EFFICACY OF *STRONGYLOIDES STERCORALIS* PEPTIDES ANTIGEN FOR SEROLOGICAL DIAGNOSTIC PURPOSE

THITIMAKORN NAMHONG

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Translational Medicine
Suranaree University of Technology
Academic Year 2022

ประสิทธิภาพเปปไทด์แอนติเจนของพยาธิสตรองจิลอยเดส สเตอร์โคราลิส สำหรับวัตถุประสงค์ในการวินิจฉัยทางเซรุ่มวิทยา

นางสาวฐิติมากรณ์ นามโฮง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเวชศาสตร์ปริวรรต
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2565

EFFICACY OF *STRONGYLOIDES STERCORALIS* PEPTIDES ANTIGEN FOR SEROLOGICAL DIAGNOSTIC PURPOSE

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

Thesis Examining Committee

.....

(Assoc. Prof. Dr. Potjanee Srimanote)
Chairperson

Jeeraphong T. .....

(Asst. Prof. Dr. Jeeraphong Thanongsaksrikul)
Member (Thesis Co-advisor)

Kanyarat T. .....

(Dr. Kanyarat Thueng-in)
Member (Thesis Advisor)

.....

(Dr. Sanong Suksaweang)
Member

.....

(Assoc. Prof. Dr. Poom Adisakwattana)
Member (Thesis Co-advisor)

Chatchai Jothityangkoon .....

(Assoc. Prof. Dr. Chatchai Jothityangkoon)
Vice Rector for Academic Affairs and
Quality Assurance

.....

(Assoc. Prof. Dr. Sutham Pinjaroen)
Dean of Institute of Medicine

ฐิติมากรณ์ นามโฮง: ประสิทธิภาพเปปไทด์แอนติเจนของพยาธิสตรองจิลอยเดส สเตอร์โคราลิส สำหรับวัตถุประสงค์ในการวินิจฉัยทางเซรุ่มวิทยา (EFFICACY OF *STRONGYLOIDES STERCORALIS* PEPTIDES ANTIGEN FOR SEROLOGICAL DIAGNOSTIC PURPOSE) อาจารย์ที่ปรึกษา: อ. ทนพญ. ดร.กัญญารัตน์ ถึงอินทร์, 75 หน้า.

คำสำคัญ: พยาธิสตรองจิลอยเดส สเตอร์โคราลิส, โรคหนอนพยาธิสตรองจิลอยด์, เปปไทด์, อีไลซ่า

โรคหนอนพยาธิสตรองจิลอยด์เกิดจากการติดเชื้อพยาธิสตรองจิลอยเดส สเตอร์โคราลิส (*Strongyloides stercoralis)* ในมนุษย์ สามารถพบได้ทั่วโลกโดยเฉพาะในเขตร้อนรวมถึงประเทศไทย เป็นโรคที่ถูกละเลยและยังคงเป็นปัญหาสำคัญของด้านสาธารณสุข มีรายงานประเมินความชุกของโรคสตรองอิลอยด์ทั่วโลกในปี 2560 เป็น 8.1% ซึ่งเท่ากับผู้ติดเชื้อ 613.9 ล้านคน และก่อนหน้านี้มีการศึกษาระบาดวิทยาของโรคสตรองจิลอยด์ในโรงพยาบาลภาคตะวันออกเฉียงเหนือของประเทศไทยในปี 2557 มีอุบัติการณ์ของโรคสตรองจิลอยด์ถึง 12.9% ซึ่งนับว่าเป็นสิ่งที่สำคัญในการเฝ้าระวัง เนื่องจากพยาธิสตรองจิลอยเดสมีวงจรชีวิตที่ซับซ้อนซึ่งมันสามารถอาศัยและอยู่รอดได้ทั้งแห่งธรรมชาติที่ขาดสุขอนามัยหรือดินที่เปียกชื้นและสามารถอาศัยอยู่ในร่างกายมนุษย์ได้นานถึงปี สามารถติดเชื้อได้ทุกเพศทุกวัย ในคนทั่วไปที่มีภูมิคุ้มกันต้านทานโรคปกติการติดพยาธิสตรองจิลอยด์มักไม่ก่ออาการ ยกเว้นเมื่อติดพยาธิจำนวนมากหรือร่างกายมีภูมิคุ้มกันต้านทานโรคที่ต่ำกว่าปกติจะมีอาการตั้งแต่เฉียบพลัน เรื้อรัง ไปจนถึงรุนแรงอาจส่งผลถึงแก่ชีวิต เนื่องจากพยาธิสามารถชอนไชไปยังอวัยวะสำคัญของร่างกายได้แก่ ปอด ถุงลม ลำไส้ ไปจนถึงสมอง ซึ่งมีการติดเชื้อแบคทีเรียที่รุนแรงร่วมด้วย ซึ่งการตรวจคัดกรองและวินิจฉัยโรคหนอนพยาธิสตรองจิลอยด์ส่วนใหญ่วินิจฉัยด้วยวิธีการส่งสิ่งส่งตรวจอุจจาระส่องผ่านกล้องจุลทรรศน์ซึ่งเป็นได้ว่าอาจมีการตรวจที่คลาดเคลื่อนและมีความไวต่ำ นี่จึงเป็นเหตุผลสำคัญที่จะศึกษาและพัฒนาวิธีการตรวจที่มีประสิทธิภาพและมีความแม่นยำเพิ่มขึ้น และนอกจากนี้มีการศึกษาเกี่ยวกับการทดสอบทางภูมิคุ้มกันวิทยาของโรคสตรองจิลอยด์มีความไวและความจำเพาะที่สูง

ในการศึกษานี้เราหาประสิทธิภาพของเปปไทด์แอนติเจนที่จำเพาะต่อเชื้อพยาธิสตรองจิลอยเดส สเตอร์โคราลิส เพื่อความแม่นยำต่อการวินิจฉัยมากขึ้น ทดสอบโดยวิธีการอีไลซ่าโดยเปรียบเทียบกับแอนติเจนจากพยาธิสตรองจิลอยเดส แดทติ (*Strongyloides ratti*) ที่เพาะเลี้ยงในหนู และเลือกคัดเลือกตัวอย่างซีรั่มของผู้ป่วยที่ติดเชื้อจากพยาธิสตรองจิลอยเดส สเตอร์โคราลิส, พยาธิหลากหลายชนิด และผู้ที่ แอนติเจนจากพยาธิสตรองจิลอยเดส แดทติ (*Strongyloides ratti*) ที่เพาะเลี้ยงในหนู และเลือกคัดเลือกตัวอย่างซีรั่มของผู้ป่วยที่ติดเชื้อจากพยาธิสตรองจิลอยเดส สเตอร์โคราลิส, พยาธิหลากหลายชนิด และผู้ที่ไม่ติดพยาธิเป็นแอนติบอดี้จำนวนทั้งหมด 78 ตัวอย่าง ผลการทดลองพบว่าเปปไทด์-1 และสตรองจิลอยเดส แดทติ มีประสิทธิภาพในการแยกระหว่างกลุ่มติดเชื้อและไม่ติดเชื้อได้ดีกว่า เปปไทด์-2 โดยเปปไทด์-1,เปปไทด์-2 และ สตรองจิลอยเดส แดทติแอนติเจนมีค่าความไวประมาณ 75%, 75% และ 50% ตามลำดับ มีความจำเพาะประมาณ 75%, 86% และ 89% ตามลำดับ ซึ่งผลการทดลองสามารถใช้ได้ในระดับหนึ่ง แต่ทางเลือกที่ดีควรออกแบบเพิ่มอีพิโทปเพื่อความจำเพาะและความไวต่อเชื้อพยาธิสตรองจิลอยเดส สเตอร์โคราลิส เพิ่มจำนวนตัวอย่างการทดลองเพื่อยืนยันผลที่น่าเชื่อถือ และอาจเป็นประโยชน์อย่างยิ่งสำหรับในห้องปฏิบัติการทางคลินิกและการศึกษาการเฝ้าระวังในอนาคต

สาขาวิชา เวชศาสตร์ปริวรรต ลายมือชื่อนักศึกษา ชัชวากรณ์ นามโสว

ปีการศึกษา 2565 ลายมือชื่ออาจารย์ที่ปรึกษา Kanyarat T.

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

THITIMAKORN NAMHONG: EFFICACY OF *STRONGYLOIDES STERCORALIS* PEPTIDES ANTIGEN FOR SEROLOGICAL DIAGNOSTIC PURPOSE. THESIS ADVISOR: Kanyarat Thueng-in, Ph.D., 75 PP.

Keywords: *Strongyloides stercoralis*, Strongyloidiasis, peptides, ELISA

Strongyloidiasis is caused by *Strongyloides stercoralis* (*S. stercoralis*) infection in humans. It can be found all over the world especially in the tropical area, including Thailand. It is a neglected disease that continues to be a major public health problem. The report estimated the global prevalence of Strongyloidiasis in 2017 to be 8.1%, equivalent to 613.9 million people infected. And a previous study of the epidemiology of strongyloidiasis in hospitals in Northeastern Thailand in 2014 had an incidence of strongyloidiasis of 12.9%. This is considered important in surveillance because *Strongyloides* have a complex life cycle where they can live and survive in unsanitary environments or wet soils and can live in the human body for up to a year. *Strongyloides* infections rarely cause symptoms in people with normal immunity. Except when the immune system is compromised, symptoms range from acute to chronic to severe, perhaps lethal. Because parasites can penetrate into important organs of the body, such as the lungs, air sacs, intestines, and brain, which are associated with serious bacterial infections. The screening and diagnosis of *Strongyloides* helminthiasis is most often performed by determine the present of parasite under a microscope, which may be inaccurate and have poor sensitivity. This is why it is important to study and develop more efficient and accurate screening methods. In addition, immunohistochemical tests for *Strongyloides* have been studied with high sensitivity and specificity.

In this study, the B-cell epitope peptide antigens of fatty acid and retinal binding protein was synthesized. The efficacy of antibody detection of peptide antigen was determined using ELISA. The *Strongyloides ratti* crude antigen was use as positive control. A total of 78 serum samples were collected from different patients infected with *S. stercoralis*, other parasites, and non-infected person. The results showed that peptide-1 and *S. ratti* could differentiate among infected and non-infected groups, whereas, whereas peptide-2 could not. Peptide-1, peptide-2 and *S. ratti* antigen had sensitivity values of about 75%, 75% and 50%, respectively, and specificity values of about 75%, 86% and 89%, respectively. The experimental results can be used at some level, but a good alternative would be to design more epitopes for *S. stercoralis* and increase the number of experimental samples to confirm reliable results. This will be especially useful in routine clinical labs and surveillance investigations in the future.

School of Translational Medicine

Academic Year 2022

Student's Signature Thitimakron Namhong

Advisor's Signature Kanyarat T.

Co-Advisor's Signature

Co-Advisor's Signature Jeeraphong T.

ACKNOWLEDGEMENTS

This thesis has come to an end successfully, I would like to express my sincere gratitude to my principal advisor Dr. Kanyarat Thueng-in, for giving me the opportunity to fulfil my study and giving the opportunity to conduct experiments in her laboratory and teaching and passing on her knowledge to me for the past academic years.

I would like to express my gratitude to my all co-advisors, Assoc. Prof. Dr. Poom Adisakwattana and Asst. Prof. Dr. Jeeraphong Thanongsaksrikul for their innumerable help, suggestion, supports, and kindness. This thesis has been accomplished under their suggestions.

I would also like to express my gratitude to the chairman of the thesis committee Assoc. Prof. Dr. Potjanee Srimanote for her excellent guidance and aid with academic and research activities, as well as advice on writing a thesis revision.

I would like to extend my gratitude to the chairman of the Program in Translational Medicine, Dr. Sanong Suksaweang for his invaluable help, supports, kindness, and endless encouragement.

I would like to express my gratitude teacher of the Preclinical Department Dr. Chompunoot Wangboon and Dr. Mantana Jamklang for their innumerable help, suggestion, supports, and kindness. This thesis has been finished under their suggestions.

I would like to acknowledge Prof. Paiboon Sithithaworn for generously provided worms of *S. ratti* from Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.

Special thanks for the financial support by National Research Council of Thailand (NRCT) and Suranaree University of Technology (SUT).

I would also like to thank to my friends for providing invaluable encouragement and help each other. Finally, I would like to thank my amazing parents for their continued love, financial support, guidance, and encouragement.

THITIMAKORN NAMHONG

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (continued)

Page

TABLE OF CONTENTS (continued)

Page

LIST OF TABLES

Tables **Page**

LIST OF FIGURES

Figures **Page**

LIST OF FIGURES (continued)

Figures **Page**

LIST OF ABBREVIATIONS

ROC	Receiver operating characteristic curve
AUC	Area under the curve
P	Probability
CI	Confidence Intervals
ANOVA	Analysis of Variance
IBM SPSS Statistics	Statistical Package for the Social Sciences
ELISA	Enzyme-Linked Immunosorbent Assay
FECT	Formalin ethyl acetate concentration technique
BM	Baermann funnel
KAP	Koga agar plate culture
APCT	Agar plate culture technique
2DE	Two-dimensional gel electrophoresis
PCR	Polymerase chain reaction
LC-ESI - MS / MS	liquid chromatography-electrospray ionization-*tandem mass spectrometry*
ICT	Immunochromatographic test kit
rSsIR	Strongyloides stercoralis recombinant IgG immunoreactive antigen
Ss-ELISA	*Strongyloides stercoralis* by enzyme-linked immunosorbent assay
Sr-ELISA	*Strongyloides ratti* antigen by enzyme-linked immunosorbent assay
NIE-ELISA	Recombinant protein by enzyme-linked immunosorbent assay
IEDB	Immune Epitope Database
BLAST program	Basic Local Alignment Search Tool program
PPV	Positive Predicted Values

LIST OF ABBREVIATIONS (continued)

NPV	Negative Predicted Values
LR+	Positive likelihood ratios
LR-	Negative likelihood ratios
Se	Sensitivity
Sp	Specificity
p-value	Probability value
SD	Standard Deviation
Pos	Positive
Neg	Negative
Ag	Antigen
Ig	Immunoglobulin
IgG	Immunoglobulin G level
IgM	Immunoglobulin M level
PBS	Phosphate Buffer Saline
HRP	Anti-human horseradish peroxidase
TMB	Tetramethylbenzidine
BSA	Bovine Serum Albumin
SDS	Sodium Dodecyl Sulfate
DI	Deionized water
OD	*Optical density*
pH	Positive potential of the Hydrogen ions
M	Molar
µl	*Microliter*
ml	*Milliliter*
L	*liter*
g	Gram

LIST OF ABBREVIATIONS (continued)

µm	Micrometer
µg	Microgram
mm	Millimeter
nm	Nanometer
rpm	Revolutions per minute
cm	centimeter
°C	Degree Celsius
L1	Larva stage 1
L2	Larva stage 2
L3	Larva stage 3
AIDS	Acquired Immune Deficiency Syndrome
HIV	Human immunodeficiency virus
S. stercoralis or Ss	*Strongyloides stercoralis*
S. ratti	*Strongyloides ratti*
FRB	Fatty acid and retinol-binding protein
US or USA	United States of America

CHAPTER I

INTRODUCTION

1.1 Background

Strongyloidiasis is caused by *Strongyloides stercoralis* infection in humans. It can be found all over the world especially in the tropical area, including Thailand (Kitvatanachai et al. 2022). It is a neglected disease that continues to be a major public health problem. *S. stercoralis* has a complex life cycle and can grow in two forms: parasitic and free-living (Laoraksawong et al. 2018). Filariform larva stage is a contagious phase that can penetrate the skin and enter the circulatory system. It may enter organs such as the digestive system, respiratory system, spinal cord and brain. A nematodes infection may show little or no symptoms, cause chronic infection and may have a relatively high risk of mortality in immunocompromised people who are receiving immunosuppressants, AIDS patients (HIV) other (Sharma et al. 2022; Czersnia and Weiss 2022). In addition, the infectious factor comes from the transmission of the filariform larva through the soil. The helminths can live in the soil without host and able to mate asexual. Direct contact with soil, especially those who do a career in farmers, individuals engaged in land activities, hiker, fisherman, athletes such as soccer players, rugby players or tribal communities that have not yet been developed sanitation. It is a risk factor for helminth infections. The South-East Asia, African, and Western Pacific Regions accounted for 76.1% of the global infections (Buonfrate et al. 2020).

Diagnosis is very important for screening a group of patients, assisting patients in becoming aware of and receiving care in a timely manner. However, there is currently no gold standard method (Sharma et al. 2022). Patients with strongyloidiasis are typically assessed using microscopic stool examination. However, these methods have a relatively low sensitivity about 21% (Buonfrate et al. 2020; Mora Carpio and Meseeha 2023). The koga agar plate culture technique (Khanna et al. 2015) and

Baermann technique (De kamonsky 1993), have the sensitivity greater than microscopic stool detection. However, no infection was found in a vast number of the cases, may still be ineffective and improper in reaching a diagnosis. In early 2020, the Strongyloidiasis ICT Kit was built as a fast test kit used to detect antibodies in the serum (Sadaow et al. 2020). This is highly sensitive, but service prices continue to be higher than the demands of service patients. The specificity still needs to be developed due to cross-reactivity with other nematode parasites (Tamarozzi et al. 2021). Immunological approaches have the advantage of being more sensitive than parasitological procedures (Levenhagen et al. 2021). And demonstrated a specificity of almost 100% (Buonfrate et al. 2015). Enzyme-Linked Immunosorbent Assay (ELISA) is an immunological assay commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples.

In this study, B-cell epitope antigen of fatty acid and retinol binding protein was synthesized and tested for their efficacy, which could be useful for *S. stercoralis* immunodiagnostic.

1.2 Research hypotheses

Peptide antigens of *S. stercoralis* could be useful for antibody detection to *S. stercoralis* infection and should not be cross-reactive with other parasite infection.

1.3 Objective

The aim of this study is to produce and determine the efficacy of peptide antigen of *S. stercoralis* for antibody detection to *S. stercoralis* infection.

CHAPTER II

LITERATURE REVIEWS

2.1 *Strongyloides stercoralis*

Strongyloides stercoralis (*S. stercoralis*) is causative agent of Strongyloidiasis in humans of all ages. The parasite is a major neglected common tropical disease with the potential of causing lifelong infection and mortality that causes serious public health problem (Balachandra et al. 2021). It is a small nematode belonging to Genus Strongyloides, classified as facultative parasite (Chavez et al. 2021). "Strongyloidiasis was first described in French troops who had returned from modern day Vietnam during the late 19th century who were sick with severe and ongoing diarrhea. Patients infected with nematodes, or roundworms through the skin such as bare feet in the rainforest. Most people who are infected with Strongyloides do not know they are infected and have no symptoms" (Viney 2017).

2.1.1 Characteristics and stages of *S. stercoralis*

Wannapinovosheep, S., et al. (2015). reported that the *S. stercoralis* are found all over the world in regions with optimum temperatures and poor sanitation. It can survive both in host and independently. In the host, it can live in the small intestine, or it can be found in the bile ducts and lungs. In addition, free-living is found in damp and waterlogged soils (Wannapinyosheep S., 2015; Chavez et al. 2021).

2.1.1.1 Eggs. The characteristics of *S. stercoralis* eggs are oval-shaped small size, about 50-58 × 30-40 µm. It has a clear thin resemble like *hookworm* eggs. Most of the time, the eggs incubate in the uterus or they in incubate a few hours can lava hatch from the eggs in the intestines of infected patients. But in some cases, eggs are detected in the feces of patients with acute diarrhea only **(Figure 2.1A)**.

2.1.1.2 Rhabditiform larva (L1-L2). In the stage 1-2 it has a slender, cylindrical shape, approximately 20 × 380 μm in size. It has rounded head and tail, has a bulbous esophagus. The stage can be detected in the feces, which can be diagnosed in infected patients **(Figure 2.1B)**.

2.1.1.3 Filariform larva (L3). It has a thin, slender shape, approximately 16 × 630 μm in size. It has a sharp head and tail it has a bulbous esophagus. It can be infected from soil to humans **(Figure 2.1C)**.

2.1.1.4 Adult free-living. It has a short chubby shaped female size approximately 0.05-0.75 × 1.0-1.7mm. It has a rounded head and a straight pointed tail. It has a bulbous esophagus both female and male. There are 2 sets of reproductive organs. And male size: 0.04-0.05 × 0.7-1.0 mm. It has rounded heads and bent tail. There is 1 set of reproductive organs. This phase can stay in the soil **(Figure 2.1D** and **Figure 2.1E)**.

2.1.1.5 Parasitic female. It has a slender shape, approximately 0.04 × 2 mm in size. It is larger than free living and it has a bulbous esophagus. There is 2 set of reproductive organs found only in females in infected patients and it is asexual reproduction **(Figure 2.1F)**.

One prevalent misperception in medical and public health is that *S. stercoralis* looks like other intestinal nematodes like *hookworms* (Page, Judd, and Bradbury 2018). Accurate endoscopic diagnosis may necessitate the use of expert researchers. Although parasites are difficult to differentiate, there are size variations between the mouth cavity and the esophagus **(Figure 2.2)**.

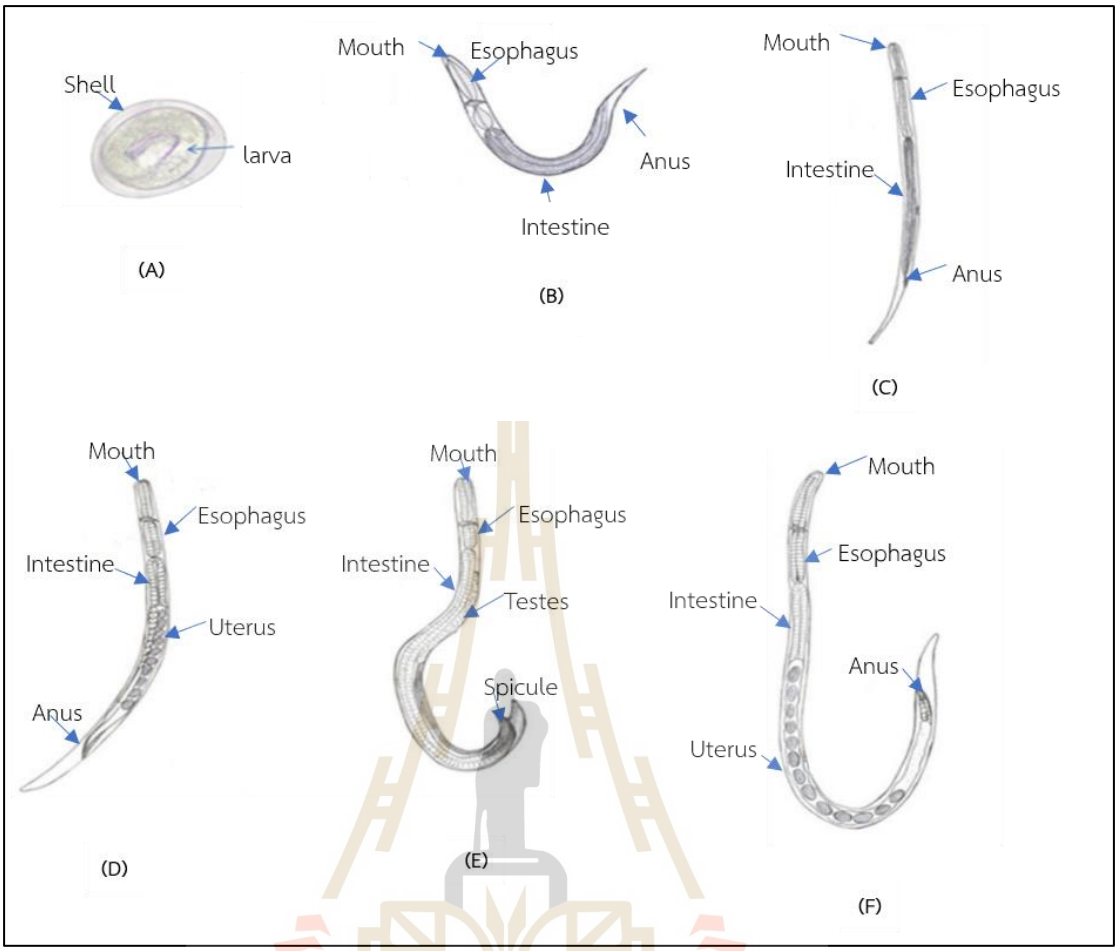

Figure 2.1 Characteristics and stages of *S. Stercoralis*. **(A)** Eggs, **(B)** Rhabditiform larva (L1-L2), **(C)** Filariform larva (L3), **(D)** Free living female, **(E)** Free living male, **(F)** Parasitic female (Huachiew Chalermprakiet Science and Technology Journal, 2015).

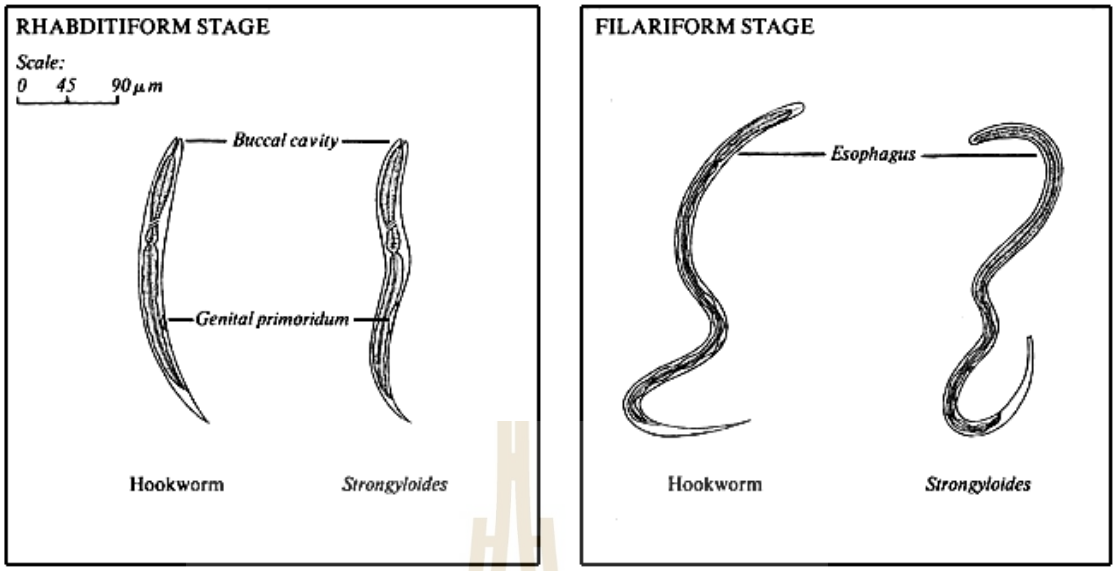

Figure 2.2 Characteristics of *S. stercoralis* and *hookworm.* This figure describes the rhabditiform larvae of *Hookworm* and *S. stercoralis* have unequal mouth sizes, *Hookworm* has a mouth that is wider and longer a little *S. stercoralis.* The genital primordium of *Strongyloides* has a wider niche than the *Hookworm.* The filariform stage differs from the esophagus *Strongyloides* is longer than the *Hookworm* (Centers for Disease Control (CDC), 2017).

2.1.2 *Strongyloides stercoralis* life cycle

The *S. stercoralis* can survive both host and free-living. Therefore, there are many pathways of life cycles: direct, indirect and automatic.

The rhabditiform larva are excreted in the stool of a person infected with the parasite into soil or environment. It later developed into filariform larva (direct cycle) and grow into adult free-living males and females that mate and produce eggs from which rhabditiform larvae and incubates. The later of them can develop into free-living adults (indirect cycle) or develop into filariform larva. This stage can be transmitted to people by means of penetration of the skin into the bloodstream. They are then transported to the lungs into the lung cavity. They are transported through the trachea, into the pharynx, are swallowed and then reached the small

intestine. It develops into a parasitic female adult and incubates develop into rhabditiform larva and can go to the large intestine through stool. In autoinfection, if the patient has been constipated for a long time or within 18 hours may cause automatic infection. (Autoinfection cycle) the rhabditiform larva developed into filariform larva. It is in the intestine penetrating the intestinal wall to the capillaries into the bloodstream through the liver, the heart to the lungs. It crawls into the trachea and is swallowed up into the esophagus, fights the small intestine and continues to mature as an adult, or they may disseminate widely in the body (CDC., 2019), **(Figure 2.3)**.

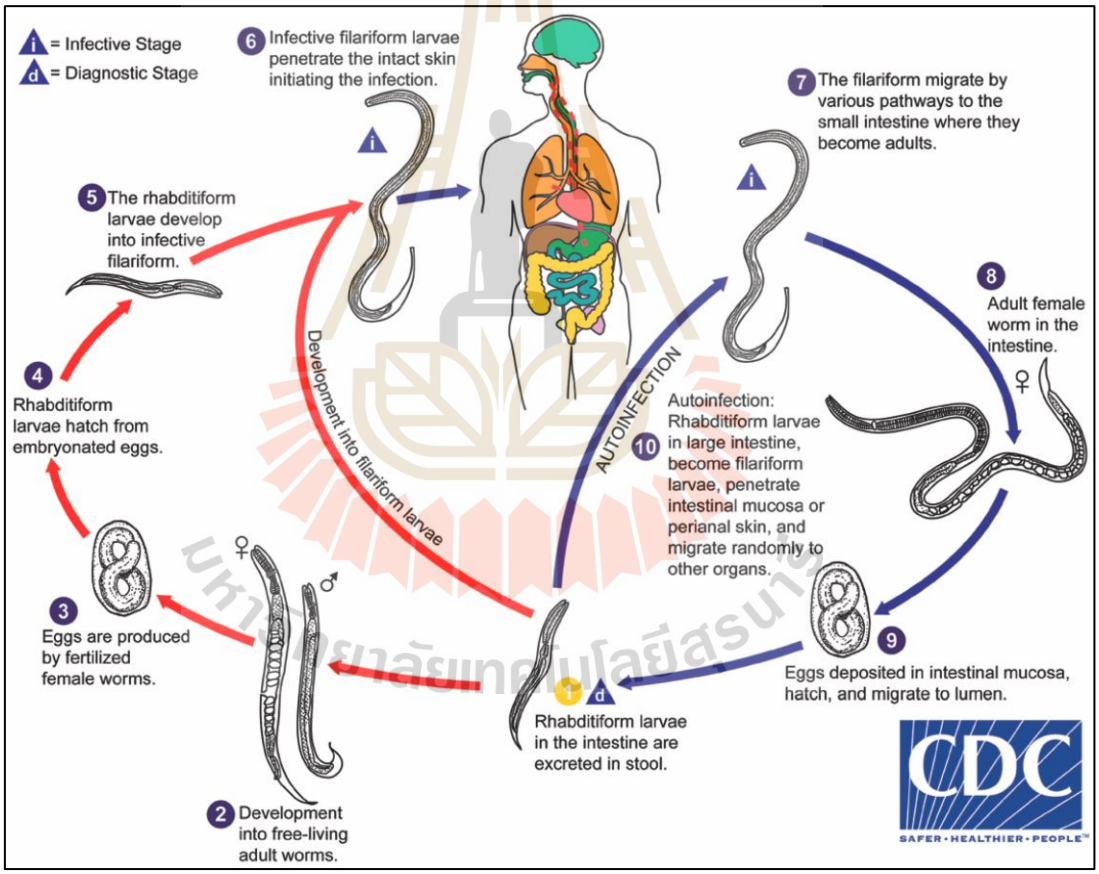

Figure 2.3 *Strongyloides stercoralis* life cycle (CDC., 2019).

Strongyloides stercoralis, a human parasite, and *Strongyloides ratti*, a rat parasite, are both members of this genus (Chavez et al. 2021).

2.1.3 Symptoms and risk groups

2.1.3.1 (Cutaneous symptoms) the filariform larva can penetrate the skin can cause mild symptoms such as itching, swelling and redness around the skin. In some cases, symptoms may last for several weeks. In some patients, if the infection is recurrent, allergic reactions may occur.

2.1.3.2 (Pulmonary symptoms) when parasitic enter the lungs in some hosts with low immunity, the patient may have pneumonia, cough with sputum, shortness of breath, fever. However, some patients do not have any symptoms.

2.1.3.3 (Intestinal symptoms) when worms enter the digestive tract, they usually have symptoms within 2 weeks after infection. It has symptoms such as nausea, vomiting, abdominal pain, constipation, diarrhea. In addition, the adult worm often lives in the intestines. It can damage the intestinal wall inflammation and affect the intestines, down the efficiency of absorption cause malnourished (Nutman 2017).

2.1.4 Risk groups requiring diagnosis

2.1.4.1 People who are receiving immunosuppressants, especially steroids and chemotherapy drugs (Muhi et al. 2022; Erdem Kivrak et al. 2017).

2.1.4.2 Patients whose cellular immunity has changed.

2.1.4.3 Tumor patient, hematologic cancer especially lymphoma leukemia.

2.1.4.4 Organ transplant recipients such as kidney transplantation.

2.1.4.5 Collagen vasculardisease.

2.1.4.6 Malnutrition.

2.1.4.7 End-stage renal disease patients.

2.1.4.8 Diabetes patients.

2.1.4.9 Elderly.

2.1.4.10 Acquired Immune Deficiency Syndrome (AIDS) patients, human immunodeficiency virus (HIV) (Sarangarajan et al. 1997).

2.1.4.11 People traveling to and from epidemic areas.

2.1.4.12 Groups of people living in confined spaces and poor environmental sanitation such as prisoners, neurological hospitals, mental retardation centers.

2.2 Epidemiology of *Strongyloides stercoralis*

2.2.1 The global prevalence

Strongyloidiasis is found worldwide, especially in hot and humid tropical areas or in areas that are not yet receiving proper healthcare assistance. This is because the helminths can be hostile and natural. It can live last several weeks and parasites can increase the number and spread a wide area. For this reason, there are reports of infectious patients commonly found.

In 2017, the estimated prevalence of *S. stercoralis* was around 613.9 million people. It was found that in Southeast Asia had the highest number of infections, approximately 237.3 million, followed by the Western Pacific Region with 133.2 million and the African with 108 million, respectively making up 76.1% of the total infected population worldwide. However, the current epidemic information in Thailand is still rare and needs further research (Buonfrate et al. 2020).

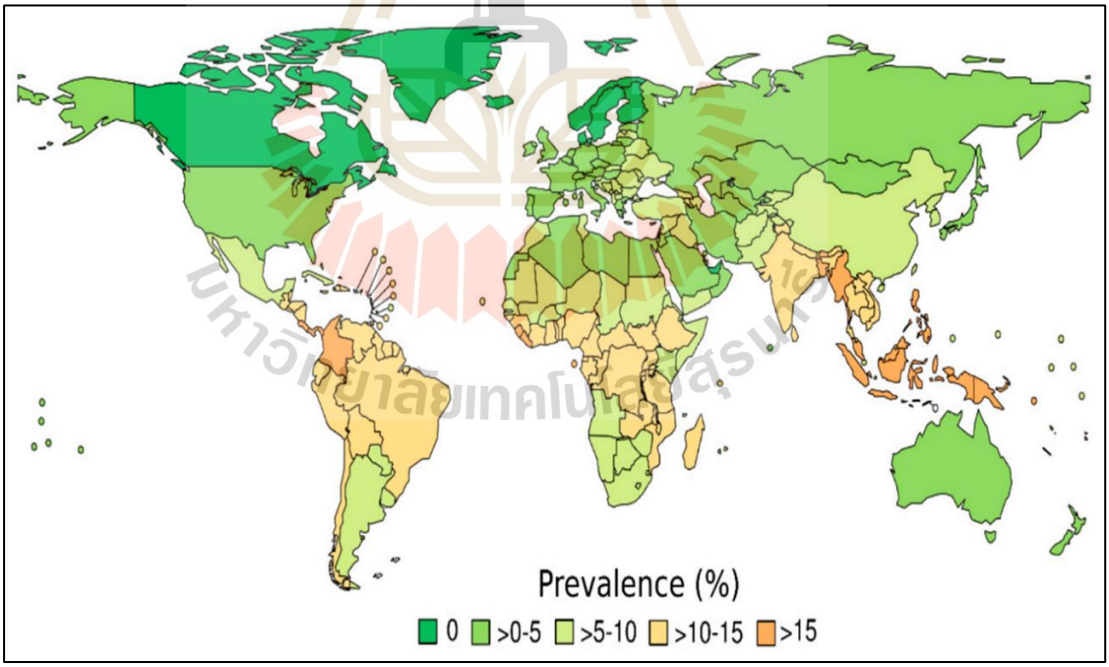

Figure 2.4 Estimate the best statistical prevalence of strongyloidiasis disease (Buonfrate et al. 2020).

2.2.2 The prevalence of helminthiasis in Thailand

A national situational survey under the name of the Department of Disease Control reported the prevalence of helminths in Thailand. According to a study of prevalence from 1957 to 2014, there was a decrease in the trend. And from the latest time, a survey was conducted in 2014 from 9,919 people in 76 provinces. The results showed that the mean prevalence of helminthiasis was 8.2%, in which one person was infected with one or more types of helminths in the same person (Division of Innovation and Research, 2019).

Previous research on the epidemiology of Strongyloidiasis in northeastern Thailand was conducted. From 2004 to 2014, prevalence data were obtained on 3889 patients (17.4%). In 2004, the prevalence incidence was 22.8%, which subsequently decreased until it was 11.2% in 2013 and remained consistent at 12.9% in 2014 (Prasongdee et al. 2017).

By using FECT technique detection of *Strongyloides stercoralis* infection in 19 provinces in northeast Thailand. Four provinces had the highest prevalence: Sisaket Province, Mukdahan Province, Roi Et Province, Kalasin Province **(Figure 5)**.

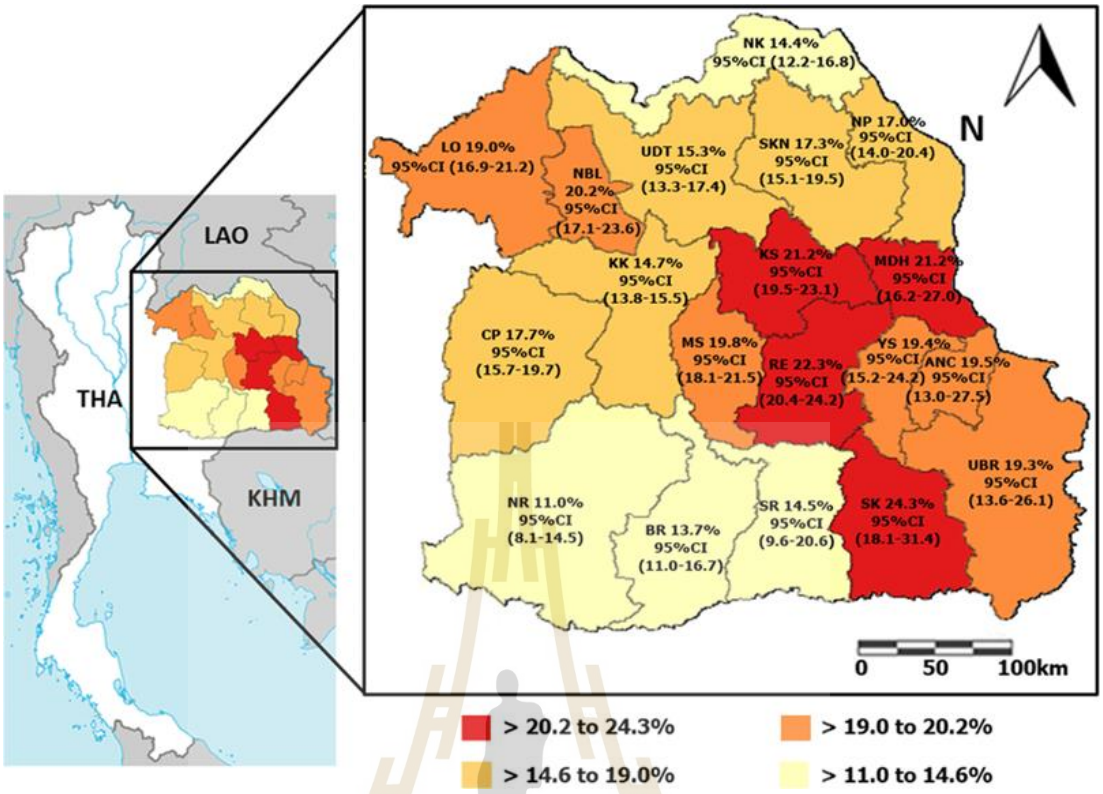

Figure 2.5 Situation of the prevalence of helminthiasis in Northeast Thailand, 2004-2014 (Prasongdee et al. 2017).

2.3 Diagnosis of strongyloidiasis

2.3.1 Parasitological techniques

In previous and present Strongyloidiasis and other parasite infection diagnostic processes, microscopy is used to detect parasite from fecal samples. However, the method has a sensitivity of only 21% and to increase the sensitivity with other concentration methods. Stool concentration methods, such as the Baermann funnel technique (72% sensitive) Formalin ether acetate concentration technique (52% sensitive) or agar plate culture (89% sensitive), were studied (Mora Carpio and Meseeha 2023). Each technique has protocols and steps as the followings.

2.3.1.1 Harada-Mori filter paper culture (HMFPC). The method is used to culture nematode larvae by incubating stool on a filter paper strip in a test tube containing water. After incubation at 24–28°C for up to 10 days, Strongyloides rhabditiform larvae migrate to the water and transform into filariform larvae. The water sediment is screened daily under a low magnification for living larvae, which should be differentiated from those of hookworm (Martín-Rabadán P et al. 1999), **(Figure 2.6)**.

Advantages: This is a method of obtaining helminths that are undiluted to the substance.

Disadvantages: This technique was time consuming, because must be incubated for at 10 days.

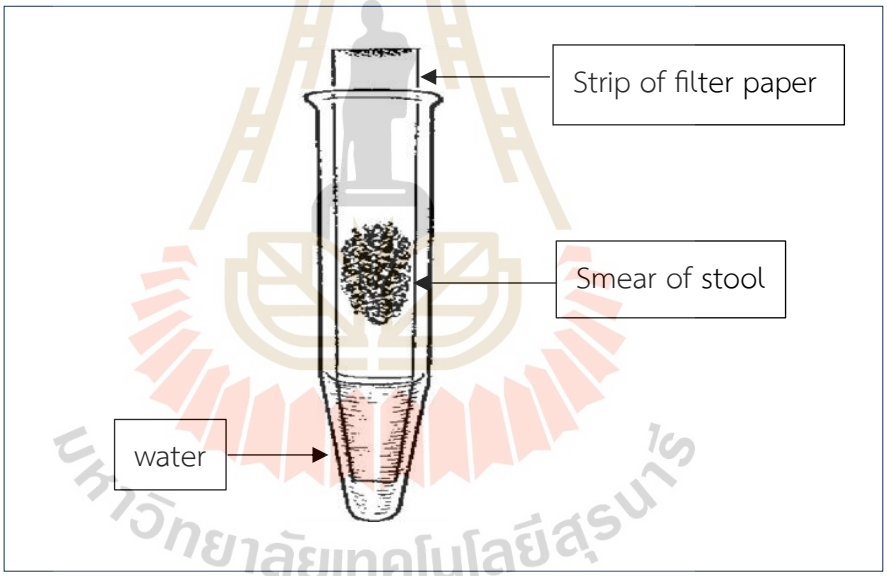

Figure 2.6 Diagram of Harada-Mori whether *Strongyloides* larvae are present (Yimam Getaneh, Abaineh Munishae, and Endalkachew Nibret. 2015).

2.3.1.2 Baermann funnel (BM). This method is used to separate larvae from stool material. The method is as follows; Measure approximately 5-10 grams of stool material place in the center of the cheesecloth and wrap the cloth. Tie the skewers to the prepared wrapping cloth so that the pouch can be suspended. Place the pouch containing the stool material in the funnel to the prepared and fit a short piece of tubing to the stem, close the tubing. Fill the funnel with lukewarm water. Leave the apparatus to stand for 24 hours. Draw off a few milliliters of fluid from the stem of the funnel into a test tube and centrifuge to collect larvae. Check sedimented sample in a petri dish for the presence of larvae (Khumpool G. 2013), **(Figure 2.7)**.

Advantages: Baermann funnel technique is more sensitive than the Formalin-ether acetate concentration technique.

Disadvantages: This technique is having labor intensive.

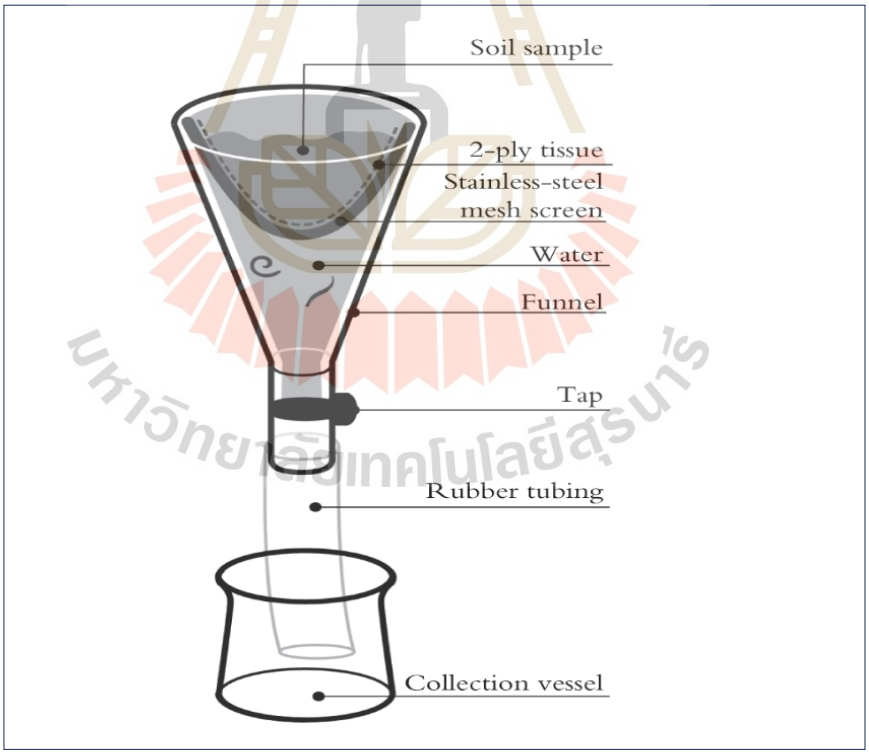

Figure 2.7 Baermann funnel technique (Cambridge University Press, 2019).

2.3.1.3 Formalin ether acetate concentration technique (FECT). Scoop approximately 3 grams of feces with 10 ml of 10% Formalin, mix well and strain. With filter sieve Place the test tube, add 3 ml of Ether and close the test tube, shake for 20 seconds. It was centrifuged at a speed of 1,500 rpm for 5 minutes. The in vitro mixture was separated into 4 layers: The Ether layer, the waste layer, the Formalin layer and the sludge. Use wood to remove waste to detach from the inner wall of the spinning tube and dumped with Ether and Formalin, leaving only the sediment. Then, fecal sediment is taken into the volume measuring platform on the Microscope Slide. Add 10% Formalin mixed with sediment and drip with 1% Iodine, cover with Cover Slip and inspect under a microscope (Prateepsuk W. 2019), **(Figure 2.8)**.

Advantages: This method has a limit for low sensitivity.

Disadvantages: The chemical component used for this technique has been considered hazardous by the US Environmental Protection Agency. Many states environmental agencies and it might also not be suitable in limited resource settings (Requena-Mendez et al. 2013).

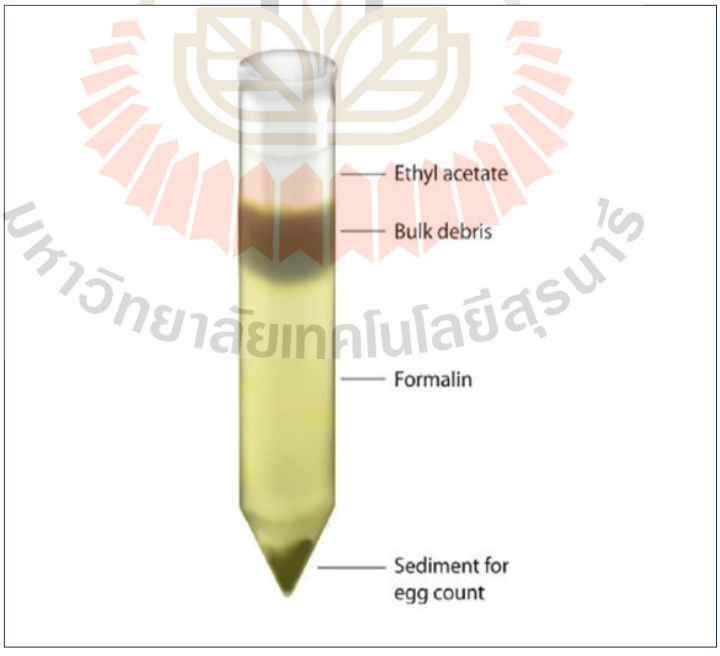

Figure 2.8 Formalin-ether acetate concentration technique (Bin Xu et al., 2012).

2.3.1.4 Koga agar plate culture (KAP). The nutrient agar was used to culture *S. stecoralis* by placing stool onto agar plate and seal plate lid. The agar plate is maintained at room temperature for at least 2 days (Khanna et al. 2015), **(Figure 2.9)**.

Advantages: This technique can be employed for isolation and preservation of *hookworm* such as *Ankylostoma duodenale*. When compared to other methods such as Harada-Mori, Bearman's techniques and agar plate culture, our technique had better yield of parasites.

Disadvantages: Lab workers or people who touch may be infected with the worms.

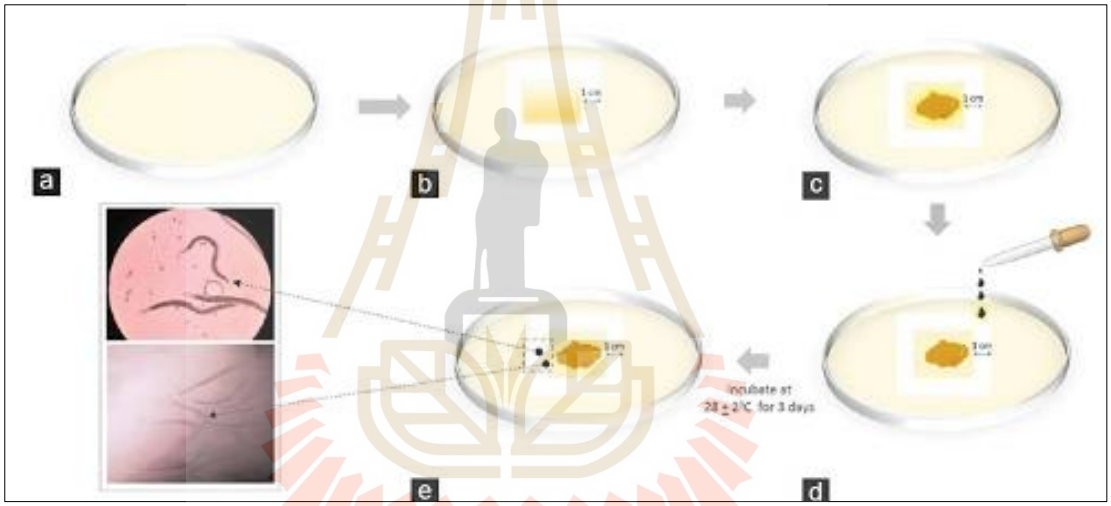

Figure 2.9 Model for *S. stercoralis* culture method: **(a)** Nutrients plate **(b)** 1 x 1 cm well cut in the nutrient agar. **(c)** Inoculation of stool at the center. **(d)** Well filled with Distilled water/Normal saline. **(e)** Microscopic observstion under low power (x10) (Khanna v *et al.,* 2015).

2.3.2 Molecular techniques

Molecular diagnostics, such as the conventional PCR-based and RT-PCR techniques, are later utilized in parasitology as a more sensitive and specific procedure, but they are not commonly available (Mora Carpio and Meseeha 2023).

Polymerase Chain Reaction (PCR) is a DNA amplification technique based on the DNA Replication principle, which is the in vitro synthesis of new DNA strands from prototype DNA in a short amount of time and the formation of a million times more new strands of DNA (Garibyan and Avashia 2013). The technique was developed in 1985 by Kary Mullis and colleagues at Cetus Corporation. Nowadays, PCR techniques have been improved and developed in many areas until it is recognized as a very important technology for molecular biomolecules. It can be utilized in both biomolecular and genetic engineering research such as gene cloning, gene sequencing, DNA probe and applied research such as mRNA gene expression studies, in vitro mutagenesis, point mutations and deletions, etc.

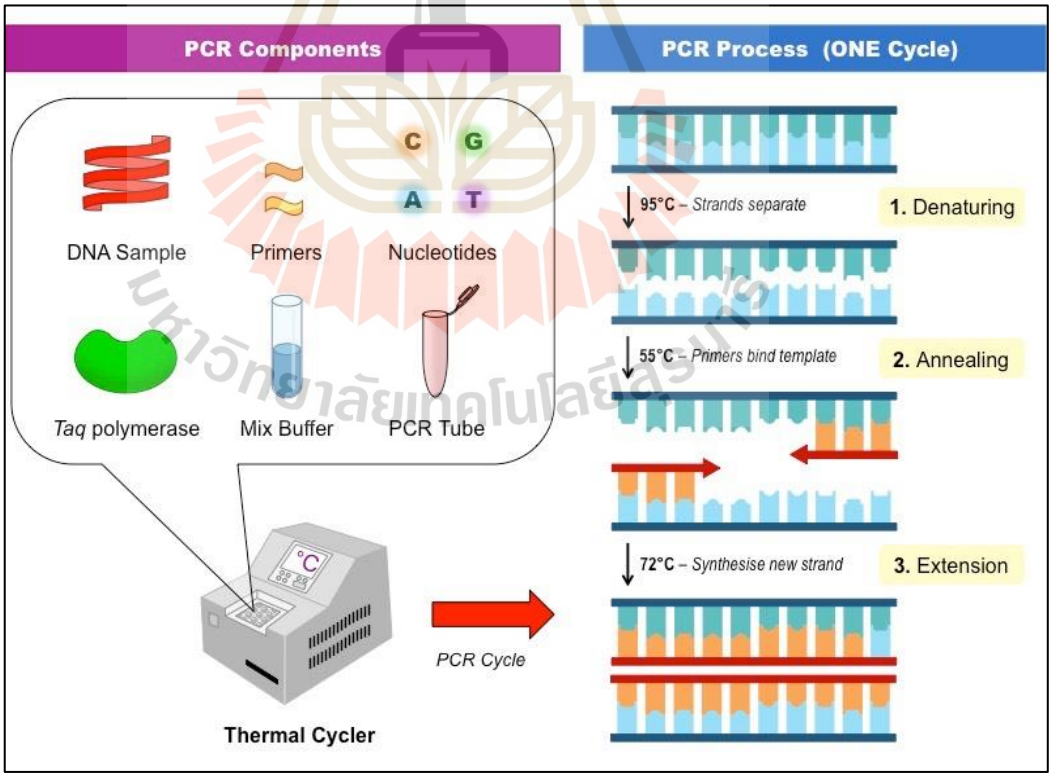

Figure 2.10 An overview of the PCR technique's work (Keagile Bati, 2018).

Advantages:

- Sensitive: PCR is a very useful technique when the amount of DNA sample is limited because it allows the detection of even a single copy of a specific DNA template (Rodu 1990).
- Versatile: The PCR technique can be used for various applications like genetic testing, criminal investigations, and paternity tests.
- Rapid and efficient: PCR can efficiently and rapidly amplify a small amount of DNA sample to million copies in just a few hours.

Disadvantages:

- Contamination: The PCR technique is very susceptible to contamination from other sources of DNA or RNA or the environment. This can mislead data interpretation.
- Cost and complexity: PCR can be expensive and requires expert knowledge for high-throughput projects.
- Lack of novel information: Since PCR can only amplify and target specific DNA sequences targeted by the primers, PCR provides limited information and cannot detect novel DNA sequences.
- Inhibition from sample content: The whole PCR cycle can be disrupted by inhibitors that co-purify with DNA, such as heme from blood samples, reducing the sensitivity of the process.
- Errors in amplification: Base substitutions, indels, and other alterations in DNA sequences can lead to inaccurate amplification and hence, false results (Garibyan and Avashia 2013).

Overall, PCR significantly impacts many research areas but careful quality measures should be performed while designing and interpreting PCR experiments.

2.3.3 Immunological techniques

At present, one strategy is being used efficiently to control strongyloidiasis by establishing a better diagnostic test, particularly using an immunological assay. Several serological tests have been reported to improve the accuracy of strongyloidiasis diagnosis (Eamudomkarn et al. 2023). The Enzyme-Linked Immunosorbent Assay (ELISA) is an immunological assay commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples. ELISA principles can be applied in experiments involving quantitative studies and the quality of antigens and antibodies, such as diagnosis of infectious diseases such as microorganisms, bacteria, viruses, parasites, fungi, microbial toxins and drugs.

2.3.3.1 Antigens and antibodies work on the same basis

In immunology, an antigen (Ag) are primarily foreign molecules in the body, an allergen, proteins, peptides (amino acid chains), antigens exist on normal cells, cancer cells, parasites, viruses, fungi, and bacteria. They stimulate the immune system to produces antibodies to remove the antigens.

Immunoglobulin (Ig), commonly known as antibodies. Plays a key role in the immune defense against pathogens. After antigen activation Antibodies secreted by B cells called plasma cells recognize specific antigens and protect against pathogens outside the body (Kapingidza, Kowal and Chruszcz 2020). The antibody is located at the top end of a "Y" shaped molecule called the epitope. The epitope binds to the antigen's paratope. This allows these two structures to bind to each other in a specific way (Ahmad et al. 2012).

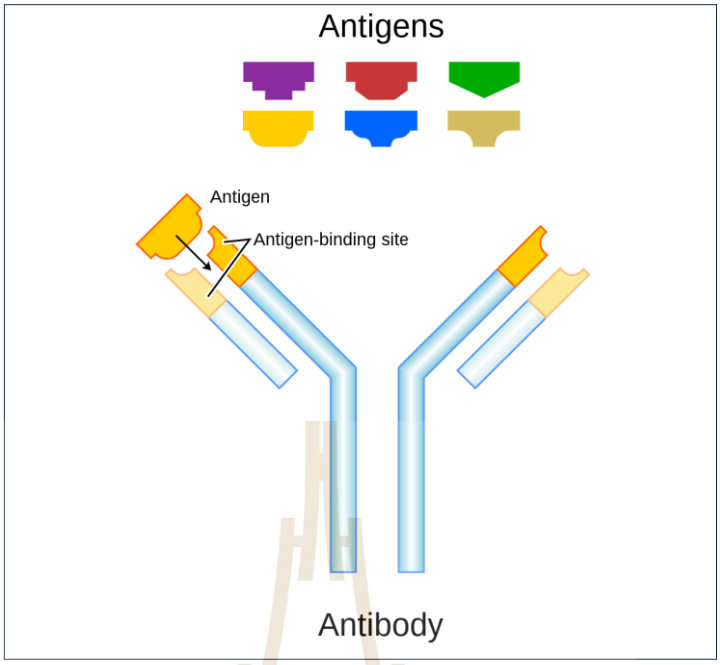

Figure 2.11 Model of antigens and antibody. An illustration that shows how antigens induce the immune system response by interacting with an antibody that matches the molecular structure of an antigen.

2.3.3.2 Enzyme-linked immunosorbent assay (ELISA)

The principle of ELISA method is to use antigen or antibody to coat the surface of ELISA plate by applying enzyme label on the antigen or antibody. Then, when the substrate is added, it reacts with the enzyme and changes color when it becomes the product. Then measure the effect of the resulting color by reading the absorbance with a microplate reader. It can check both the quantity and quality of the substance that we want to check (Tabatabaei and Ahmed 2022).

There are 4 types of ELISA testing principles:

2.3.3.2.1 Direct ELISA. In a direct ELISA, an antigen or sample is immobilized directly on the solid plate and a conjugated detection antibody binds to the target protein. Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample **(Figure 2.12).**

Advantages:

- Simple protocol, time-saving, and reagents-saving.
- No cross-reactivity from secondary antibody.

Disadvantages:

- Less specific since you are only using 1 antibody.
- Potential for high background if all proteins from a sample are immobilized in well.

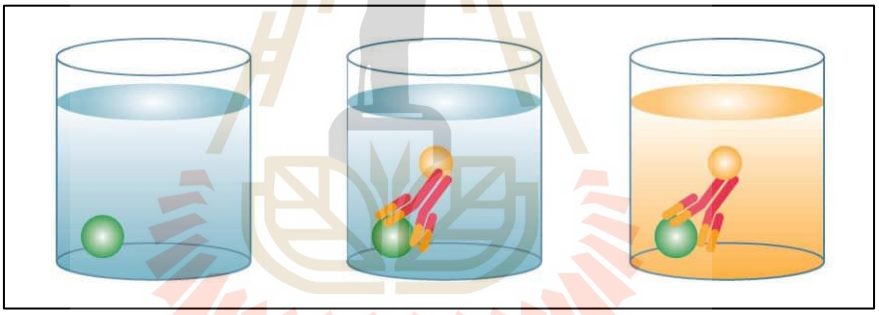

Figure 2.12 Direct ELISA assay (Bio-Techne, 2023**).**

2.3.3.2.2 Indirect ELISA. An indirect ELISA is like similar to a direct ELISA in that an antigen is immobilized on the solid plate. An unconjugated primary detection antibody is added and binds to the specific antigen. A conjugated secondary antibody directed against the host species of the primary antibody is then added. Substrate then produces a signal proportional to the amount of antigen bound in the well **(Figure 2.13)**.

Advantages:

- Signal amplification, since one or more secondary antibodies can be used to bind to the primary antibody.
- High flexibility since, since the same secondary antibody can be used for various primary antibodies.

Disadvantages:

- Complex protocol compared with direct ELISA.
- Cross-reactivity from secondary antibody.

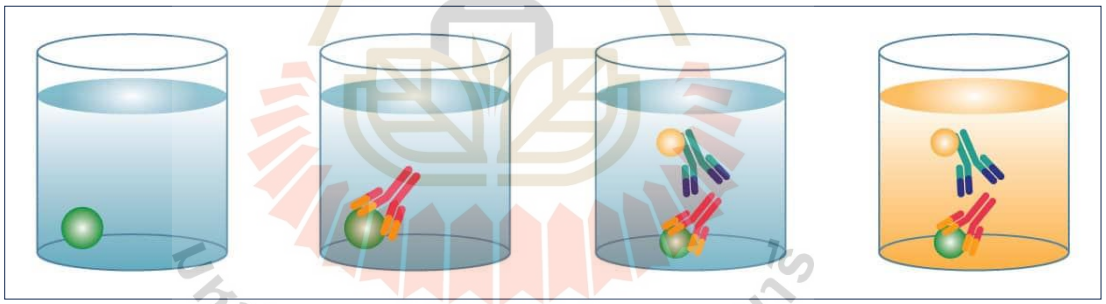

Figure 2.13 Indirect ELISA assay (Bio-Techne, 2023)**.**

2.3.3.2.3 Sandwich ELISA. Antibody was coated on the surface of the solid **plate** and react with the desired antigen. After washing, enzyme-labeled antibodies are applied. This antibody is also specific to the antigen to be tested. Wash and add the substrate and quantify the resulting color intensity **(Figure 2.14)**.

Advantages:

- High flexibility.
- High sensitivity.
- High specificity since different antibodies bind to the same antigen for detection.

Disadvantages:

- The antigen of interest must be large enough so that two different antibodies can bind to it at different epitopes.
- It's sometimes difficult to find two different antibodies that recognize different epitopes on the antigen of interest.

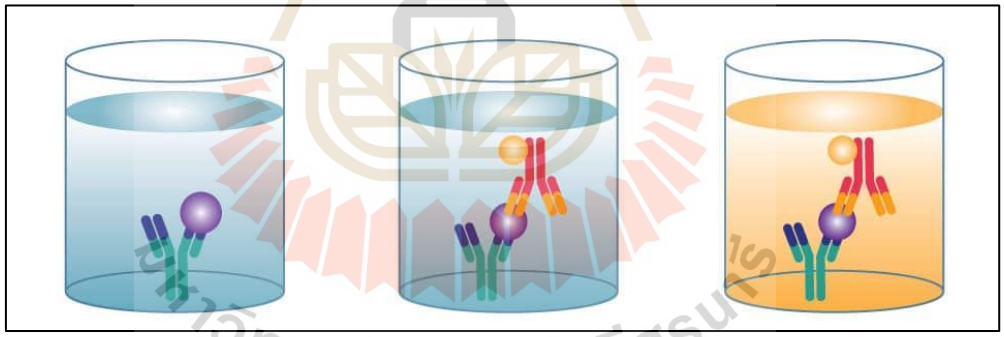

Figure 2.14 Sandwich ELISA assay **(**Bio-Techne, 2023).

2.3.3.2.4 Competitive ELISA. Competitive ELISAs are commonly used for small molecules, when the protein of interest is too small to efficiently sandwich with two antibodies. a capture antibody is coated on a microplate. Instead of using a conjugated detection antibody, a conjugated antigen is used to complete for binding with the antigen present in the sample. To detect and enzymatically labeled antigens, wash and add the substrate and quantify the resulting color intensity **(Figure 2.15)**.

Advantages:

- High flexibility.
- High sensitivity.
- Best for the detection of small antigens, even when they are present in low concentrations.

Disadvantages:

- Less specific since you are only using 1 antibody.
- Requires a conjugated antigen.

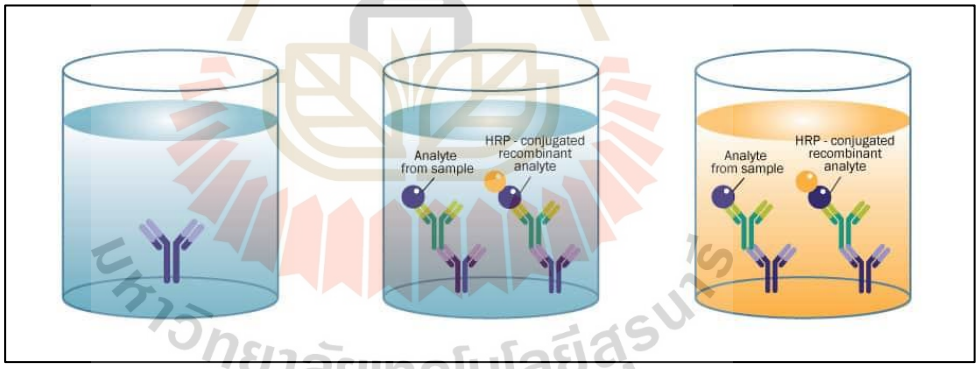

Figure 2.15 Competitive ELISA **(**Bio-Techne, 2023).

2.3.4 Experiments chosen for this study

To identify strongyloidiasis-specific antibodies indirect ELISA was used in this study. A novel fatty acid and retinol-binding port was chosen as antigen (Masoori et al. 2019). Fatty acid and retinol-binding protein is nematode-specific proteins that is involved in the development, reproduction, and infection of nematodes (Ding et al. 2019). Fatty acids are required for lipid biosynthesis, neurological functions, and parasite cuticle building. Furthermore, it has previously been discovered that fatty acid binding is abundant in the fluid surrounding the embryo in eggs and may be vital in nutritional acquisition and the maintenance of the growing larvae's eggshell; consequently, fatty acid binding is recognized as a critical molecule in larva development. Fatty acid and retinol-binding protein also engages in intracellular and intercellular lipid signaling, as well as host defense systems and nematode disease. Furthermore, fatty acid and retinol-binding protein has been demonstrated to be useful for immunodiagnostic and to have good potency in experimental vaccinology (Masoori et al. 2019). As a result, they have the potential to have a considerable impact on the host immune system.

Synthesized peptides have proven to be useful tools in the laboratory for viral diagnosis (Gomara and Haro 2007). This method can give antigens that are homogenous and chemically well-defined for antibody analysis, reduce variance both between and within experiments. The primary purpose of the peptide-based diagnostic assay was to detect distinct antibodies triggered by *S. stercoralis.* In recent years, there has been investigated into the use of two synthetic peptides, C10 and D3, that mimic specific epitopes of the infection-causing proteins of strongyloidiasis. The results revealed that the sensitivity was 95% and the specificity was 89.2% and 92.5%, respectively (Faliciano et al. 2016). The success of this method is determined by the ability of synthetic peptides to replicate immunodominant epitopes of antigens ((Gomara and Haro 2007).

The difficulty in obtaining infective *S. stercoralis* larvae is the principal restriction discovered in the standardization of more specific serological testing. As a result, standardization and the usage of heterologous antigens from *S. ratti* have been convenient (Levenhagen and Costa-Cruz 2014), because antigens can be easily and safely prepared (Eamudomkarn et al. 2015). Even more than that Masoori et al. (2019) study revealed the similarity of the inferred fatty acid and retinol-binding protein amino acid sequences with fatty acid and retinol-binding protein homologues from other nematode species, with 93% similarity between *S. stercoralis* and *S. ratti*, which was the main reason for using *S. ratti* as a control group for this experiment **(Figure 2.15)**.

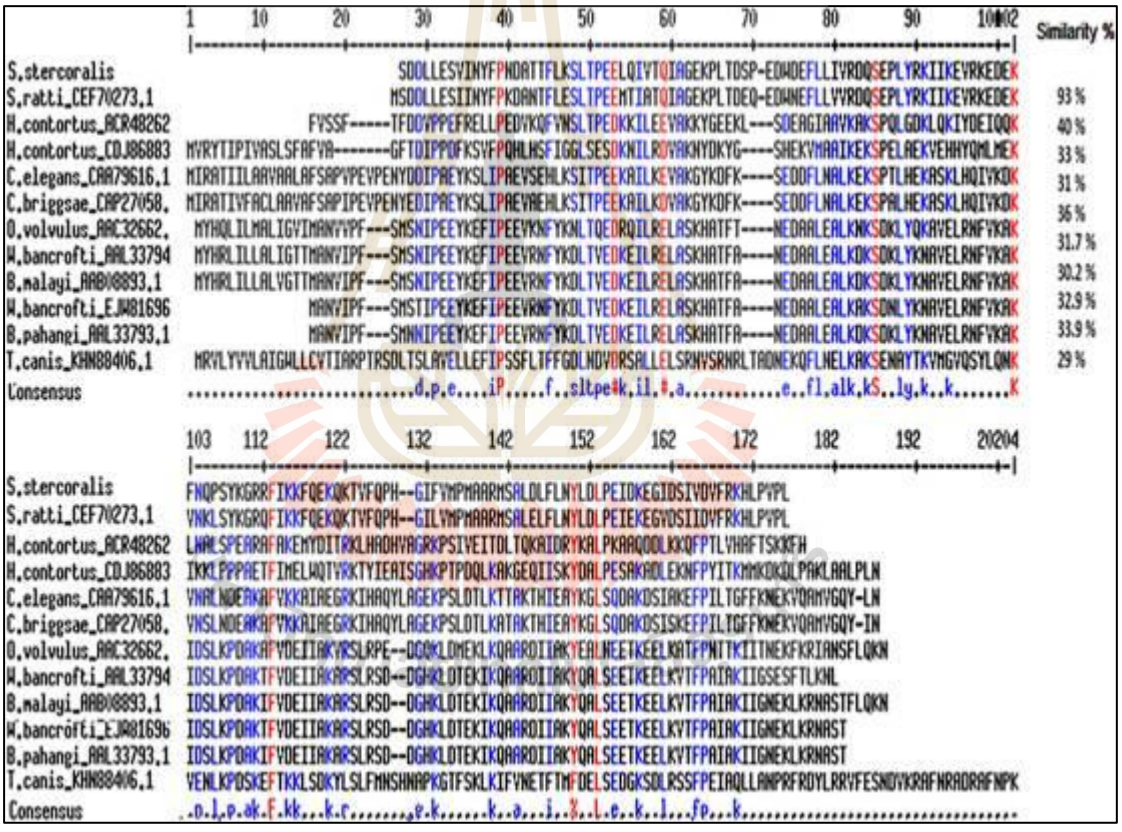

Figure 2.16 Multiple sequence alignment of fatty acid and retinol-binding protein (FAR) of *S. stercoralis* (Masoori et al. 2019).

2.5 Associated research

Journal of Fernando S., et al., (2014) say about "Usefulness of *Strongyloides stercoralis* Serology in the Management of Patients with Eosinophilia." Overall, 147 patients were included, 89 (60.5%) patients had a positive *S. stercoralis* serology. The *S. stercoralis* larvae were detected only in 15 (10.2%) patients.

Buonfrate D., et al., (2015) say about "Accuracy of five serologic tests for the follow up of *Strongyloides stercoralis* infection." The samples were tested with two commercially available ELISA tests (IVD, Bordier), two techniques based on a recombinant antigen (NIE-ELISA and NIE-LIPS) and one in-house IFAT. A high proportion of samples demonstrated for each test. In particular, IVD-ELISA, in particular (almost 90% samples demonstrated relevant decline) and IFAT (almost 87%) had the best performance. Considering only samples with a complete negativization, NIE-ELISA showed the best performance (72.5% sera-reversion).

Eamudomkarn et al. (2015) study on comparative evaluation of *S. ratti* and *S. stercoralis* larval antigen for diagnosis of strongyloidiasis in an endemic area of Opisthorchiasis. In this study, they are performance of ELISA based on *S. ratti* was compared with that based on *S. stercoralis* for diagnosis of strongyloidiasis in areas where *O. viverrini* is co-endemic in Thailand. The results showed that in total 107 individuals, 50 (46.7 %) were positive for *S. stercoralis* by agar culture method and by ELISA; 82 (76.6 %) and 81 (75.7 %) were seropositive using *S. ratti* and *S. stercoralis* antigens, respectively. The levels of parasite-specific IgG to *S. ratti* and *S. stercoralis* antigen were significantly proportionally correlated ($P < 0.001$). The findings imply that the *S. ratti* antigen is a suitable choice for diagnosing strongyloidiasis in an Opisthorchiasis endemic area, with sensitivity comparable to the *S. stercoralis* antigen, and provides a foundation for efficient strongyloidiasis control efforts.

Rutchanee R., et al., (2017) say about "Identification of antigenic proteins in *Strongyloides stercoralis* by proteomic analysis." This study reported the biochemical properties of antigenic protein spots derived from *S. stercoralis* L3 using immunoproteomic and mass spectrometry techniques. Samples were collected by Harada Mori filter paper culture, Protein extraction and protein concentration was measured, two-dimensional gel electrophoresis (2DE) was performed, and the data

was analyzed by LC-ESI - MS / MS. Twenty-six protein antigen-related points were found in Coomassie staining gel is closely related to *Haemonchus, Caenorhabditis elegans, Caenorhabditis remanei* and *Strongyloides ratti.*

Eamudomkarn C, et al., (2018) say about "Diagnostic performance of urinary IgG antibody detection: A novel approach for population screening of strongyloidiasis." They used FECT and APCT techniques to screen samples. ELISA is also used to detect antigens specific to antibodies. Find the cross reactivity to other parasites. The results showed that in the urine and serum there was a cross-reaction with *Opisthorchis viverrine 2/5, 3/5 respectively.*

Serological testing is currently being developed regarding the subject "Development of immunochromatographic device as a point-of-care tool for serodiagnosis of human strongyloidiasis cases." (Sadaow L., *et al*., 2020). They have set up an immunochromatographic (ICT) test kit for the assay. serodiagnosis Rapidly in humans with sepsis of Strongyloidiasis. The antigens used in ICT series are extracted from the embryos of *S. stercoralis*. The diagnostic efficacy of this kit was assessed using human serum samples from patients with Strongyloidiasis. healthy people and people with other parasites. A cutoff level of 0.5 or higher was used, diagnostic sensitivity, specificity, and positive and negative prognostic values at 34.4% infection prevalence were 93.3%, 83.7%, 76.7% and 95.6%, respectively. This ICT kit is easy to use and three to know the results in 15 minutes."

Masoori et al. (2019) study on Fatty acid and retinol-binding protein: A novel antigen for immunodiagnosis of human strongyloidiasis. They have designed a diagnostic method more specific to *S. stercoralis* by creating a recombinant form of this protein. RNA was extracted from *S. stercoralis* L3 and cDNA synthesis. The coding sequence of *S. stercoralis* fatty acid and retinol-binding protein (SsFAR) was cloned into a pET28a(+) vector expressed as *E. coli* BL21 and purified using ELISA and immunoblotting. To determine the specificity and sensitivity of rSsFAR using a defined set of sera. The experimental results were the cloned SsFAR has an open reading frame of 447 bp encoding 147 amino acids and a determined molecular mass of 19 kD. The SsFAR amino acid sequence was 93% identical to FAR of *S. ratti*. For

differential immunodiagnosis of strongyloidiasis, rSsFAR exhibited 100% sensitivity and 97% specificity.

Patcharaporn B., et al., (2020) say about "Effectiveness of Strongyloides Recombinant IgG Immunoreactive Antigen in Detecting IgG and IgG4 Subclass Antibodies for Diagnosis of Human Strongyloidiasis Using Rapid Immunochromatographic Tests." He developed a new rapid immunochromatography (ICT) test kit. *S. stercoralis* recombinant IgG immunoreactive antigen (GenBank: AAB97359.1; rSsIR-based ICT kit) is used for the detection of IgG and IgG4 antibodies. Result prevalence of 36.4% infection, the sensitivity and accuracy of the IgG ICT set using rSsIR is 91.7. % 83.8%, 76.4%, 94.6% and 86.7%, respectively, and the rSsIR-based IgG4 ICT series were 78.3%, 84.8%, 74.6%, 87.3% and 82.4%, respectively.

Eamudomkarn, C., et al., (2023). They studied the epidemiology of Strongyloides in Northeast Thailand. Detection of urinary anti-Strongyloides IgG by enzyme-linked immunosorbent assay (ELISA). Different antigens were including crude *S. stercoralis* antigen (Ss-ELISA), crude *S. ratti* antigen (Sr-ELISA) and recombinant NIE antigen (NIE-ELISA) and fecal examination by agar plate-culture (APCT) technique and formalin-ethyl acetate concentration technique (FECT). Urine and fecal samples were collected from 966 individuals. As a result, the prevalence rate of strongyloidiasis (58.9-65.1%) was significantly higher than that of the feces (19.7%).

According to the research, many of the serologic tests that are available are quite sensitive but cross-react with other filarial parasites, schistosomes, and Ascaris lumbricoides, decreasing the specificity of the tests. This makes it possible to see the gaps in the work. More sensitive and specific serological tests are still needed using more specific antigens. It may be a good alternative to develop a diagnosis of Strongyloidiasis.

CHAPTER III
MATERIALS AND METHODS

3.1 Ethics statement

This study was approved by Suranaree University of Technology International Standard Human Ethics Committee, with project code EC-64-57. Patient samples were identified by an anonymous code **(Appendix A)**.

3.2 Study area and specimen collection

This study is an experimental laboratory cross-sectional research. Samples were collected from volunteers in hospitals in Nakhon Ratchasima Province, including NonSung Hospital. Maharat Nakhon Ratchasima Hospital, Suranaree University of Technology Hospital. Additional serum sample was also provided by Assoc. Prof. Dr. Poom Adisakwattana in Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand. And calculate the sample size in **(Appendix B)**. Stool and blood samples were collected from a total of 78 individuals. Screening people infected with parasites by examining their stools under a microscope and collecting blood samples, where medical technicians or professional nurses collect blood samples for laboratory testing. Serum was stored at -20°C until use. The individuals were assigned to 3 groups based on the results of fecal examinations. Group 1 was *S. stercoralis*- infected (n = 12), Group 2 was infected with other parasitic (n = 37), including *Gnathostoma spinigerum* (n = 4), Trichinella spiralis (n = 4), *Necator americanus* (n = 3), *Ascaris lumbricoides* (n = 4), *Trichuris trichiura* (n = 5), *Opisthorchis viverrini* (n = 8), *Taenia spp.* (n = 8), *Enterobius vermicularis* (n = 1), and group 3 was parasite-negative (n = 29).

3.3 Peptides designing and synthesis

The amino acid sequence of fatty acid and retinol-binding protein of *S. stercoralis* was retrieved from database (accession number BBB03675.1) (Masoori et al. 2019). The position of linear B-cell epitope region was analyzed using the Immune Epitope Database (IEDB) Analysis Resource program (http://tools.iedb.org/bcell/). The amino acid sequences predicted from all linear B-cell epitope predictions were put into the BLAST program. Selecting amino acid sequences that match up to 100% similarity *S. stercoralis* in the GenBank database yielded 2 peptides. The two peptides; peptide-2 were selected and synthesized (GenScript USA, Inc. United States).

The sequences of peptide are as follows;

Peptide-1: CKSLTPEELQI VTQIAGEKPL TDSPEDWDEF

Peptide-2: CLYRKIIKEVR KEDEKFNQPS YKGRRFIKKF

Molecular weight and purity were 3519.85 and 93.1% and 3948.66 and 89.8% for peptide-1 and peptide-2, respectively.

3.4 The *S. ratti* crude extracted antigens

The *S. ratti* larvae were extracted and used as antigen. The filariform larva (L3) of *S. ratti* were generously provided from Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. The *S. ratti* crude extracted antigens were prepared according to the protocol previously described (Eamudomkarn et al., 2018). Briefly, the dead worms of *S. ratti* were washed in normal saline and stored at -20 °C. The larvae were dispersed in 2 ml of phosphate-buffered saline (PBS) pH 7.4, frozen them at -76 °C for 30 minutes and thawed, repeat again 4-5 times. Then, created a subsequent disruption of L3 by Ultrasonic Processors (Cole Parmer-CPX 750, Inc. USA) and homogenized, stored overnight at 4°C, then centrifuged at 15,000×g for 30 minutes at 4°C. The supernatant was taken. The crude protein extracted was measure using NanoDrop (Thermo scientific, Inc. USA) and kept at -20°C until use **(Appendix D)**.

3.5 Titration on a checkerboard to optimize ELISA immunoassays

The peptides–antibodies interactions were examined using the checkerboard method in the 96-well plate. A recommended starting concentration of antigen was previously described by (Eamdomkarn et al. 2023; Umair et al. 2016). Five µg/mL of peptide 1, peptide 2 in 0.05 M carbonate buffer pH 9.6 were individually immobilized into microtiter plate, kept at 4°C overnight. The coated plates were washed with washing buffer (1xPBS pH 7.4, containing 0.05% Tween-20) one time. After that, the plates were blocked with 1% Bovine Serum Albumin (BSA) (© Capricorn Scientific 2023, Inc. USA) in 1X PBS pH 7.4 for 1 hour at room temperature. After washing three times, positive and negative serum samples were diluted (1:10, 1:100, 1:1000) and duplicate, in the abovementioned sample diluent, diluted sera were transferred to coated plates at 100 µl per well and the plate was incubated for 2 hours at room temperature. After washing 4 times, 100 µl/well of 1:3,000 peroxidase-conjugated goat anti-human IgG was added into each wells, incubated for 1 hour at room temperature. Repeat the washing steps and add 100 µl/well ABTS® Peroxidase Substrate (KPL, Inc. USA) kept for 15 minutes at room temperature in the dark. Add 100 µl/well of 1% Sodium Dodecyl Sulfate (SDS, Affymetrix, USA) in 1X PBS pH 7.4 for Stop the reaction and read OD at *405 nm* **(Appendix D)**.

Table 3.1 A microtiter plate with checkerboard to estimate interaction of serum samples.

	Positive of *S. stercoralis*						Negative parasite					
	1	2	3	4	5	6	7	8	9	10	11	12
A	1:10	1:100	1:1000	1:10	1:100	1:1000	1:10	1:100	1:1000	1:10	1:100	1:1000
B	1:10	1:100	1:1000	1:10	1:100	1:1000	1:10	1:100	1:1000	1:10	1:100	1:1000
C	Blank	Blank	Blank	Blank								
D												
E												
F												
G												
H												

Note: = Peptide-1, = Peptide-2

3.6 The enzyme-linked immunosorbent assay (ELISA) condition optimization

Five µg/mL of peptide-1, peptide-2 and *S. ratti* crude extracted in 0.05 M carbonate buffer pH 9.6 were individually immobilized into microtiter plate, kept at 4°C overnight. Duplicate wells were performed of each antigen. The proper blocking reagent, buffer, concentration of secondary antibody was determined. Briefly, the coated plates were washed with washing buffer (1xPBS pH 7.4, containing 0.05% Tween-20) one time. After that, the plates were blocked with 1% BSA in 1X PBS pH 7.4 for 1 hour at room temperature. After washing three times, 1:100 diluted serums of each patient were added individually into each wells and incubated for 2 hours. After washing 4 times, 100 µl/well of 1:3,000 peroxidase-conjugated goat anti-human IgG was added into each wells, incubated for 1 hour at room temperature. Repeat the washing steps and add 100 µl/well ABTS® Peroxidase Substrate kept for 15 minutes at room temperature in the dark. Add 100 µl/well 1% SDS in 1X PBS pH 7.4 for Stop the reaction and read OD at *405 nm*.

3.7 Statistical analysis

The receiver operating characteristic curve (ROC) were used to evaluate the diagnostic performance of peptide antigens. The area under the curve (AUC), the best cut-off point, sensitivity, specificity was calculated using MedCalc software version 20.218 (MedCalc Software, Ostend, Belgium). Probability (P) values < 0.05 were regarded as significant and 95% confidence intervals (CI 95%) were provided. Analysis Of Variance (ANOVA) was used to determine differences in peptide reactivity among groups. Statistical analyses were performed with IBM SPSS Statistics version 26 (© Copyright IBM Corporation Institute, USA). Automatically added multiple pairwise comparisons analysis using GraphPad Prism software 9.5.1 (GraphPad Software Inc., San Diego, USA).

CHAPTER IV

RESULTS

4.1 Molecular Immunology Database of the peptides antigen

4.1.1 Predicting Linear B-Cell Epitopes

Identification of B-cell epitopes in target antigens is one of the key steps in epitope-driven subunit peptides design, immunodiagnostic tests, and antibody production. It can be seen that the method for predicting the epitope of successive antibodies from the FAR protein of *S. stercoralis* sequence obtained (Masoori et al. 2019) gives us know the location of the B-cell epitope. In general, a window size of 5 to 7 is appropriate for finding regions that may potentially be antigenic. The higher the score assigned to a residue is, the more likely it belongs to a linear B-cell epitope **(Figure 4.1)**, **(Appendix C)**. In addition, only the short peptide fragments were predicted, and we were only interested in selecting the two longest peptide fragments **(Table 4.1).**

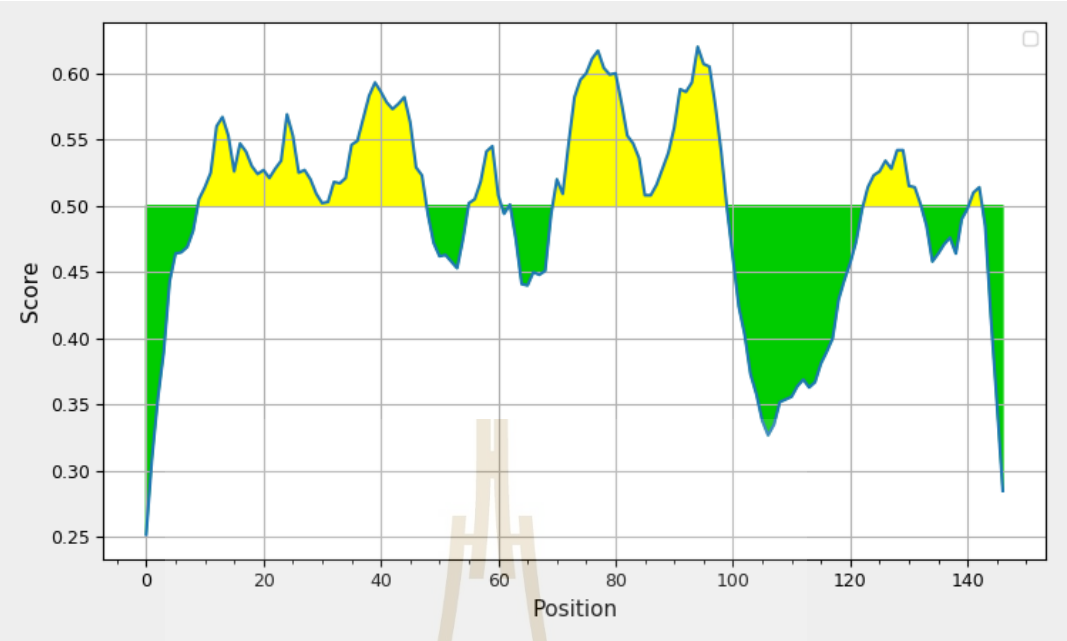

Figure 4.1 Propensity scale profiles for fatty acid and retinol-binding protein of *S. stercoralis* regions with scores above the yellow graph are more likely to contain linear B-cell epitopes. On the graphs, the Y-axes depicts for each residue the correspondent score, while the X-axes depicts the residue positions in the sequence.

Table 4.1 Predicted peptides

No.	Start	End	Peptide	Length
1	10	48	NYFPNDATTFLKSLTPEELQIVTQIAGEKPLTDSPEDWD	39
2	56	61	DQSEPL	6
3	63	63	R	1
4	71	99	KEDEKFNQPSYKGRRFIKKFQEKQKTVFQ	29
5	124	133	LPEIDKEGID	10
6	142	143	HL	2

4.1.2 The Basic Local Alignment Search Tool (BLAST)

BLAST finds regions of similarity between biological sequences. The program compares protein sequences to sequence databases and calculates the statistical significance.

Sequence Peptide 1 is KSLTPEELQI VTQIAGEKPL TDSPEDWDEF

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	immunogenic fatty acid and retinol binding protein [Strongyloides stercoralis]	Strongyloides st…	100	100	100%	5e-22	100.00%	147	BBB03675.1
☐	Nematode fatty acid retinoid binding family-containing protein [Strongyloides ratti]	Strongyloides ratti	74.0	74.0	96%	8e-13	79.31%	148	XP_024507379.1
☐	DNA-directed DNA polymerase I [Candidatus Bathyarchaeota archaeon]	Candidatus Bat…	39.7	39.7	73%	0.75	50.00%	884	RLI27215.1
☐	DNA mismatch repair ATPase msh1 [Mortierella sp. AM989]	Mortierella sp. A…	38.8	38.8	76%	1.5	50.00%	1074	KAF9109420.1
☐	GMP synthase [Halomonas sp.]	Halomonas sp.	38.4	38.4	86%	2.0	60.71%	254	MAZ06469.1
☐	hypothetical protein DFH09DRAFT_1314576 [Mycena vulgaris]	Mycena vulgaris	38.4	38.4	40%	2.1	83.33%	790	KAJ6565788.1
☐	hypothetical protein [Pirellulaceae bacterium]	Pirellulaceae ba…	38.0	38.0	76%	2.9	60.87%	392	MCH2125027.1
☐	hypothetical protein [Luteimonas panaciterrae]	Luteimonas pan…	37.1	37.1	80%	5.5	62.50%	235	WP_226467225.1
☐	hypothetical protein [Candidatus Omnitrophota bacterium]	Candidatus Om…	37.1	37.1	43%	5.7	84.62%	522	MDD4910604.1
☐	hypothetical protein C0604_09835 [Clostridiales bacterium]	Clostridiales bac…	37.1	37.1	93%	5.7	52.94%	705	PLX29444.1
☐	HD domain-containing protein [Candidatus Micrarchaeota archaeon]	Candidatus Micr…	37.1	37.1	80%	5.7	53.85%	930	MCC7571559.1
☐	hypothetical protein [Syntrophomonadaceae bacterium]	Syntrophomona…	36.7	36.7	80%	7.1	42.86%	85	MBC7075948.1
☐	cell division protein SepF [bacterium]	bacterium	36.7	36.7	90%	7.6	51.85%	184	MBT5855396.1
☐	formate dehydrogenase subunit alpha [Azospirillum soli]	Azospirillum soli	36.7	36.7	60%	8.0	66.67%	962	WP_209875793.1
☐	hypothetical protein SynA15127_01056 [Synechococcus sp. A15-127]	Synechococcus …	36.3	36.3	86%	10	45.95%	100	QNI94138.1
☐	antitoxin YezG family protein [Thermobifida alba]	Thermobifida alba	36.3	36.3	80%	11	58.33%	156	WP_248591035.1
☐	hypothetical protein [Bdellovibrionales bacterium]	Bdellovibrionale…	36.3	36.3	96%	11	62.07%	183	MCB0333089.1
☐	hypothetical protein [Chryseobacterium shandongense]	Chryseobacteriu…	36.3	36.3	60%	11	66.67%	183	WP_123850794.1
☐	SDR family NAD(P)-dependent oxidoreductase [Acidimicrobiaceae bacterium]	Acidimicrobiace…	36.3	36.3	76%	11	57.14%	241	MCY4422423.1
☐	glycoside hydrolase family 15 protein [Kitasatospora sp. MY 5-36]	Kitasatospora s…	36.3	36.3	76%	11	60.87%	243	WP_049661656.1
☐	selenium metabolism-associated LysR family transcriptional regulator [Oscillibacter hominis]	Oscillibacter ho…	36.3	36.3	76%	11	56.52%	317	WP_187333828.1
☐	glycoside hydrolase family 15 protein [Kitasatospora sp. MY 5-36]	Kitasatospora s…	36.3	36.3	76%	11	60.87%	331	WP_234348294.1

immunogenic fatty acid and retinol binding protein, partial [Strongyloides stercoralis]

Sequence ID: BBB03675.1 Length: 147 Number of Matches: 1

Range 1: 21 to 50 GenPept Graphics Next Match Previous Match

Score	Expect	Identities	Positives	Gaps
100 bits(229)	5e-22	30/30(100%)	30/30(100%)	0/30(0%)

```
Query  1   KSLTPEELQIVTQIAGEKPLTDSPEDWDEF  30
           KSLTPEELQIVTQIAGEKPLTDSPEDWDEF
Sbjct  21  KSLTPEELQIVTQIAGEKPLTDSPEDWDEF  50
```

Figure 4.2 The statistics of local sequence comparison from peptide-1 sequence. The results for the similarity between peptide-1 and *S. stercoralis* showed about 100% identity.

Sequence Peptide 2 is LYRKIIKEVR KEDEKFNQPS YKGRRFIKKF

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
immunogenic fatty acid and retinol binding protein [Strongyloides stercoralis]	Strongyloides sterc…	102	102	100%	9e-23	100.00%	147	BBB03675.1
Nematode fatty acid retinoid binding family-containing protein [Strongyloides ratti]	Strongyloides ratti	80.0	80.0	100%	7e-15	86.67%	148	XP_024507379.1
toll/interleukin-1 receptor domain-containing protein [uncultured Nostoc sp.]	uncultured Nostoc sp.	40.9	40.9	80%	0.27	51.61%	597	WP_298906710.1
hypothetical protein J6590_025018 [Homalodisca vitripennis]	Homalodisca vitripe…	39.2	39.2	60%	1.1	56.00%	1081	KAG8302743.1
LOW QUALITY PROTEIN: vigilin [Macrosteles quadrilineatus]	Macrosteles quadrili…	39.2	39.2	60%	1.1	56.00%	1240	XP_054285585.1
vigilin [Homalodisca vitripennis]	Homalodisca vitripe…	39.2	39.2	60%	1.1	56.00%	1248	XP_046678921.1
ATP-binding protein [Antarcticibacterium]	Antarcticibacterium	38.8	38.8	83%	1.5	56.00%	804	WP_139066408.1
uncharacterized protein LOC111126952 [Crassostrea virginica]	Crassostrea virginica	38.4	38.4	43%	2.0	84.62%	161	XP_022327613.1
prephenate dehydrogenase/arogenate dehydrogenase family protein [Ignicoccus islandicus]	Ignicoccus islandicus	37.1	37.1	60%	5.6	73.68%	348	WP_075050188.1
SAM-dependent methyltransferase [Nitrospirota bacterium]	Nitrospirota bacterium	36.7	36.7	43%	7.3	84.62%	113	MBI4680521.1
PH domain-containing protein [Abyssisolibacter fermentans]	Abyssisolibacter fer…	36.7	36.7	43%	7.4	76.92%	128	WP_066498175.1
ribosomal protein S3 [Heterostelium pallidum]	Heterostelium pallid…	36.7	36.7	100%	7.9	54.84%	389	YP_209585.1
zinc finger protein 300-like isoform X2 [Microcaecilia unicolor]	Microcaecilia unicolor	36.7	36.7	60%	7.9	38.46%	574	XP_030060946.1
zinc finger protein 300-like isoform X1 [Microcaecilia unicolor]	Microcaecilia unicolor	36.7	36.7	60%	8.0	38.46%	596	XP_030060936.1
alanyl-tRNA synthetase [Candidatus Paceibacter sp.]	Candidatus Paceib…	36.7	36.7	46%	8.0	73.33%	638	MCC2630868.1
zinc finger protein 2 homolog [Geotrypetes seraphini]	Geotrypetes seraphini	36.7	36.7	60%	8.0	38.46%	687	XP_033775123.1
helicase-exonuclease AddAB subunit AddB [Lachnospiraceae bacterium]	Lachnospiraceae b…	36.7	36.7	53%	8.0	76.47%	1132	MDE6313259.1
glutamine ABC transporter ATP-binding protein [Opitutae bacterium SCGC AG-212-L18]	Opitutae bacterium…	36.3	36.3	86%	11	55.17%	261	OAI50229.1
ATP-dependent DNA helicase RecQ [Melioribacter roseus]	Melioribacter roseus	36.3	36.3	50%	11	68.75%	847	WP_014856588.1
hypothetical protein [Hydrogenobacter hydrogenophilus]	Hydrogenobacter h…	35.8	35.8	93%	13	53.57%	65	WP_096603088.1
flavin reductase family protein [Streptococcus sanguinis]	Streptococcus sang…	35.8	35.8	50%	15	52.00%	191	WP_002922108.1
flavin reductase family protein [Streptococcus sanguinis]	Streptococcus sang…	35.8	35.8	50%	15	52.00%	191	WP_061589262.1

immunogenic fatty acid and retinol binding protein, partial [Strongyloides stercoralis]

Sequence ID: BBB03675.1 Length: 147 Number of Matches: 1

Range 1: 61 to 90 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Positives	Gaps
102 bits(234)	9e-23	30/30(100%)	30/30(100%)	0/30(0%)

```
Query  1   LYRKIIKEVRKEDEKFNQPSYKGRRFIKKF  30
           LYRKIIKEVRKEDEKFNQPSYKGRRFIKKF
Sbjct  61  LYRKIIKEVRKEDEKFNQPSYKGRRFIKKF  90
```

Figure 4.3 The statistics of local sequence comparison from peptide-2 sequence. The results for the similarity between peptide-2 and *S. stercoralis* showed about 100% identity representation as well.

After the use of *S. ratti* as a control group. We notice suitability as a control group alignment to determine by (Masoori et al. 2019), showing that *S. ratti* (sequence ID: CEF70273.1) is similar with *S. stercoralis* (sequence ID: BBB03675.1) about 93% **(Figure 2.15)**. When attempting to locate the similarity between the peptides and the direct amino acid sequence of *S. ratti*, the similarity between *S. ratti* and peptide-1 was found to be about 79% **(Figure 4.4)** and compared to peptide-2, the similarity was about 87% **(Figure 4.5)**.

unnamed protein product

Sequence ID: Query_44765 Length: 30 Number of Matches: 1

Range 1: 2 to 30 Graphics ▼ Next Match

Score	Expect	Identities	Positives	Gaps
74.0 bits(167)	3e-24	23/29(79%)	25/29(86%)	0/29(0%)

```
Query  2   SLTPEEMTIATQIAGEKPLTDEQEDWNEF  30
           SLTPEE+ I TQIAGEKPLTD  EDW+EF
Sbjct  2   SLTPEELQIVTQIAGEKPLTDSPEDWDEF  30
```

Figure 4.4 Comparison of amino acid sequence positions between *S. ratti* with peptide-1

unnamed protein product

Sequence ID: Query_62403 Length: 30 Number of Matches: 1

Range 1: 1 to 30 Graphics ▼ Next Match

Score	Expect	Identities	Positives	Gaps
80.0 bits(181)	1e-26	26/30(87%)	26/30(86%)	0/30(0%)

```
Query  1   LYRKIIKEVRKEDEKVNKLSYKGRQFIKKF  30
           LYRKIIKEVRKEDEK N  SYKGR FIKKF
Sbjct  1   LYRKIIKEVRKEDEKFNQPSYKGRRFIKKF  30
```

Figure 4.5 Comparison of amino acid sequence positions between *S. ratti* with peptide-2**.**

4.2 Standardization of serum ELISA

ELISA used serial dilution of *S. stercoralis*-positive serum. The maximum dilution of serum that retained OD405 above the cut-off value of not more than 1 was 1:100 **(Figure 4.6** and **Figure 4.7)**.

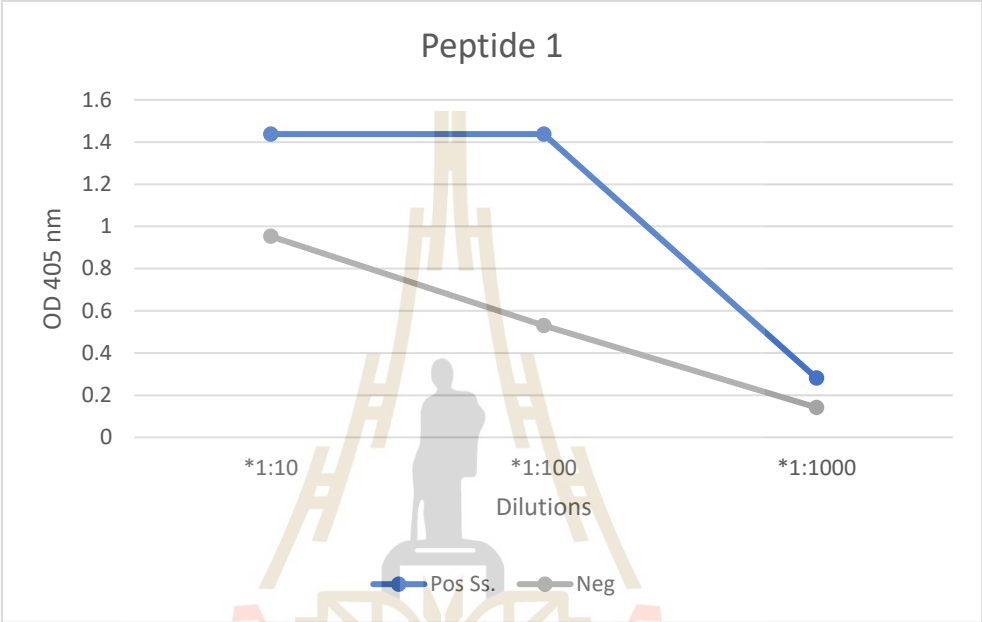

Figure 4.6 Results of checkerboard titration assays used to optimize peptide-1 concentrations and serum dilution. Serial dilutions from 1:10 to 1:1000 of *S. stercoralis* positive serum and negative serum were tested by peptide 1 ELISA.

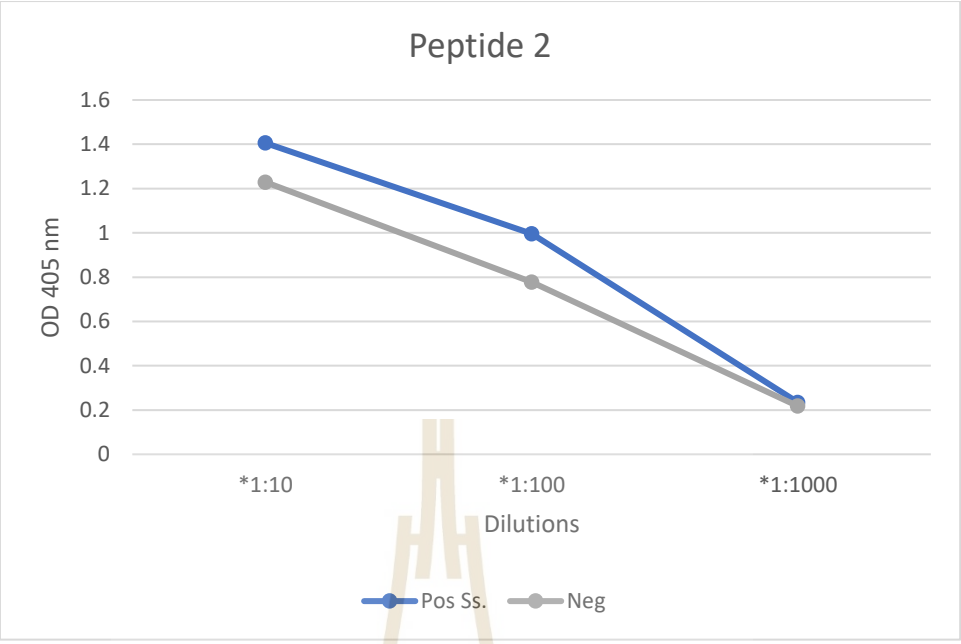

Fig 4.7 Results of checkerboard titration assays used to optimize peptide-2 concentrations and serum dilution. Serial dilutions from 1:10 to 1:1000 of *S. stercoralis* positive serum and negative serum were tested by peptide-2 ELISA.

4.3 The receiver operating characteristic curve (ROC) of three antigen groups

A ROC curve is a plot of the true positive rate (Sensitivity) in the function of the false positive rate (100-Specificity) for different cut-off points between the *S. stercoralis*-infected and non-infected parasites groups of the peptide-1. The ROC curve showed the value of AUC of about 0.724 with sensitivity of about 75% and specificity of about 75.86%. It demonstrates that the test's AUC overall diagnostic accuracy meets acceptable criteria **(Figure 4.8)**. The cut-off optical density, positive and Negative Predicted Values (PPV and NPV), positive and negative likelihood ratios (LR+, LR-) and intersections are shown in **(Table 4.2)**.

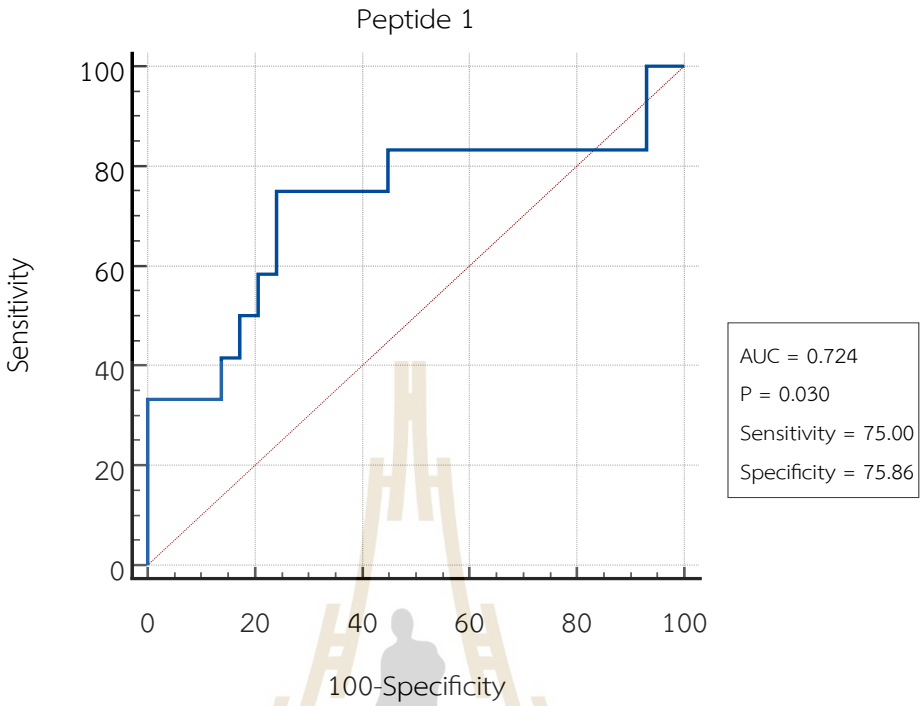

Figure 4.8 ROC curve of peptide-1. Select between serum samples from patients with strongyloidiasis (Ss., n=12) and negative parasitic infections (Neg, n=29) analyzed by ELISA. Receiver operating characteristic curve (ROC) indicating area under the curve (AUC), sensitivity (Se) and specificity (Sp), and p-value 0.05. The empirical ROC curve that is obtained by joining the points represented by the sensitivity and 100−specificity for the different cut points and the chance diagonal represented by the 45-degree line drawn through the coordinates 0,0 and 1,1.

The ROC curve of peptide-2 shown the value of AUC about 0.632 with sensitivity of 50.00 % and specificity of 86.21 %. The overall diagnostic accuracy of the test's AUC has been demonstrated to be within an almost acceptable range **(Figure 4.9)**.

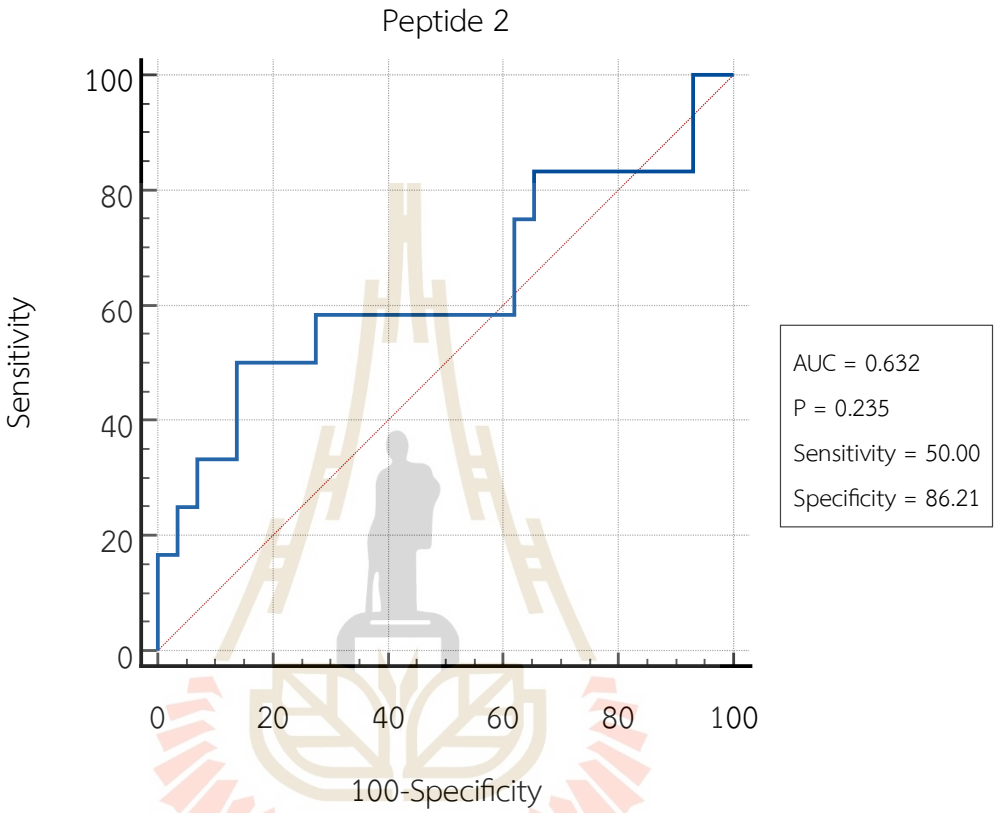

Figure 4.9 ROC curve of peptide-2. Select between serum samples from patients with strongyloidiasis (Ss., n=12) and negative parasitic infections (Neg, n=29) analyzed by ELISA. Receiver operating characteristic curve (ROC) indicating area under the curve (AUC), sensitivity (Se) and specificity (Sp), and p-value 0.05.

ROC curve combines peptide-1 with peptide-2 shown the value of AUC about 0.694 with sensitivity of 66.70 % and specificity of 72.41 %. The overall diagnostic accuracy of the test's AUC has been demonstrated to be within an almost acceptable range **(Figure 4.10)**.

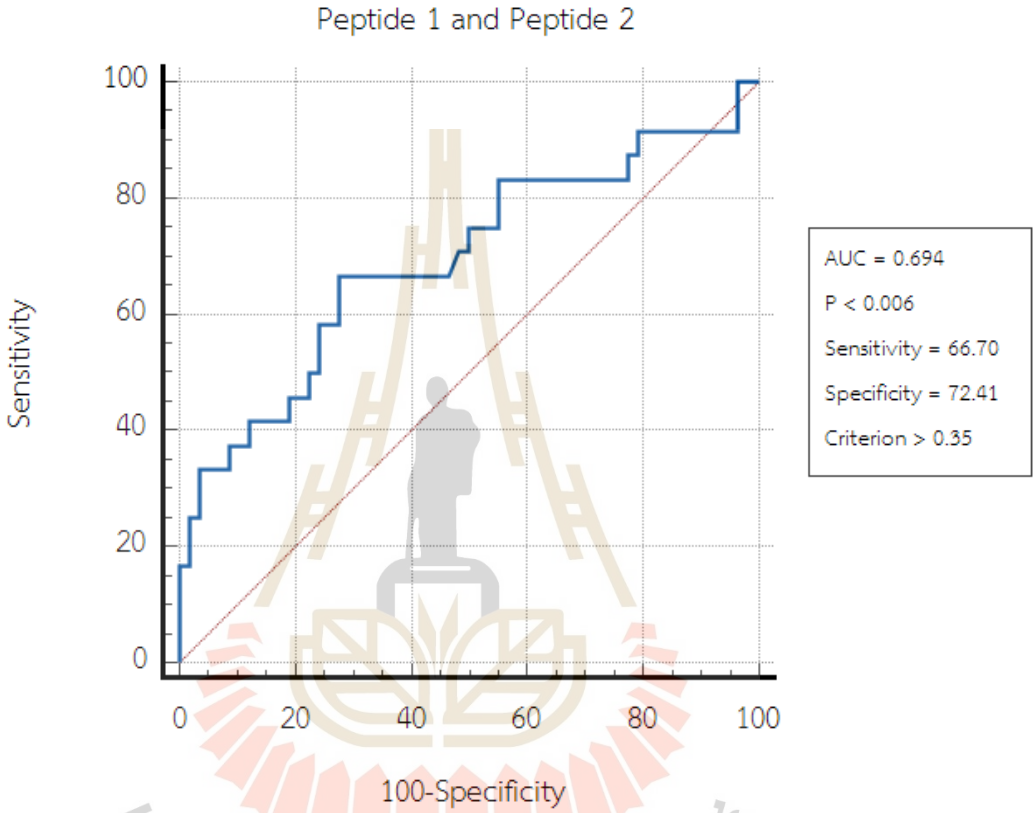

Figure 4.10 ROC curve combines peptide-1 with peptide-2. Select between serum samples from patients with strongyloidiasis (Ss., n=24) and negative parasitic infections (Neg, n=58) analyzed by ELISA.

The ROC curve of *S. ratti* antigen show the value of AUC about 0.825 with sensitivity of 75.00 % and specificity of 89.66 %. The overall diagnostic accuracy of the AUC of the test has been demonstrated to be within the excellent range **(Figure 11)**.

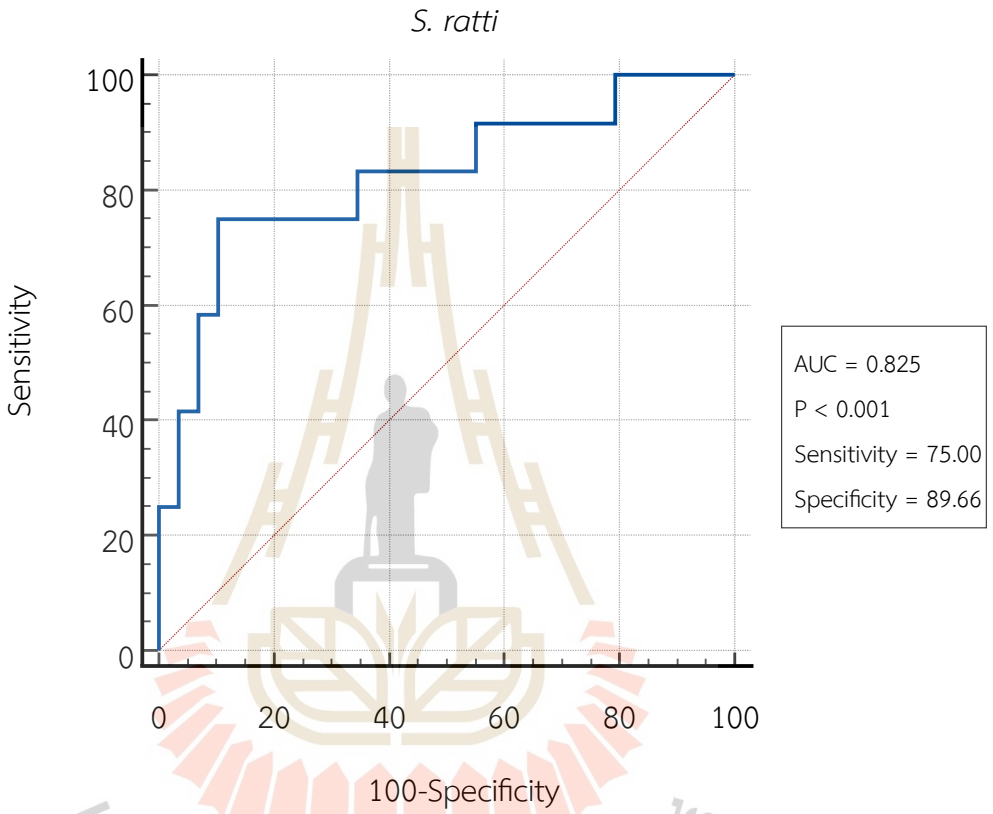

Figure 4.11 ROC curve of *S. ratti* antigen. Select between serum samples from patients with strongyloidiasis (Ss., n=12) and negative parasitic infections (Neg, n=29) analyzed by ELISA. Receiver operating characteristic curve (ROC) indicating area under the curve (AUC), sensitivity (Se) and specificity (Sp), and p-value 0.05.

The ROC curve (AUC) shows the comparison between the efficacy of peptide-1, peptide-2 and *S. ratti* antigen in *S. stercoralis*-infected and non-infected parasites groups. It could be that the *S. ratti* curve had a higher AUC than that of peptide-1 and peptide-2, but that peptide-1 was within tolerance as well **(Figure 4.12)**.

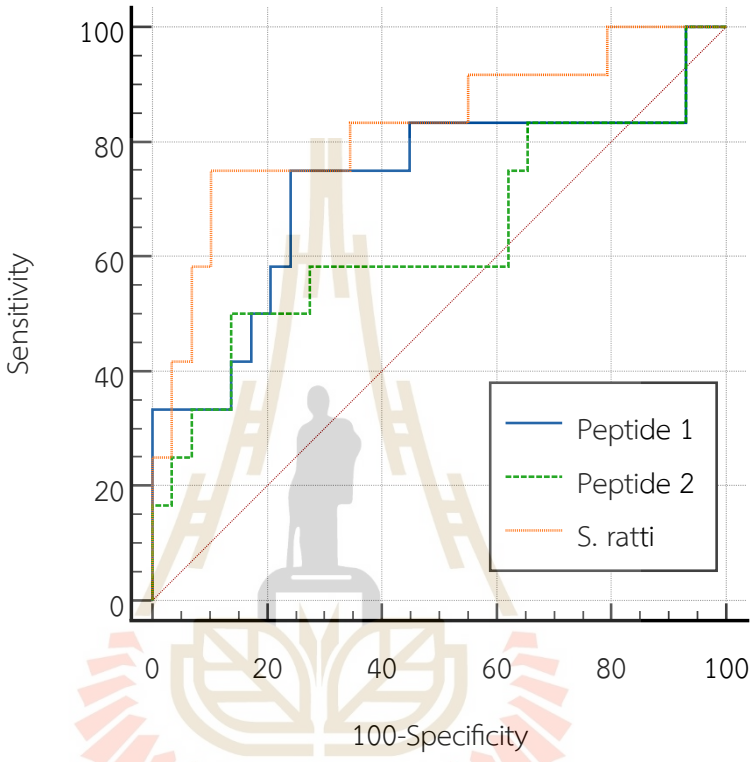

Figure 4.12 Compare ROC curve of the peptide-1, peptide-2 and *S. ratti* antigen. Select between serum samples from patients with strongyloidiasis (Ss., n=12) and negative parasitic infections (Neg, n=29) analyzed by ELISA. The blue dashed line represents peptide-1, green tan instead peptide-2, and orange instead of *S. ratti.*

Table 4.2 Diagnostic performance of antibody detection by the serum assay compared peptide-1, peptide-2 and *S. ratti* antigen.

ELISA tests	Cut-off	AUC	Sensitivity (%) and 95 % CI	Specificity (%) and 95 % CI	Predictive value (%)		+LR	-LR
					Pos	Neg		
Peptide-1	>0.34	0.724	75.00 42.80 - 94.50	75.86 56.50 - 89.70	56.2	88.0	3.11	0.33
Peptide-2	>0.49	0.632	50.00 21.10 - 78.90	86.21 68.30 - 96.10	60.0	80.6	3.62	0.58
S. ratti	>1.06	0.828	75.00 42.80 - 94.50	89.66 72.60 - 97.80	75.0	89.7	7.25	0.28

4.4 Performance of the peptide's antigen for ELISA tests

Comparing mean values among the *S. stercoralis*-infected, other infected parasites, and non-infected parasites of peptide-1 had a mean ± SD of about 0.504 ± 0.320, 0.344 ± 0.202, and 0.279± 0.141, respectively. The results of the mean difference test were obtained with the F-test statistical value of about 5.009 and P-value of about 0.008. It demonstrates that the mean of the three serum groups differs by at least one pair. Scheffe's test results showed that the difference between the SS-infected groups and non-infected groups was statistically significant at the 0.05 level. At the same time, the SS-infected groups and other infected groups were not different, and other infected groups and non-infected groups were not different **(Figure 4.13** and **Fig 4.14)**.

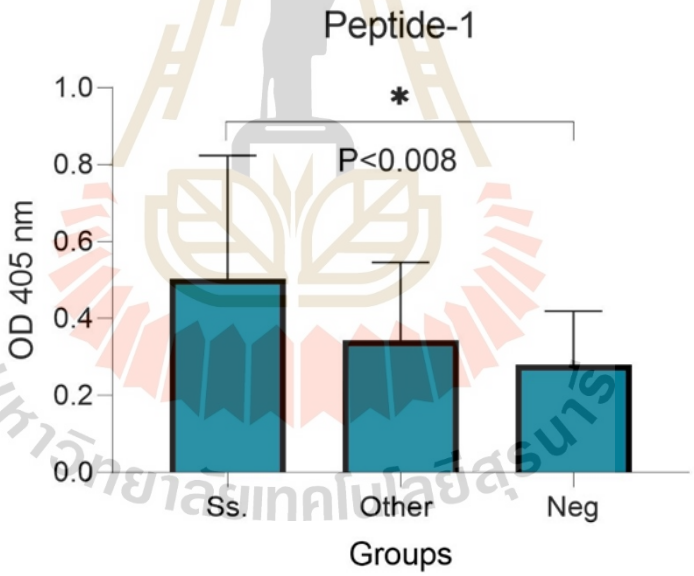

Figure 4.13 Mean ± SD of three groups assigned using peptide-1 as the antigen. One - way ANOVA test of the optical density (OD absorbance at 405 nm) values (mean + SD) of all serum groups. Serum samples from patients with strongyloidiasis (Ss., n=12) with other parasitic infections (Other, n=37) and negative parasitic infections (Neg, n=29) analyzed by ELISA. Scatter dot-plots with ELISA reactivity ANOVA (*P < 0.05). (Note that the significant value is marked with an asterisk *).

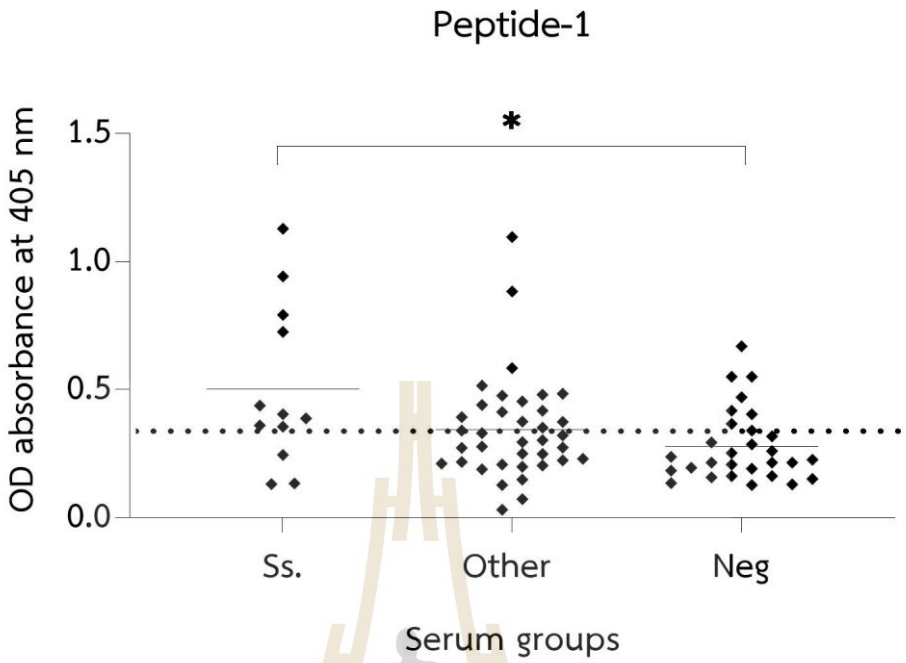

Figure 4.14 Scatter dot plot showed the ELISA result of individual sample from three groups using peptide-1 as the antigen. The cut-off value for OD absorbance at 405 nm to discriminate between positive and negative results, according to the peptide-1 ROC curve, was 0.34.

The peptide-2 had a mean ± SD of *S. stercoralis*-infected, other parasites infected, and non-infected parasites about 0.523 ± 0.307, 0.366 ± 0.204, and 0.359 ± 0.175 respectively. The F-test statistical value of about 2.869 and P-value of about 0.063. It demonstrates that the mean of the three serum groups was not different **(Figure 4.15** and **Figure 16)**.

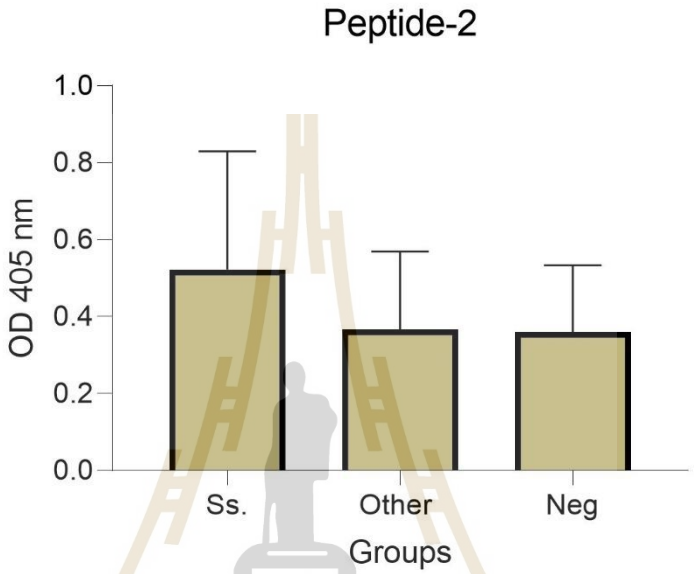

Figure 4.15 Mean ± SD of the three groups assigned using peptide-2 as the antigen. One - way ANOVA test of the optical density (OD absorbance at 405 nm) values (mean + SD) of all serum groups. Serum samples from patients with strongyloidiasis (Ss., n=12) with other parasitic infections (Other, n=37) and negative parasitic infections (Neg, n=29) analyzed by ELISA. Scatter dot-plots with ELISA reactivity.

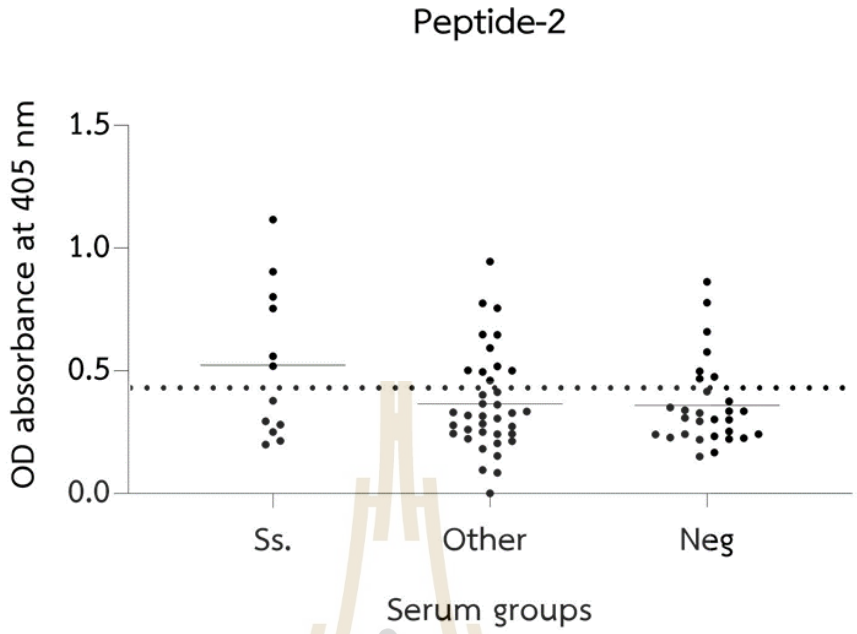

Fig 4.16 Scatter dot plot showed the ELISA result of individual sample from three groups using peptide-2 as the antigen. The cut-off value for OD absorbance at 405 nm to discriminate between positive and negative results, according to the peptide-2 ROC curve, was 0.49.

The *S. ratti antigen* had a mean ± SD of *S. stercoralis*-infected, other parasites infected, and non-parasites infected about 1.346 ± 0.559, 1.164 ± 0.711, and 0.748 ± 0.349 respectively. The F-test statistical value of about 6.232 and P-value of about 0.003. It demonstrates that the mean of the three serum groups differs by at least one pair. Scheffe's test results showed that the difference between the *S. stercoralis*-infected and non-infected was statistically significant at the 0.05 level, and other parasites infected and non-parasites infected was statistically significant at the 0.05 level. At the same time, *S. stercoralis*-infected and other parasites infected was not different **(Figure 4.17** and **Figure 4.18)**.

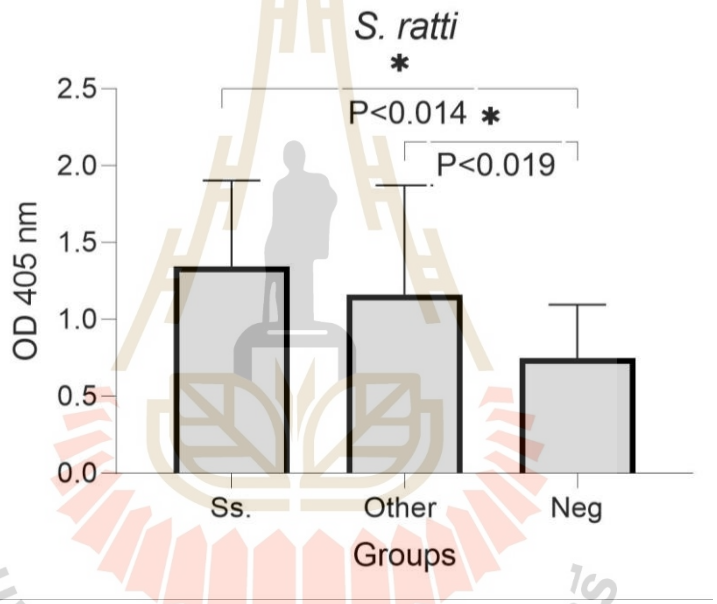

Figure 4.17 Mean ± SD of the three groups assigned using *S. ratti* as the antigen. One - way ANOVA test of the optical density (OD absorbance at 405 nm) values (mean + SD) of all serum groups. Serum samples from patients with strongyloidiasis (Ss., n=12) with other parasitic infections (Other, n=37) and negative parasitic infections (Neg, n=29) analyzed by ELISA. Scatter dot-plots with ELISA reactivity ANOVA (*P < 0.05). (Note that the significant value is marked with an asterisk *).

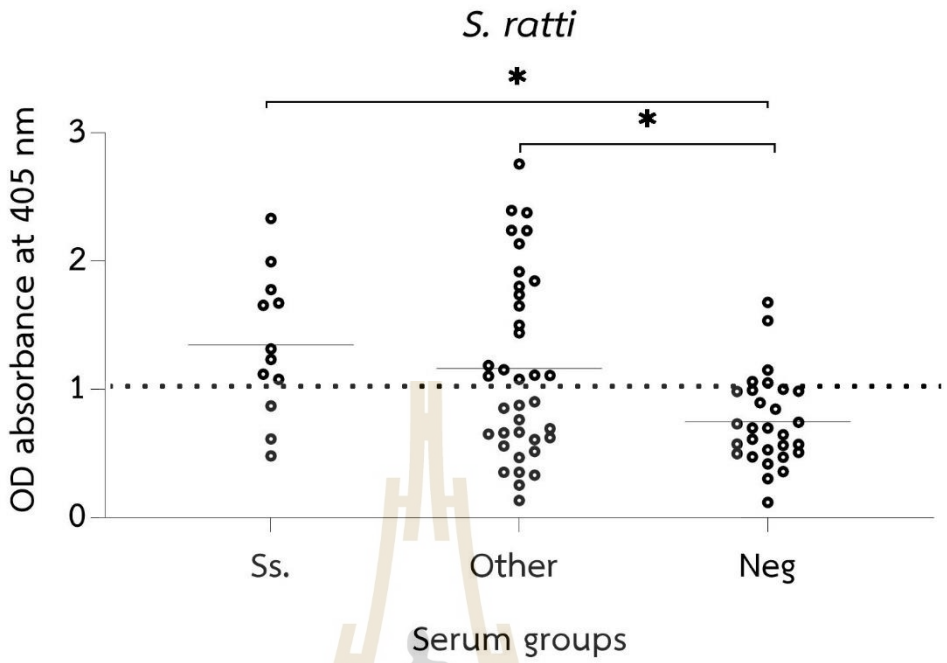

Figure 4.18 Scatter dot plot showed the ELISA result of individual sample from three groups using *S. ratti* as the antigen. The cut-off value for OD absorbance at 405 nm to discriminate between positive and negative results, according to the *S. ratti* antigen ROC curve, was 1.06.

CHAPTER V
DISCUSSION AND CONCLUSION

The *S. stercoralis* infection still have the problem, especially in immunocompromised individual. Most of diagnosis is based on the present of parasite in stool under microscopy. However, this method had low sensitivity. Stool concentration methods may increase the sensitivity of the test, such as the Baermann funnel technique (72% sensitive) Formalin ether acetate concentration technique (52% sensitive) or agar plate culture (89% sensitive), etc. (Mora Carpio and Meseeha 2023). The low sensitivity of the test may result in lower amount of infected patient than real situation. Many research tried to describe the methodology for detection S. stercoralis infection with higher sensitivity and specificity such as PCR (Iamrod et al. 2021), chromatographic strip test (Sadaow et al. 2020), ELISA (Eamudomkarn et al. 2018) etc. Most of antibody to *S. stercoralis* detection used crude protein extracted of *S. ratti* as the antigen. The reasons are this parasite can culture in rat and have little harmful to human when compare to culture *S. stercoralis*. However, this method needs to use animal model; rat. The new era of genetic engineering allowed us to manipulate gene, DNA, RNA and protein; bypass using animal model. Thus, this study aimed to design the peptide of fatty acid and retinol-binding protein of *S. stercoralis* in order to use as the antigen for ELISA testing. The 78 total samples; 12 positive *S. stercoralis* infection, 37 positives for other parasites infection include 4 *Gnathostoma spinigerum*, 4 Trichinella spiralis, 3 *Necator americanus*, 4 *Ascaris lumbricoides*, 5 *Trichuris trichiura*, 8 *Opisthorchis viverrini*, 8 *Taenia spp.*, 1 *Enterobius vermicularis*, and 29 negative parasites infection were used to determine the efficacy of synthesized peptide antigens. Most of the antibody specific to *S. stercoralis* detection used crude protein extracted of *S. ratti* as the antigen. *S. ratti* can culture in rat and little harmful to human as culture *S. stercoralis* (Eamudomkarn et al. 2023; Viney and Kikuchi 2017). Even more than that Masoori et al. (2019) study revealed the

similarity of the inferred fatty acid and retinol-binding protein (FAR) amino acid sequences with fatty acid and retinol-binding protein homologues from other nematode species, with 93% similarity between *S. stercoralis* and *S. ratti*, which was the main reason for using *S. ratti* as a control group for this experiment. The molecular era especially genetic engineering allowed us to manipulated gene and protein. The advantages of peptide antigen are easy to synthesize and get high purity. The ELISA result found that the OD of test using crude protein extracted of *S. ratti* was the highest when compared to peptide-1 and 2. The lower OD value of peptide 1 and 2 may be caused by only one epitope was presented in the reaction. Some patients have no B-cell clone that produce antibody specific to that epitope. The combined two epitope may increase the OD value. The sensitivity of peptide-1, peptide-2 and crude protein extracted of *S. ratti* were 75%, 50%, and 75%, respectively. The specificity of peptide 1, peptide 2 and crude protein extracted of *S. ratti* were 75%, 86%, and 89%, respectively. The peptide-1 could be used to differentiate among infected group from non-infected group but peptide-2 could not. When the experimental results of peptide 1 and peptide 2 were combined, it was found that sensitivity and specificity were 66.70%, and 72.41%, respectively. Which is still considered too low for the standard. The ROC curve of peptide-1, peptide-2 and crude protein extracted of *S. ratti* showed the value of AUC of about 0.724, 0.632, and 0.825, respectively. The reason may be that the number of n samples is small and the number of samples between positive and negative groups is not equal. Therefore, the positive prediction value and negative prediction value was changed, resulting in the sensitivity and specificity being inappropriate values. The reason may be that the number of n samples is small because it was previously in the situation of spreading coronavirus. As a result, hospitals or government offices refrain from accessing the service and refrain from all activities, making it impossible to collect all samples. However, cross-reactivity of antibody still present from experiment. Worms of another parasite may have FAR proteins because FAR proteins are nematode-specific proteins and similar proteins may exist but are not yet available in the BLAST database. In order to increase the sensitivity and specificity, more peptide epitope should be included to the test. And recombinant proteins may contribute to higher

sensitivity and specificity (Mohd-Hassan, Noordin, and Arifin 2020). Moreover, the amount of sample should be increase for more accuracy testing.

The conclusion, we have designed and synthesized two epitope antigens (peptide-1 and peptide-2). The experimental results can be used at some level but a good alternative would be to design more epitopes for *S. stercoralis* specificity, increase the number of experimental samples to differentiate the experimental groups and examine their sensitivity and specificity for accuracy and reliability. In addition, to find ways for more efficient diagnosis. This will be especially useful in routine clinical labs and surveillance investigations in the future.

REFERENCES

REFERENCES

Ahmad, Z. A., S. K. Yeap, A. M. Ali, W. Y. Ho, N. B. Alitheen, and M. Hamid. (2012). 'scFv antibody: principles and clinical application', Clin Dev Immunol, 2012: 980250.

Balachandra, D., H. Ahmad, N. Arifin, and R. Noordin. (2021). 'Direct detection of *Strongyloides* infection via molecular and antigen detection methods', Eur J Clin Microbiol Infect Dis, 40: 27-37.

Bio-Techne. (2023). What is an ELISA. Retrieved from https://www.rndsystems.com /resources/what-is-an-elisa-and-elisa-types?gclid=CjwKCAjw-b-kBhB-EiwA4fvKrGbeMA_%20diV5pnKFhviSwFpgN4Idn-xQI46M_b5sWIFgGJluS_jBu0xoCq7sQAvD_%20BwE&gclsrc=aw.ds0

Buonfrate, D., D. Bisanzio, G. Giorli, P. Odermatt, T. Fürst, C. Greenaway, M. French, R. Reithinger, F. Gobbi, A. Montresor, and Z. Bisoffi. (2020). 'The Global Prevalence of *Strongyloides stercoralis* Infection', Pathogens, 9.

Buonfrate, D., F. Formenti, F. Perandin, and Z. Bisoffi. (2015). 'Novel approaches to the diagnosis of *Strongyloides stercoralis* infection', Clin Microbiol Infect, 21: 543-52.

Centers for Disease Control and prevention (CDC). (2017). Hookworm pada. Retrieved from https://www.cdc.gov/dpdx/hookworm/index.html

Centers for Disease Control and prevention (CDC). (2019). Life Cycle of *Strongyloides stercoralis*. Retrieved from https://www.cdc.gov/ dpdx/strongyloidiasis/ index.html

Chavez, I. N., T. M. Brown, A. Assié, A. S. Bryant, B. S. Samuel, and E. A. Hallem. (2021). 'Skin-penetrating nematodes exhibit life-stage-specific interactions with host-associated and environmental bacteria', BMC Biol, 19: 221.

Czeresnia, J. M., and L. M. Weiss. (2022). *'Strongyloides stercoralis'*, Lung, 200: 141-48.

De Kaminsky, R. G. 1993. 'Evaluation of three methods for laboratory diagnosis of *Strongyloides stercoralis* infection', J Parasitol, 79: 277-80.

Ding, S. W., D. W. Wang, Y. Xiang, C. L. Xu, and H. Xie. (2019). 'Identification and Characterization of a Fatty Acid- and Retinoid-Binding Protein Gene (Ar-far-1) from the Chrysanthemum Foliar Nematode, Aphelenchoides ritzemabosi', Int J Mol Sci, 20.

Eamudomkarn, C., S. Ruantip, J. Sithithaworn, A. Techasen, K. Y. Kopoolrat, C. Worasith, P. Wongphutorn, J. M. Bethony, T. Laha, and P. Sithithaworn. (2023). 'Epidemiology of strongyloidiasis determined by parasite-specific IgG detections by enzyme-linked immunosorbent assay on urine samples using *Strongyloides stercoralis*, *Strongyloides ratti* and recombinant protein (NIE) as antigens in Northeast Thailand', PLoS One, 18: e0284305.

Eamudomkarn, C., P. Sithithaworn, C. Kamamia, A. Yakovleva, J. Sithithaworn, S. Kaewkes, A. Techasen, W. Loilome, P. Yongvanit, C. Wangboon, P. Saichua, M. Itoh, and M. Bethony J. (2018). 'Diagnostic performance of urinary IgG antibody detection: A novel approach for population screening of strongyloidiasis', PLoS One, 13: e0192598.

Khanna V, Tilak K, Prakash PY., Mukhopadhyay C. (2015). Modified agar plate culture method for culture of Strongyloides stercoralis. Trop Parasitol. 5(2):136-138.

Khumpool G. (2013). Larval Culture of *Strongyloides papillosus* for PERL Chamber System. Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok, Thailand.

Eamudomkarn, C., P. Sithithaworn, J. Sithithaworn, S. Kaewkes, B. Sripa, and M. Itoh. (2015). 'Comparative evaluation of *Strongyloides ratti* and *Strongyloides stercoralis* larval antigen for diagnosis of strongyloidiasis in an endemic area of opisthorchiasis', Parasitol Res, 114: 2543-51.

Erdem Kivrak, E., E. Atalay Şahar, H. Can, M. Döşkaya, M. Yilmaz, H. Pullukçu, A. Caner, H. Töz, A. Y. Gürüz, and M. Işikgöz Taşbakan. (2017). 'Screening of immunocompromised patients at risk of strongyloidiasis in western Turkey using ELISA and real-time PCR', Turk J Med Sci, 47: 897-901.

Division of Innovation and Research. (2019). Research Plan 2019-2021, Department of Disease Control. Retrieved from http://irem.ddc.moph.go.th/book/ detail/92

Feliciano, N. D., V. S. Ribeiro, H. T. Gonzaga, F. A. Santos, P. T. Fujimura, L. R. Goulart, and J. M. Costa-Cruz. (2016). 'Short epitope-based synthetic peptides for serodiagnosis of human strongyloidiasis', Immunol Lett, 172: 89-93.

Garibyan, L., and N. Avashia. (2013). 'Polymerase chain reaction', J Invest Dermatol, 133: 1-4.

Gómara, M. J., and I. Haro. (2007). 'Synthetic peptides for the immunodiagnosis of human diseases', Curr Med Chem, 14: 531-46.

Iamrod, K., A. Chaidee, R. Rucksaken, K. Y. Kopolrat, C. Worasith, P. Wongphutorn, K. Intuyod, S. Pinlaor, J. Sithithaworn, P. Sithithaworn, and N. Hongsrichan. (2021). 'Development and Efficacy of Droplet Digital PCR for Detection of *Strongyloides stercoralis* in Stool', Am J Trop Med Hyg, 106: 312-19.

Kapingidza, A. B., K. Kowal, and M. Chruszcz. (2020). 'Antigen-Antibody Complexes', Subcell Biochem, 94: 465-97.

Keagile Bati. (2018). Polymerase Chain Reaction: Innovation that Revolutionized Molecular Biology. Retrieved from https://longroadtoinnovation.wordpress.com/2018/11/01/polymerase-chain-reaction-innovation-that-revolutionized-molecular-biology/

Khanna, V., K. Tilak, P. Y. Prakash, and C. Mukhopadhyay. (2015). 'Modified agar plate culture method for culture of *Strongyloides stercoralis*', Trop Parasitol, 5: 136-8.

Kitvatanachai, S., K. Kritsiriwutthinan, A. Taylor, and P. Rhongbutsri. (2022). 'Modified Nonnutrient Agar Plate Culture for the Diagnosis of *Strongyloides stercoralis* and Hookworm Infections in La-Ngu District, Satun Province, Southern Thailand', J Parasitol Res, 2022: 1117400.

Laoraksawong, P., O. Sanpool, R. Rodpai, T. Thanchomnang, W. Kanarkard, W. Maleewong, R. Kraiklang, and P. M. Intapan. (2018). 'Impact of the health education and preventive equipment package (HEPEP) on prevention of *Strongyloides stercoralis* infection among rural communities in Northeast Thailand: a cluster randomized controlled trial', BMC Public Health, 18: 1184.

Levenhagen, M. A., and J. M. Costa-Cruz. (2014). 'Update on immunologic and molecular diagnosis of human strongyloidiasis', Acta Trop, 135: 33-43.

Levenhagen, M. A., P. T. Fujimura, F. A. A. Santos, L. Vecchi, R. P. Alves-Balvedi, A. G. B. Madurro, J. M. Madurro, J. M. Costa-Cruz, and L. R. Goulart. (2021). 'Innovative approaches to improve serodiagnosis of *Strongyloides stercoralis* infection', Braz J Infect Dis, 25: 101545.

Martín-Rabadán P, Muñoz P, Palomo J, Bouza E. (1999). Strongyloidiasis: the Harada-Mori test revisited. Clin Microbiol Infect; 5(6):374-376. doi: 10.1111/j.1469-0691.1999.tb00157.x. PMID: 11856283.

Masoori, L., A. R. Meamar, M. Bandehpour, A. Hemphill, E. Razmjou, K. Mokhtarian, M. Roozbehani, A. Badirzadeh, N. Jalallou, L. Akhlaghi, and R. Falak. (2019). 'Fatty acid and retinol-binding protein: A novel antigen for immunodiagnosis of human strongyloidiasis', PLoS One, 14: e0218895.

Mohd-Hassan, N. H., R. Noordin, and N. Arifin. (2020). 'Enhanced production of short-peptide tagged Ss3a recombinant protein as a novel potential biomarker for strongyloidiasis', Trop Biomed, 37: 578-86.

Mora Carpio, A. L., and M. Meseeha. (2023). *'Strongyloides stercoralis* Infection.' in, StatPearls (StatPearls Publishing Copyright © 2023, StatPearls Publishing LLC.: Treasure Island (FL) ineligible companies. Disclosure: Marcelle Meseeha declares no relevant financial relationships with ineligible companies.).

Muhi, S., D. K. Ko, S. L. McGuinness, B. A. Biggs, S. Mahanty, and C. Delany. (2022). 'Mixed-method analysis of screening for *Strongyloides stercoralis* prior to immunosuppression: a problem of limited bandwidth?', Intern Med J, 52: 790-99.

Nutman, T. B. (2017). 'Human infection with Strongyloides stercoralis and other related *Strongyloides* species', Parasitology, 144: 263-73.

Page, W., J. A. Judd, and R. S. Bradbury. (2018). 'The Unique Life Cycle of *Strongyloides stercoralis* and Implications for Public Health Action', Trop Med Infect Dis, 3.

Prasongdee, T. K., P. Laoraksawong, W. Kanarkard, R. Kraiklang, K. Sathapornworachai, S. Naonongwai, P. Laummaunwai, O. Sanpool, P. M. Intapan, and W. Maleewong. (2017). 'An eleven-year retrospective hospital-based study of epidemiological data regarding human strongyloidiasis in northeast Thailand', BMC Infect Dis, 17: 627.

Prateepsuk W. (2019). Comparison of Efficacy of Formalin– Ether Concentration Technique and Formalin– Ethyl Acetate Concentration Technique to Identify Species and Number of Gastrointestinal Eggs in Goats. Faculty of Veterinary Science, Western University, Thailand.

Requena-Méndez, A., P. Chiodini, Z. Bisoffi, D. Buonfrate, E. Gotuzzo, and J. Muñoz. (2013). 'The laboratory diagnosis and follow up of strongyloidiasis: a systematic review', PLoS Negl Trop Dis, 7: e2002.

Rodu, B. (1990). 'The polymerase chain reaction: the revolution within', Am J Med Sci, 299: 210-6.

Sadaow, L., O. Sanpool, R. Rodpai, P. Boonroumkaew, W. Maleewong, and P. M. Intapan. (2020). 'Development of immunochromatographic device as a point-of-care tool for serodiagnosis of human strongyloidiasis cases', Eur J Clin Microbiol Infect Dis, 39: 465-70.

Sarangarajan, R., A. Ranganathan, A. H. Belmonte, and V. Tchertkoff. (1997). *'Strongyloides stercoralis* infection in AIDS', AIDS Patient Care STDS, 11: 407-14.

Sharma, N., K. Zivari, D. Yunina, M. Grunwald, O. Azar, R. Rahmani, and K. Tin. (2022). *'Strongyloides stercoralis* Infection in Immunocompromised Host', Cureus, 14: e21217.

Tabatabaei, M. S., and M. Ahmed. (2022). 'Enzyme-Linked Immunosorbent Assay (ELISA)', Methods Mol Biol, 2508: 115-34.

Tamarozzi, F., S. S. Longoni, C. Mazzi, S. Pettene, A. Montresor, S. Mahanty, Z. Bisoffi, and D. Buonfrate. (2021). 'Diagnostic accuracy of a novel enzyme-linked immunoassay for the detection of IgG and IgG4 against *Strongyloides stercoralis* based on the recombinant antigens NIE/SsIR', Parasit Vectors, 14: 412.

Umair, S., Q. Deng, J. M. Roberts, R. J. Shaw, I. A. Sutherland, and A. Pernthaner. (2016). 'Identification of Peptide Mimics of a Glycan Epitope on the Surface of Parasitic Nematode Larvae', PLoS One, 11: e0162016.

Viney, M. (2017). 'Strongyloides', Parasitology, 144: 259-62.

Viney, M., and T. Kikuchi. (2017). *'Strongyloides ratti* and *Strongyloides venezuelensis* - rodent models of *Strongyloides* infection', Parasitology, 144: 285-94.

Wannapinyosheep S. (2015). Strongyloidiasis: skin- penetration nematodes, the near dangers. Faculty of Science and Technology, Huachiew Chalermprakiet University. Samutprakarn, Thailand.

Yimam Getaneh, Abaineh Munishae, and Endalkachew Nibret. (2015). Helminthic infections in relation to Nutritional status and CD4+ T- cell count among HIV/AIDS patients. Retrieved from https://www.researchgate.net/figure/Diagram-of-Harada-Mori-whether-hookworm-Strongyloides-or-Trichostrongylus-larvae-are_fig6_292605696

APPENDICES

APPENDIX A

COA No. 49/2564 1st Renew

Human Research Ethics Committee, Suranaree University of Technology

Certificate of Approval

Human Researches Ethics Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand, has Expedited the following study which is to be carried out in compliance with the International guidelines for human research protection as Declaration of Helsinki, The Belmont Report, CIOMS Guideline, International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

Title of Project : Efficacy of Strongyloides stercoralis peptide antigen for diagnosis

Project Code : EC-64-57

Principal Investigator : Miss Thitimakorn Namhong

Department : Institute of Medicine

Review Method : Expedited

Continuing Report : At least once annually or submit the final report if finished

Document Reviewed : Protocol, Information Sheet, Informed Consent, Questionnaire (version 2.0, 9 June 2021)

Signature.....Chairman
(Asst. Prof. Pattama Torgdee, MD)
Human Researches Ethics Committee, Suranaree University of Technology

Date of Approval : 10 June 2022

Approval Expiry Date : 9 June 2023

Approval is granted subject to the following conditions : (see back of this Certificate)

ผ่านการพิจารณาจาก
คณะกรรมการจริยธรรมการวิจัยในมนุษย์
มหาวิทยาลัยเทคโนโลยีสุรนารี แล้ว

Suranaree University of Technology 111 University Avenue, Sub District Suranaree, Muang District, Nakhon Ratchasima 30000, Thailand
Tel. +66-4422-3000 Fax. +66-4422-4070

APPENDIX A (cont.)

All approved investigators must comply with the following conditions:

1. Strictly conduct the research as stated by the protocol.
2. Use only the information sheet, consent form (and recruitment material, if any), interview outlines and/or questionnaires bearing the ethics committee seal of approval.
3. Report to the Ethics Committee any serious adverse event or any changes in the research activity within the timeframe started in the standard operating procedures.
4. Provide progress reports to the Ethics Committee within the specified time period or upon request
5. If the study cannot be finished within the expired date of the approval certificated, the investigator is obliged to reapply for approval at least 30 days to the expiration date.
6. The expiry date of every approved document is based on the expiration date of the origin approved protocol (EC-64-57)
7. Complete and submit the final report form to the Human Research Ethics Committee, as soon as possible after the completeness of research.

ผ่านการพิจารณาจาก
คณะกรรมการจริยธรรมการวิจัยในมนุษย์
มหาวิทยาลัยเทคโนโลยีสุรนารี แล้ว

APPENDIX B

Sample size calculation

N of this study will be calculated from population of 9,919 (8.2%) Helminths in Thailand in 2014 (Division of Innovation and Research, 2019)

The following sample formula used $\boxed{n = \frac{Z^2 P(1-P)}{d^2}}$

$$n = \frac{(1.96)^2\ 0.082(1-0.082)}{(0.05)^2}$$

$$n = 115.67 \approx 116$$

n = Sample positive parasite infections

Z = Confidence level, (95%) = 1.96

P = Population of Helminths infections in Thailand, (8.2%) = 0.082

d = Precision, (5%) = 0.05

APPENDIX C

Predicted residue scores:

Position	Residue	Score	Assignment
0	S	0.252	.
1	D	0.31	.
2	D	0.355	.
3	L	0.39	.
4	L	0.443	.
5	E	0.464	.
6	S	0.465	.
7	V	0.469	.
8	I	0.481	.
9	N	0.505	E
10	Y	0.514	E
11	F	0.525	E
12	P	0.56	E
13	N	0.567	E
14	D	0.553	E
15	A	0.526	E
16	T	0.547	E
17	T	0.541	E
18	F	0.53	E
19	L	0.524	E

APPENDIX C (cont.)

Position	Residue	Score	Assignment
20	K	0.527	E
21	S	0.521	E
22	L	0.528	E
23	T	0.534	E
24	P	0.569	E
25	E	0.553	E
26	E	0.525	E
27	L	0.527	E
28	Q	0.52	E
29	I	0.509	E
30	V	0.502	E
31	T	0.503	E
32	Q	0.518	E
33	I	0.517	E
34	A	0.521	E
35	G	0.546	E
36	E	0.549	E
37	K	0.566	E
38	P	0.583	E
39	L	0.593	E
40	T	0.586	E
41	D	0.578	E
42	S	0.573	E
43	P	0.577	E

APPENDIX C (cont.)

Position	Residue	Score	Assignment
44	E	0.582	E
45	D	0.563	E
46	W	0.529	E
47	D	0.523	E
48	E	0.494	.
49	F	0.472	.
50	L	0.462	.
51	L	0.463	.
52	I	0.458	.
53	V	0.453	.
54	R	0.475	.
55	D	0.502	E
56	Q	0.505	E
57	S	0.518	E
58	E	0.541	E
59	P	0.545	E
60	L	0.508	E
61	Y	0.494	.
62	R	0.501	E
63	K	0.475	.
64	I	0.441	.
65	I	0.44	.
66	K	0.45	.
67	E	0.448	.

APPENDIX C (cont.)

Position	Residue	Score	Assignment
68	V	0.451	.
69	R	0.493	.
70	K	0.52	E
71	E	0.509	E
72	D	0.547	E
73	E	0.582	E
74	K	0.595	E
75	F	0.6	E
76	N	0.611	E
77	Q	0.617	E
78	P	0.604	E
79	S	0.599	E
80	Y	0.6	E
81	K	0.578	E
82	G	0.553	E
83	R	0.547	E
84	R	0.536	E
85	F	0.508	E
86	I	0.508	E
87	K	0.516	E
88	K	0.528	E
89	F	0.54	E
90	Q	0.559	E
91	E	0.588	E

APPENDIX C (cont.)

Position	Residue	Score	Assignment
92	K	0.586	E
93	Q	0.593	E
94	K	0.62	E
95	T	0.607	E
96	V	0.605	E
97	F	0.576	E
98	Q	0.541	E
99	P	0.498	.
100	H	0.461	.
101	G	0.424	.
102	I	0.403	.
103	F	0.373	.
104	V	0.358	.
105	M	0.338	.
106	P	0.327	.
107	M	0.335	.
108	A	0.352	.
109	A	0.354	.
110	R	0.356	.
111	M	0.364	.
112	S	0.369	.
113	A	0.363	.
114	L	0.367	.
115	D	0.381	.

APPENDIX C (cont.)

Position	Residue	Score	Assignment
116	L	0.39	.
117	F	0.4	.
118	L	0.429	.
119	N	0.444	.
120	Y	0.457	.
121	L	0.473	.
122	D	0.497	.
123	L	0.514	E
124	P	0.523	E
125	E	0.526	E
126	I	0.534	E
127	D	0.528	E
128	K	0.542	E
129	E	0.542	E
130	G	0.515	E
131	I	0.514	E
132	D	0.501	E
133	S	0.485	.
134	I	0.458	.
135	V	0.464	.
136	D	0.471	.
137	V	0.476	.
138	F	0.464	.

APPENDIX C (cont.)

Position	Residue	Score	Assignment
139	R	0.49	.
140	K	0.498	.
141	H	0.51	E
142	L	0.514	E
143	P	0.485	.
144	V	0.414	.
145	P	0.351	.
146	L	0.285	.
139	R	0.49	.
140	K	0.498	.
141	H	0.51	E
142	L	0.514	E
143	P	0.485	.
144	V	0.414	.
145	P	0.351	.
146	L	0.285	.

APPENDIX D

Reagents for indirect ELISA

1. Coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6)

Dissolve 3.7 g sodium bicarbonate (NaHCO3) and 0.64 g sodium carbonate (Na2CO3) in 1 L of distilled water, and adjust pH 9.6. The solution was sterilized by autoclaving.

2. Phosphate buffered saline (1X PBS in DI pH 7.4)

One L of deionized water was added PBS (Sigma, USA) and measure the pH 7.4. The solution was sterilized by autoclaving.

3. PBS-T buffer (0.05% Tween-20 in PBS)

One L of PBS was sterilized by autoclaving and added with 0.05 ml of Tween-20 (Omni Pur®, USA).

4. Bovine Serum Albumin (1% BSA in PBS)

The blocking was prepared by dissolving 1 g of BSA (© Capricorn Scientific 2023, Inc. USA) in 100 ml of PBS sterilized.

5. Sodium Dodecyl Sulfate (1% SDS in DI water)

The stop color reaction by dissolving 1 g of SDS (SDS, Affymetrix, USA) in 100 ml of DI water sterilized.

CURRICURUM VITAE

Miss Thitimakorn Namhong was born on February 8, 1995. Place of birth at Muang District, Kalasin province, Thailand. Graduated with a bachelor of science degree (Public Health) Vongchavalitkul University, Nakhon Ratchasima province, Thailand, 2017. Home address details 180 Aphai road, Muang district, Kalasin province, Thailand. Publication/ presentation of the 10th national and the 8th International conference on research and innovation: research and innovation development for developing sustainable communities in titer efficacy of *Strongyloides stercoralis* peptides antigen for serological diagnosis.