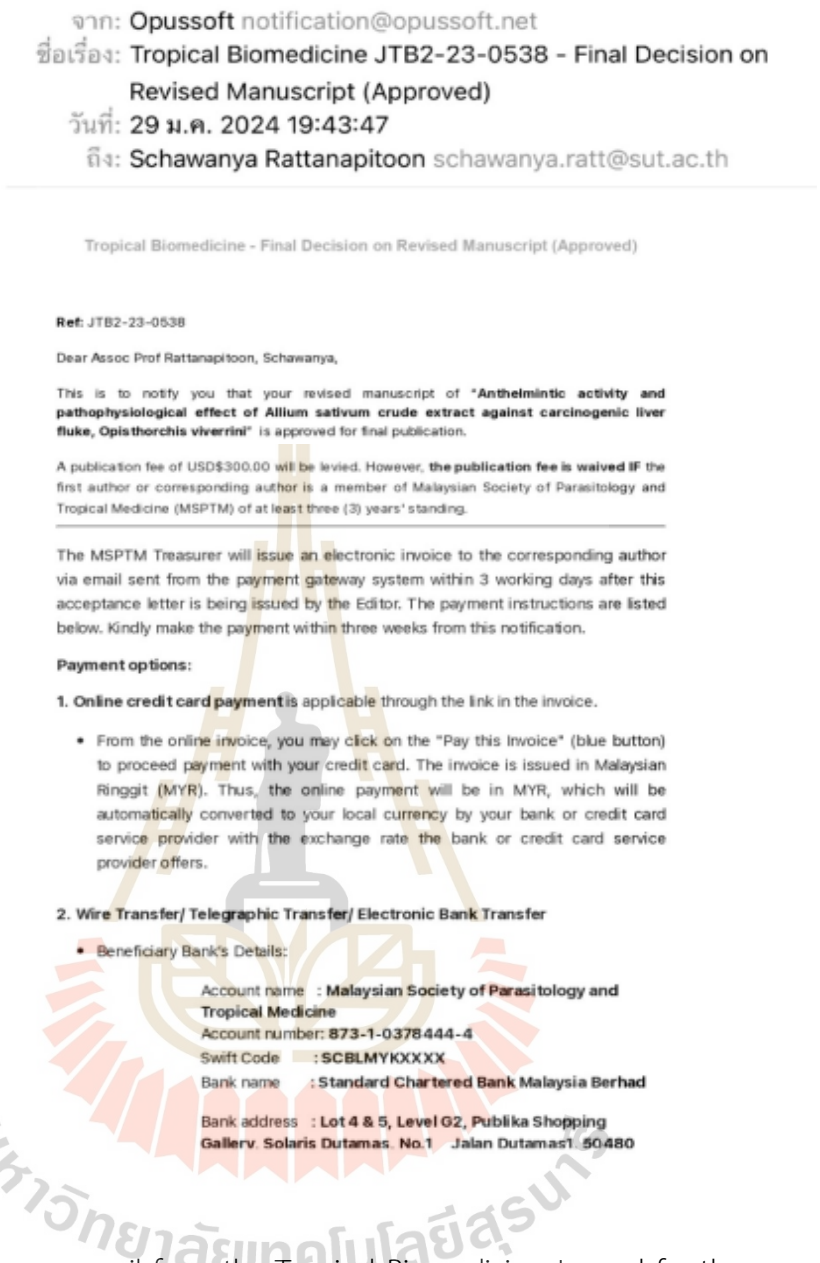
Figure 2G Acceptance email from The Natural Products Journal for the manuscript titled "Papaya Seeds Extract Combined Lauryl Glucoside Against Human Parasites Contaminated in Fresh Vegetables," for publication.



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วันที่: 29 ม.ค. 2024 19:43:47
ถึง: Schawanya Rattanapitoon schawanya.ratt@sut.ac.th

Tropical Biomedicine - Final Decision on Revised Manuscript (Approved)

Ref: JTB2-23-0538

Dear Assoc Prof Rattanapitoon, Schawanya,

This is to notify you that your revised manuscript of "**Anthelmintic activity and pathophysiological effect of Allium sativum crude extract against carcinogenic liver fluke, Opisthorchis viverrini**" is approved for final publication.

A publication fee of USD$300.00 will be levied. However, **the publication fee is waived IF** the first author or corresponding author is a member of Malaysian Society of Parasitology and Tropical Medicine (MSPTM) of at least three (3) years' standing.

The MSPTM Treasurer will issue an electronic invoice to the corresponding author via email sent from the payment gateway system within 3 working days after this acceptance letter is being issued by the Editor. The payment instructions are listed below. Kindly make the payment within three weeks from this notification.

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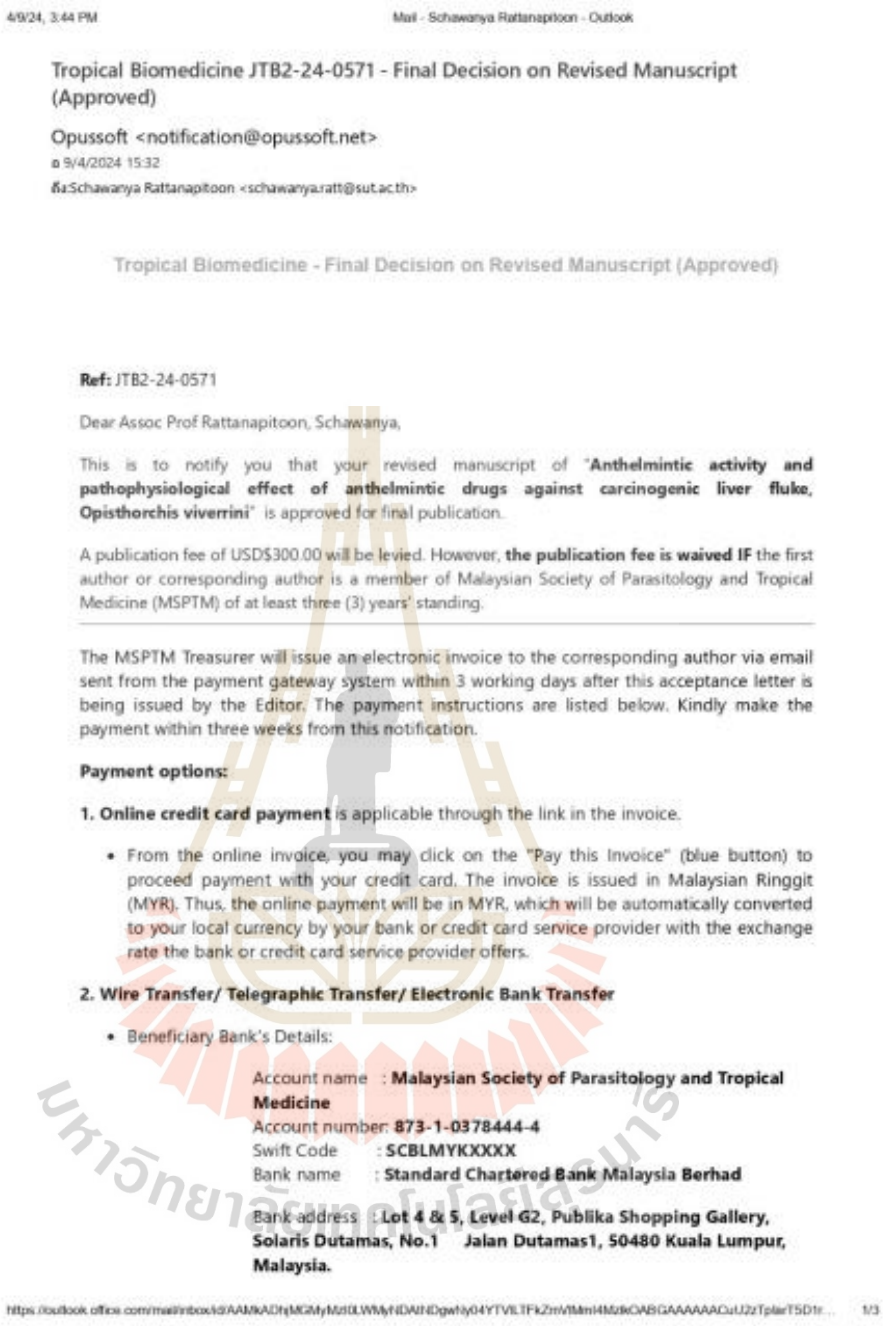
Swift Code : **SCBLMYKXXXX**
Bank name : **Standard Chartered Bank Malaysia Berhad**
Bank address : **Lot 4 & 5, Level G2, Publika Shopping Gallery, Solaris Dutamas, No.1 Jalan Dutamas1 50480**

Figure 3G Acceptance email from the Tropical Biomedicine Journal for the manuscript titled "Anthelmintic activity and pathophysiological effect of *Allium sativum* crude extract against the carcinogenic liver fluke, *Opisthorchis viverrini*," for publication.



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Tropical Biomedicine JTB2-24-0571 - Final Decision on Revised Manuscript (Approved)

Opussoft <notification@opussoft.net>
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ถึง:Schawanya Rattanapitoon <schawanya.ratt@sut.ac.th>

Tropical Biomedicine - Final Decision on Revised Manuscript (Approved)

Ref: JTB2-24-0571

Dear Assoc Prof Rattanapitoon, Schawanya,

This is to notify you that your revised manuscript of "**Anthelmintic activity and pathophysiological effect of anthelmintic drugs against carcinogenic liver fluke, Opisthorchis viverrini**" is approved for final publication.

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2. Wire Transfer/ Telegraphic Transfer/ Electronic Bank Transfer

- Beneficiary Bank's Details:

Account name : **Malaysian Society of Parasitology and Tropical Medicine**
Account number: **873-1-0378444-4**
Swift Code : **SCBLMYKXXXX**
Bank name : **Standard Chartered Bank Malaysia Berhad**
Bank address : **Lot 4 & 5, Level G2, Publika Shopping Gallery, Solaris Dutamas, No.1 Jalan Dutamas1, 50480 Kuala Lumpur, Malaysia.**

https://outlook.office.com/mail/inbox/id/AAMkADhjMGMyMzI0LWMyNDAtNDgwNy04YTVlLTFkZmVlMmI4MzdkOABGAAAAAACuU2zTplarT5D1r... 1/3

Figure 4G Acceptance email from the Tropical Biomedicine Journal for the manuscript titled "Anthelmintic activity and pathophysiological effect of anthelmintic drugs against carcinogenic liver fluke, *Opisthorchis viverrini.*" for publication.

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RESEARCH ARTICLE

Oral parasitic protozoan *Entamoeba gingivalis* in periodontal disease patients, northeastern Thailand

Boonsuya, A.[1], Chitpitaklert, P.[1,2], Pechdee, P.[1,3], Srithongklang, W.[1], Thanchonnang, C.[1], La, N.[1], Gordon, C.N.[4], Rattanapitoon, N.K.[1,5], Arunsan, P.[1,3], Rattanapitoon, S.K.[1,6*]

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ABSTRACT

Entamoeba gingivalis is present in the oral cavity of humans and is associated with periodontal disease. Consequently, this study aimed to comprehensively investigate the *E. gingivalis* infection and the associated risk factors among individuals suffering from periodontal conditions. A cross-sectional descriptive study was carried out within a cohort of periodontal patients. Dental plaque specimens were meticulously collected and subsequently subjected to thorough examination using the polymerase chain reaction (PCR)-based technique targeting the small subunit ribosomal RNA (SrRNA) gene of the organism. The occurrence of risk factors for *E. gingivalis* infection was analyzed by the chi-square test and binary logistic regression. Out of the 230 participants, 60 were clinically diagnosed with periodontitis, while 170 were afflicted with gingivitis. Out of the 230 patients, 25 (10.9%) tested positive for *E. gingivalis* infections. An in-depth analysis unveiled that a significant majority of infections were recorded within subgroups characterized by a marital status (15.45%), manifestation of periodontitis (25.00%), and concomitant presence of underlying disease (20.83%). Furthermore, the high risk factor associated with *E. gingivalis* infection was the female (OR_{adj} = 13.65, 95% CI = 1.08-173.21), followed by periodontitis (OR_{adj} = 3.30, 95% CI = 1.21-9.00), respectively. The study employs a molecular diagnostic approach to screen for *E. gingivalis* enrichment within a subset of periodontal patients with advancing disease. The findings emphasize the necessity for further research to elucidate the pathogenesis of *E. gingivalis* and advocate for vigilant surveillance within a substantial population of periodontal patients.

Keywords: *Entamoeba gingivalis*; polymerase chain reaction (PCR); small subunit ribosomal RNA (SrRNA) gene; Thailand; periodontal disease.

INTRODUCTION

Entamoeba gingivalis, an amoebic protozoan, inhabits the oral cavity of individuals exhibiting inadequate oral hygiene practices. It is detected within dental plaques on gingival and tooth surfaces, interdental spaces, and carious lesions (Alhammaa Abbass *et al.*, 2020). While the amoeba's trophozoite form could potentially be transmitted, the infective stage exclusively spreads through direct droplet exposure or intimate contact such as kissing (Bonner *et al.*, 2018; Mielnik-Błaszczak *et al.*, 2018). *E. gingivalis* scavenges dental plaques within the oral cavity, yet the accuracy of its impact on oral hygiene remains inconsistent (Smith & Barrett, 1915; Craig, 1916). Prior investigations have reported *E. gingivalis* as an opportunistic pathogen that aggravates periodontitis within the complex molecular milieu shaped by periodontal disease (Ponce de León *et al.*, 2001). Acting synergistically with symbiotic bacteria (*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*), *E. gingivalis* contributes to the onset of periodontal disease in immunocompromised hosts (Chen *et al.*, 2001; Socransky & Haffajee, 2005; Dubar *et al.*, 2020). Moreover, studies employing progressive molecular methodologies have explored the prevalence of *E. gingivalis* in both healthy individuals and those affected by oral cavity diseases, effectively capturing th
the organism (Badri *et al.*, 2021). The occ
prevalence has been examined across di
the highest rates documented in Jordan (8
lower rates observed in Portugal (3%). I
small-scale study in Suphanburi Province re
participants (9.5%) had tested positive for *E. gingivalis* infection in dental plaque samples. The infection rates were 9.7% for males and 9.4% for females (Siriba *et al.*, 2016). Among diverse diagnostic techniques, molecular approaches (51%) and other methodologies (36%) have exhibited the highest combined prevalence (Badri *et al.*, 2021). Furthermore, research efforts have documented *E. gingivalis*

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Figure 5E Research article titled 'Oral parasitic protozoan *Entamoeba gingivalis* in periodontal disease patients, northeastern Thailand' has been published in the Tropical Biomedicine Journal.

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RESEARCH ARTICLE

Molecular detection of oral *Trichomonas tenax* in periodontal disease patients by polymerase chain reaction -based 18S rRNA gene

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ABSTRACT

Trichomonas tenax, an oral flagellated protozoon found in humans, potentially associated with the inflammation of periodontal tissues and decreased immunity that causes the tissue damage and tooth loss from chronic infection. Currently, there is a lack of data regarding the prevalence of *T. tenax* infection in Thailand. Therefore, this study aimed to measure prevalence of *T. tenax* in periodontal disease patients by using polymerase chain reaction (PCR) to amplify the 18S ribosomal RNA (18S rRNA) gene and to determine the factors associated with the presence of this protozoan. A cross-sectional descriptive study was conducted among 230 patients with periodontal disease, who visited the oral health center of Suranaree University of Technology Hospital, Thailand from 2021 to 2022. Dental plaque specimens were collected and examined to identify the presence of *T. tenax* using the PCR-based 18S rRNA gene. The occurrence of factors associated with *T. tenax* infection was analyzed by the chi-square test and binary logistic regression. The prevalence of *T. tenax* infection was 13.48% (31/230), in patients, including 96.77% (30/31) and 3.23% (1/31) in periodontitis and gingivitis patients, respectively. The presence of *T. tenax* was associated with periodontal disease ($p<0.001$) and the Periodontal Screening and Record (PSR) index ($p=0.001$). The significant risk factors for *T. tenax* infection were periodontitis (OR_{adj}=239.89, 95% CI=23.801-2417.746), no-underlying disease (OR_{adj}=0.31, 95% CI=0.099–0.942), and male sex (OR_{adj}=0.25, 95% CI=0.062-0.981). Dentists should be concerned about this oral protozoan in periodontitis patients. Furthermore, epidemiologic studies of *T. tenax* are still needed to investigate the mechanism of pathogenesis from *T. tenax* infection.

Keywords: Oral protozoa; *Trichomonas tenax*; periodontal disease; polymerase chain reaction (PCR); Thailand.

INTRODUCTION

Trichomonas tenax is a motile-flagellated protozoan is one of the risk factors for inflammation of periodontal tissues. It was first considered to be a commensal protozoan in the oral cavity and nasopharyngeal cavity (Hamadto *et al*., 2014) until the 1940s, when Dobell (1939) and Wenrich (1944) described the oral flagellate with the name *T. tenax* (Honigberg & Lee, 1959). It may be found in the intraoral cavity, such as in dental plaque, calculus, saliva of periodontal disease patients (Ribeiro *et al*., 2015), and in the extraoral cavity, such as in the respiratory tract, lung, maxillary sinus in compromised patients, other organs, and tissue (Mallat *et al*., 2004; Marty *et al*., 2017). For several decades, studies in oral protozoans have been of interest, and some authors have reported that *T. tenax* has a potential pathogenic role and is a coinfection pathogen in various infections (Socransky & Haffajee, 1992; Ribeiro *et al*., 2015; Dybicz *et al*., 2018). The occurrence of *T. tenax* has been observed in the oral cavity of patients with pulmonary disease and rheumatoid arthritis as well as in immunosuppressive patients. The functions of the immune system are impaired due to the main disease (Kikuta *et al*., 1997; Marty *et al*., 2017). Moreover, immunosuppressive patients can develop opportunistic parasitic disease (Dybicz *et al*., 2018). In various studies, *T. tenax* has recently been reported to damage mammalian epithelial cells, and it behaves similarly to and is closely related *Trichomonas vaginalis*, and pathogenic *Trichomonas* species of the genitourinary tract, thus satisfying the requirements to be considered a parasite (Ribeiro *et al*., 2015).

Periodontal disease is a chronic illness in humans that is characterized by inflammation and the loss of both soft and hard tissue supporting the teeth, as shown in Figure 1. In addition, periodontal disease associated to worsening systemic disorders such as diabetes, atherosclerosis, and cardiovascular diseases

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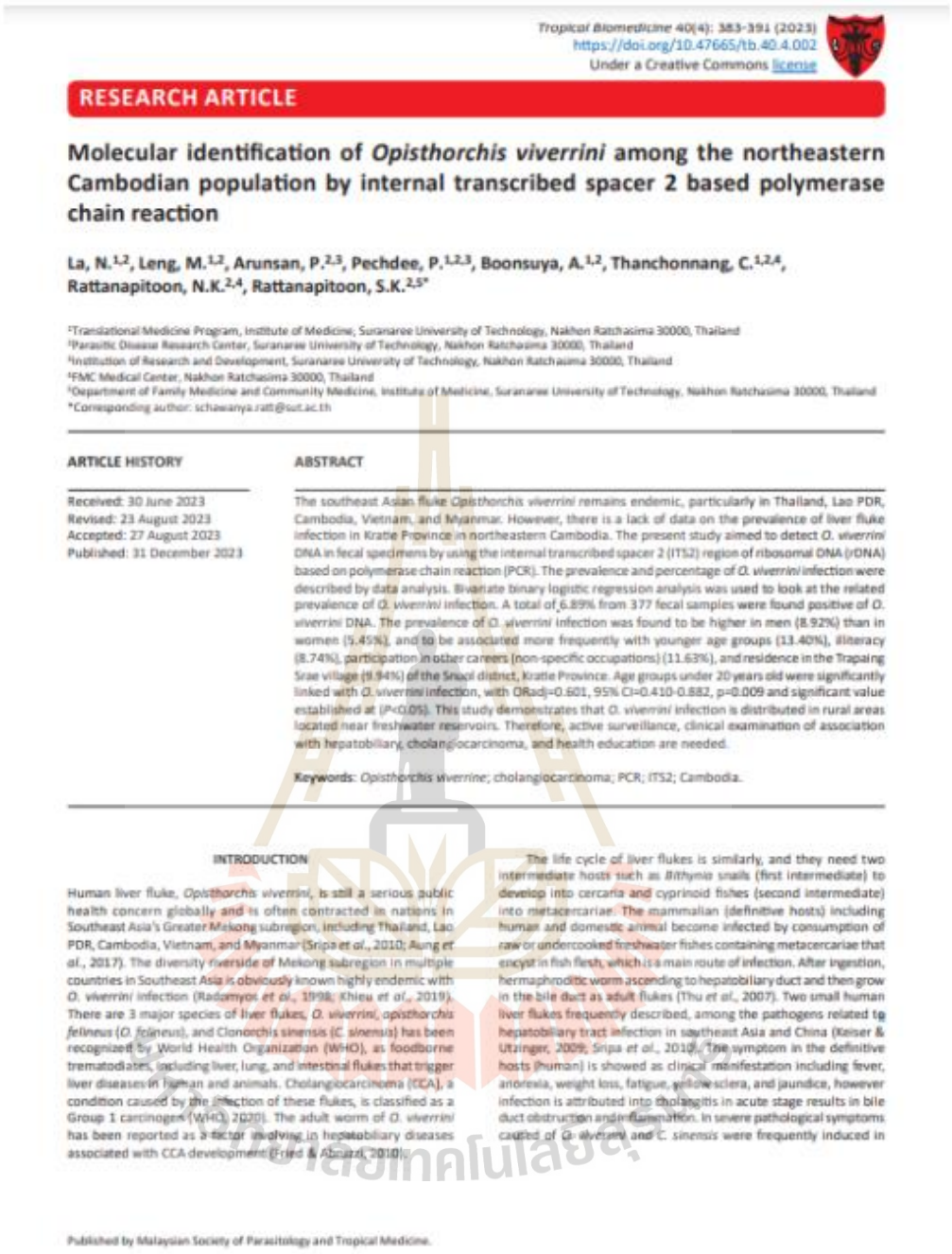
Figure 6G Research article titled “Molecular detection of oral *Trichomonas tenax* in periodontal disease patients by polymerase chain reaction based 18S rRNA gene.” has been published in the Tropical Biomedicine Journal.



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RESEARCH ARTICLE

Molecular identification of *Opisthorchis viverrini* among the northeastern Cambodian population by internal transcribed spacer 2 based polymerase chain reaction

La, N.[1,2], Leng, M.[1,2], Arunsan, P.[2,3], Pechdee, P.[1,2,3], Boonsuya, A.[1,2], Thanchonnang, C.[1,2,4], Rattanapitoon, N.K.[2,4], Rattanapitoon, S.K.[2,5*]

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ABSTRACT

The southeast Asian fluke *Opisthorchis viverrini* remains endemic, particularly in Thailand, Lao PDR, Cambodia, Vietnam, and Myanmar. However, there is a lack of data on the prevalence of liver fluke infection in Kratie Province in northeastern Cambodia. The present study aimed to detect *O. viverrini* DNA in fecal specimens by using the internal transcribed spacer 2 (ITS2) region of ribosomal DNA (rDNA) based on polymerase chain reaction (PCR). The prevalence and percentage of *O. viverrini* infection were described by data analysis. Bivariate binary logistic regression analysis was used to look at the related prevalence of *O. viverrini* infection. A total of 6.89% from 377 fecal samples were found positive of *O. viverrini* DNA. The prevalence of *O. viverrini* infection was found to be higher in men (8.92%) than in women (5.45%), and to be associated more frequently with younger age groups (13.40%), illiteracy (8.74%), participation in other careers (non-specific occupations) (11.63%), and residence in the Trapaing Srae village (9.94%) of the Snuol district, Kratie Province. Age groups under 20 years old were significantly linked with *O. viverrini* infection, with ORadj=0.601, 95% CI=0.410-0.882, p=0.009 and significant value established at ($P<0.05$). This study demonstrates that *O. viverrini* infection is distributed in rural areas located near freshwater reservoirs. Therefore, active surveillance, clinical examination of association with hepatobiliary, cholangiocarcinoma, and health education are needed.

Keywords: *Opisthorchis viverrine*; cholangiocarcinoma; PCR; ITS2; Cambodia.

INTRODUCTION

Human liver fluke, *Opisthorchis viverrini*, is still a serious public health concern globally and is often contracted in nations in Southeast Asia's Greater Mekong subregion, including Thailand, Lao PDR, Cambodia, Vietnam, and Myanmar (Sripa *et al.*, 2010; Aung *et al.*, 2017). The diversity riverside of Mekong subregion in multiple countries in Southeast Asia is obviously known highly endemic with *O. viverrini* infection (Radomyos *et al.*, 1998; Khieu *et al.*, 2019). There are 3 major species of liver flukes, *O. viverrini*, *opisthorchis felineus* (*O. felineus*), and Clonorchis sinensis (*C. sinensis*) has been recognized by World Health Organization (WHO), as foodborne trematodiases, including liver, lung, and intestinal flukes that trigger liver diseases in human and animals. Cholangiocarcinoma (CCA), a condition caused by the infection of these flukes, is classified as a Group 1 carcinogen (WHO, 2020). The adult worm of *O. viverrini* has been reported as a factor involving in hepatobiliary diseases associated with CCA development (Fried & Abruzzi, 2010).

The life cycle of liver flukes is similarly, and they need two intermediate hosts such as *Bithynia* snails (first intermediate) to develop into cercaria and cyprinoid fishes (second intermediate) into metacercariae. The mammalian (definitive hosts) including human and domestic animal become infected by consumption of raw or undercooked freshwater fishes containing metacercariae that encyst in fish flesh, which is a main route of infection. After ingestion, hermaphroditic worm ascending to hepatobiliary duct and then grow in the bile duct as adult flukes (Thu *et al.*, 2007). Two small human liver flukes frequently described, among the pathogens related to hepatobiliary tract infection in southeast Asia and China (Keiser & Utzinger, 2009; Sripa *et al.*, 2010). The symptom in the definitive hosts (human) is showed as clinical manifestation including fever, anorexia, weight loss, fatigue, yellow sclera, and jaundice, however infection is attributed into cholangitis in acute stage results in bile duct obstruction and inflammation. In severe pathological symptoms caused of *O. viverrini* and *C. sinensis* were frequently induced in

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Figure 7G Research article titled "Molecular identification of *Opisthorchis viverrini* among the northeastern Cambodian population by internal transcribed spacer 2 based polymerase chain reaction." has been published in the Tropical Biomedicine Journal.

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RESEARCH ARTICLE

Intestinal parasitic infections and risk factors among the population in Cambodia

La, N.[1], Leng, M.[1], Rattanapitoon, N.K.[1], Pechdee, P.[1,2], Boonsuya, A.[1], Arunsan, P.[1,2], Rattanapitoon, S.K.[1,3*]

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ABSTRACT

Many species of helminths and protozoa caused intestinal parasitic infections (IPIs). It belongs to neglected tropical diseases (NTDs) and remains a major public health problem in several Southeast Asian countries. The present study aimed to investigate the prevalence of IPIs and associated risk factors among the population in Kratie Province in northeastern Cambodia and Phnom Penh is the capital that locates in southern Cambodia. Fecal specimens (n=366) were collected in 10 villages in Kratie Province and Phnom Penh from 2019 to 2021. They were processed using the formalin ethyl-acetate concentration technique (FECT) to investigate parasites at egg and cyst stages and then examined under a light microscope. The results revealed that the prevalence of IPIs among the population in Kratie Province (n=317) and Phnom Penh (n=49) was 16.12% (n=59); of Kratie Province (n=50, 13.66%) and Phnom Penh (n=9, 2.46%), 12.02% (n=44) were helminths and 4.10% (n=15) were protozoa. The parasitic infection rate was higher in males (9.02%) than in females (7.10%) and more likely to be due to helminths (7.38%) than protozoa (1.64%). Prevalence of *Opisthorchis viverrini* was the highest (5.74%), followed by those of *Entamoeba coli* (4.10%), hookworm (3.83%), *Ascaris lumbricoides* (1.10%), *Hymenolepis nana* (1.09%), *Taenia* spp. (0.54%), *Trichuris trichiura* (0.55%), and *Enterobius vermicularis* (0.27%), respectively. Moreover, *O. viverrini* infection was the most common infection in the >20-year age group in Kratie Province. In addition, the bivariate and multivariate analyses showed that the association between gender. Gender was a significant risk factor positively associated with *O. viverrini* and hookworm infections (OR_{adj}=0.318, 95% CI=0.122-0.8270, P=0.019 and OR_{adj}=0.085, 95% CI=0.017-0.436, P=0.003, respectively). In conclusion, the IPIs were highly prevalent, especially *O. viverrini* and hookworm infections, among the population in Cambodia. These IPIs impact the public health burden but can be prevented by education regarding good sanitary practices in this community.

Keywords: Intestinal parasitic infections (IPIs); formalin ethyl-acetate concentration technique (FECT); Cambodia; prevalence; Kratie Province and Phnom Penh.

INTRODUCTION

Intestinal parasitic infections (IPIs) are a serious public health problem in several countries throughout the world. IPIs are highly prevalent in Southeast Asia (Dunn *et al.*, 2016), including Thailand, Cambodia, Lao PDR, and Myanmar (Han *et al.*, 2019). Neglected helminth and protozoa infections have affected almost 200 million people who live in poverty in countries belonging to the Association of Southeast Asian Nations (ASEAN) (Hotez *et al.*, 2015). The main cause of intestinal parasite transmission is poor hygiene conditions, such as a lack of clean water and sanitary toilets (Ziegelbauer *et al.*, 2012; Echazu *et al.*, 2015). More than 10 million people suffer from parasitic infections, including infections of liver and intestinal flukes, in Southeast Asia (Furst *et al.*, 2012). In Myanmar, the prevalence of intestinal helminth infection is approximately 11.3 million people (Hotez *et al.*, 2015; WHO, 2018). The primary socioeconomic factors related to the high prevalence of IPIs include poor food hygiene, unsanitary housing, congested living conditions, agricultural conditions, insufficient water resources, animal living near the reservoirs, and poor healthcare services (Echazu *et al.*, 2015; Hotez *et al.*, 2015; Dunn *et al.*, 2016;). Nevertheless, these parasitic infections are classified as neglected tropical diseases. The most patients are unaware/less of symptoms until progression and illness presentation, leading to individual, economic, and society burdens due to chronic infection or reinfection. Consequently, this problem has persisted. Approximately 9.3 million cases of liver fluke infection have been reported in ASEAN countries (Hotez *et al.*, 2015). A previous study reported on the prevalence of intestinal helminth infections among villages in Kratie Province, northeastern Cambodia, using fecal specimens and the Kato-Katz thick smear technique. The highest prevalence of *Opisthorchis viverrini* infections occurred in Roka Kandal A (10.4%), followed by Talous (5.9%); in both these

Figure 8G Research article titled "Intestinal parasitic infections and risk factors among the population in Cambodia." has been published in the Tropical Biomedicine Journal.

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Poster presentation

Poster presentation in titled Efficacy of High sensitively diagnosis of Gastrointestinal parasitic infections by Fully Automatic Feces Analyzer at the 59th Annual Scientific Conference of the Malaysian Society of Parasitology & Tropical Medicine. (MSPTM 2023) Organized by Malaysian Society of Parasitology and Tropical Medicine on 15-16 March 2023 at the Wembley Hotel, Penang, Malaysia.

Proceedings

Submitting your manuscript in titled "Detection of intestinal parasitic infections in the population in northeastern Thailand using the Kato-Katz Technique (proceedings) at the 3rd International Conference on Parasitology 2023 (ICP2023). Organized by Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand on 9th -10th November 2023

Publication

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