EFFECT OF GARLIC, AND LACTIC ACID FROM FERMENTED STEAMED STICKY RICE ON VIABILITY AND INFECTIVITY OF TRICHINELLA SPIRALIS

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Thesis

Entitled

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ABSTRACT

Trichinella spiralis is a cause of Trichinellosis**.** One source of this disease is eating some uncooked meat, including Naem (แหนม). Naem consists of pork mixed with salt, steamed sticky rice and garlic. This mixture is fermented until it becomes sour. This study aimed at studying garlic and lactic acid from steamed sticky rice on viability and infectivity of *Trichinella spiralis* larvae. The experimental design was the 3x3 factorial experiment. The first 3 is a quantity of garlic juice (*Allium sativum*) with concentrations at 1, 4 and 12 gm/100 ml of water (w/v) respectively. The second 3 is a quantity of steamed sticky rice transmuted into lactic acid with concentrations at 4, 10 and 20 gm respectively. Lactic acid from steamed sticky rice was prepared by fermenting 100 gm of blended pork, 1 gm of salt, garlic at 1, 4 and 12 gm, and steamed sticky rice at 4, 10 and 20 gm. These ingredients were fermented in sealed plastic for 3 days. This method was emulated from Naem as commercially produced. After that, *T. spiralis* larvae were moved into the experiment by leaving them there for 18 hours before giving it to mice, which were fed for 30 days, to examine the viability of *T. spiralis* larvae. The result showed that *T. spiralis* larvae in all groups bended in rolls and did not have any movement. The infectivity in mice was also tested. Larvae infection was not found in the group of garlic ($p = 0.005$), but in the group of lactic acid from fermented steamed sticky rice that was mixed with some garlic, larvae infection was found ($p = 0.75$ and 0.32). Thus, the garlic used in Naem may affect to the viability of larvae, but lactic acid from fermented steamed sticky rice had no effect to larvae.

KEY WORDS : TRICHINELLA SPIRALIS / VIABILITY / INFECTIVITY / GARLIC / LACTIC ACID / STEAMED STICKY RICE

58 pp.

ผลของกระเทียมและกรดแลคติกจากข้าวเหนียวต่อการรอดชีพและความสามารถในการติดเชื้อของ TRICHINELLA SPIRALIS (EFFECT OF GARLIC, AND LACTIC ACID FROM FERMENTED STEAMED STICKY RICE ON VIABILITY AND INFECTIVITY OF TRICHINELLA SPIRALIS)

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บทคัดย่อ

พยาธิทริคิเนลล่า (*Trichinella spiralis*) เป็นสาเหตุของโรคทริคิเนลโลซิส (Trichinellosis) เกิดจากการบริโภคเนื้อที่ไม่ปรุงสุก แหนมหมูเป็นอาหารประเภทเนื้อหมักและเป็นหนึ่งในอาหารที่มักบริโภคดิบ แหนมหมูประกอบด้วยเนื้อหมู ข้าวเหนียว กระเทียม เกลือ หมักรวมกันจนเกิดรสเปรี้ยวของกรดแลคติก ดังนั้นในการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของกระเทียมและกรดแลคติกจากข้าวเหนียวต่อการรอดชีพและความสามารถในการติดเชื้อของตัวอ่อน *T. spiralis* รูปแบบการวิจัยเป็นแบบแฟคทอเรียลชนิด 3×3 คือ กระเทียม 3 ระดับ และกรดแลคติกจากข้าวเหนียว 3 ระดับ น้ำกระเทียม (*Allium sativum*) จำนวน 1, 4 และ 12 กรัมต่อน้ำ 100 มิลลิลิตร กรดแลคติกผลิตโดยหมักเนื้อหมูบด 100 และเกลือ 1 กรัม ผสมกับข้าวเหนียว 4, 10 และ 20 กรัม และกระเทียมบด 1, 4 และ 12 กรัม หมักในถุงพลาสติกปิดสนิท เป็นเวลา 3 วันจะเกิดกรดแลคติกจากกระบวนการหมัก ซึ่งเลียนแบบการหมักแหนมหมูชนิดที่ผลิตเพื่อจำหน่าย จากนั้นนำตัวอ่อนของ *T. spiralis* ใส่ในสารประกอบดังกล่าว ตั้งทิ้งไว้ 18 ชั่วโมงก่อนนำไปป้อนในหนูทดลอง (*Mus musculus*) หลังจากนั้นเลี้ยงหนูต่อ 30 วัน เพื่อทดสอบการรอดชีพของตัวอ่อน ผลการทดลองพบว่า ตัวอ่อนของ *T. spiralis* ในน้ำกระเทียมและกรดแลคติกจากการหมักข้าวเหนียวขดเป็นวงและไม่เคลื่อนไหว เมื่อทดสอบความสามารถในการติดเชื้อในหนูพบว่ากลุ่มการทดลองน้ำกระเทียมไม่พบตัวอ่อนของ *T. spiralis* ในหนู (p-value = 0.005) ส่วนกลุ่มการทดลองกรดแลคติกจากการหมักข้าวเหนียวและกลุ่มที่ทดลองกรดแลคติกจากการหมักข้าวเหนียวร่วมกับกระเทียมพบตัวอ่อนของ *T. spiralis* ในหนู (p-value = 0.75 และ0.32) ตามลำดับ ดังนั้นน้ำกระเทียมที่ใช้ทำแหนมอาจมีผลต่อการรอดชีพและการติดเชื้อของตัวอ่อนของ *T. spiralis* แต่กรดแลคติคจากข้าวเหนียวไม่มีผลต่อตัวอ่อน

58 หน้า

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

Trichinellosis is a zoonotic disease that can be passed from animals, both wild and domestic ones, to humans. The causative agent is a parasitic nematode of the genus *Trichinella*. These parasites have a wide range of host species, mostly mammals. The infection is caused by eating raw or inadequately cooked meat of an infected animal. The lifecycle of *Trichinella* is domestic when the infection is passed from domestic animals and rats, and sylvatic when passed among wildlife animals living far away from human residence. Trichinellosis is endemic when the infection constantly stays in a particular area. Trichinellosis is much less prevalent in humans than in the past, but even though the infection rate in humans has declined dramatically, the relationship between pork and trichinellosis still adversely affects pork consumption. It has been a major public health problem and has been reported in many Asian countries, including China, Japan, Korea and Thailand. In Thailand, this disease is highly endemic in the Northeastern area where the wild and hill tribe pigs are fed in main reservoirs; human trichinellosis was first reported in June 1962. The causative agent of most outbreak of this disease has been identified as *T. spiralis* (1). The outbreaks of trichinosis in Thailand have been likely to come from the density of *Trichinella* contamination in domestic and wild animals, but they are also influenced by social and other factors, including people's eating habits.

Swine are the most commonly infected domestic animal. Swine are communicated to *Trichinella* by eating some uncooked garbage containing infected meat scraps or from rodent. Once ingested, the parasite would sexually be mature in the intestine and migrates to the muscle tissue. From 17 to 21 days, the larvae become encysted and infective. Encysted larvae can remain viable for many years and, in most cases, for the whole life of that animal. An infected swine will generally not show any clinical signs of illness unless there are a large number of parasites.

All trichinellosis cases showed the history of consuming raw pork in the form of “Lahb” and “Naem”, favorite dishes at the northern part of Thailand (2). Lahb is made from chopped raw pork to be mixed with lemon juice, roasted rice powder, finely cut red onion and parsley. Naem is also made from chopped raw pork to be mixed with salt, garlic and steamed sticky rice; such mixture is fermented for a few days. Some Thai dishes are proven as viable *T. spiralis* larvae sources due to cooking procedures especially Naem; raw and uncooked Naem may be sometimes eaten by a delighted consumer.

The mixture of Naem consisted of blended pork, salt, garlic and steamed sticky rice. The role of salt in fermentation is to control the microbiological spoilage and contamination, and to draw good taste while garlic has a good smell and controls microbiological spoilage too. A flavor component in the garlic compound comes from the sulfurs group, especially Allicin. Allicin is the chief thiosulfinate (about 75 percent in total) formed when fresh raw garlic is crushed, chopped or chewed. Allicin and possibly other thiosulfinates are main compounds essential to garlic’s antimicrobial, lipid-lowering, antithrombotic, fibrinolytic, antioxidant, anticancer and pro-immune effect. In the fermentation process, garlic inhibits the growth of some microbiological contamination. In addition, Allicin inhibits the growth of other protozoan parasites such as *Entamoeba histolytica* (3) *Giardia lamblia, Leishmania major, Leptomonas colosoma,* and *Crithidia fasciculate* (4) and lethal adult *Hymenolepis nana* (5). Garlic also possesses the ability of inhibiting the growth of parasites in the intestines, including amoebas which cause dysentery. It should be noted that amoebic dysentery is a potentially serious condition which requires the assistance from a trained physician. Garlic has also been used in traditional medicine in many parts of the world to treat pinworms, an annoying but generally harmless intestinal parasite (6) and has not been researched with reports about garlic to inhibit *Trichinella* spp. Moreover, the role of steamed sticky rice in the fermentation process is to harbor, provide a carbohydrate source of lactic acid bacteria to be changed to lactose and then lactic acid, at pH 5-4.5 Naem has a sour taste and inhibits growth of microbiology too, but it has not been researched with reports about inhibits parasites. So this study aimed at examining the quantity of garlic and lactic acid in fermented steamed sticky rice and its effect on viability and infectivity of *Thichinella spiralis*.

Research objectives

General objective

To study the garlic juice and lactic acid in fermented steamed sticky rice on viability and infectivity of *Trichinella spiralis* larvae.

Specific objectives

1. To study viability and infectivity of *T. spiralis* in 1 %w/v, 4 %w/v and 12 %w/v of garlic juice concentrate.

2. To study viability and infectivity of *T. spiralis* in 4 grams, 10 grams and 20 grams of steamed sticky rice.

3. To study viability and infectivity of *T. spiralis* in 1 gram, 4 grams and 12 grams of garlic combined with 4 grams, 10 grams and 20 grams of steamed sticky rice.

Hypothesis

Garlic and lactic acid in fermented steamed sticky rice have inhibited viability and infectivity of *T. spiralis* larvae.

Assumption of study

One gram of salt and 100 grams of blended pork were fixed for each group of ingredients and they were fermented.

Definitions

***Trichinella spiralis* larvae** were excysted and isolated from infected mice, digested by 1% HCl-pepsin and collected by Baermann technique.

Viability was a *T. spiralis* larvae tested in garlic juice, lactic acid from fermented steamed sticky rice and lactic acid that were combined with garlic, put in 30°C for 18 hours and checked survival under the microscope; the surviving larvae will have movement and do not infect the mice.

Infectivity was of *T. spiralis* larvae. They were tested for viability and put in mice that were fed for 30 days until larvae were found in the diaphragm of infected mice.

Infected mice were mice that were infected with *T. spiralis* larvae.

Non-infected mice were mice that were not infected with *T. spiralis* larvae.

Naem was made from blended raw pork to be mixed with salt, garlic and steamed sticky rice that were fermented for a few days.

Garlic juice was made from the blended garlic and 100 ml of water.

Lactic acid was a main acid from the fermentation of meat; it was the fermentation fluid.

A fermentation fluid was the fluid got by the fermentation of blended pork and salt, but, in some experiments, garlic and steamed sticky rice might be added and the fluid was sifted by gauze.

Positive control was the experiment group consisting of mice that were fed by 50 larvae.

Negative control was the experiment group consisting of mice that were not fed by larvae.

Control was the control group in which the larvae were fed to test the viability in fermented fluid (fermented 100 grams to be blended with pork and 1 gram of salt).

Grouping was a proof of each experiment in 3 groups: 1) group of garlic with 3 subgroups namely 1%w/v, 4%w/v and 12%w/v, 2) group of steamed sticky rice with 3 subgroups namely 4 gm, 10 gm and 20 gm, 3) group of garlic combined with steamed sticky rice with 9 subgroups namely 1 gm of garlic combined with steamed sticky rice at 4 gm, 10 gm and 20 gm., 4 gm of garlic combined with steamed sticky rice at 4 gm, 10 gm and 20 gm, and 12 gm of garlic combined with steamed sticky rice at 4 gm, 10 gm and 20 gm.

Each group was done in the triplicate experiment.

CHAPTER II
LITERATURE REVIEW

Trichinellosis is a zoonotic disease that can be passed from animals, both wild and domestic ones, to humans. The causative agent is a parasitic nematode of the genus *Trichinella*. These parasites have a wide range of host species, mostly mammals. The infection is contracted by eating raw or inadequately cooked meat of an infected animal. The tissue is then digested in the stomach. The excysted larvae then invade the intestinal mucosa, develop through four larval stages, mature, and mate by the second day. By the sixth day of infection, the female worms begin to deposit motile larvae, which are carried by the intestinal lymphatic system or mesenteric venules to the body tissues, primarily striated muscle. Deposition of larvae continues for approximately 4 weeks The life cycle of *Trichinella* is domestic when the infection is passed among domestic animals and rats, and sylvatic when passed among wildlife away from human habitation. The review items related to this study were as bellowed;

1. Situation of disease
    Epidemiology of Trichinellosis in Thailand
2. *Trichinella spiralis*
    2.1 The taxonomy
    2.2 Morphology
    2.3 Life cycle
    2.4 Clinical pathology
        2.4.1 Vasculitis
        2.4.2 Eosinophil response
        2.4.3 Clinical findings
        2.4.4 Enteral Phase
        2.4.5 Parenteral Phase

1. Situation of disease

Trichinellosis, a zoonotic disease caused by nematodes of the genus *Trichinella,* is prevalent worldwide among carnivores and omnivores. Human infections can be acquired from ingestion of meat containing infective late first-stage larvae from wild or domestic animals. In Thailand, trichinellosis is still a serious public health problem among people who prefer raw undercooked meat. At least 3,000 persons have reportedly been infected (including 85 who died), mostly in the northern part of the country, since 1962 (7).

Trichinellosis is still one of Thailand's major heath problems, especially in the north. Infection is acquired by the consumption of under-cooked meat of animals harboring the infective larvae of *Trichinella spiralis*. Northern are very fond of eating raw meat in the form of "Lahb" and "Naem" favorite dishes in northern Thailand. "Lahb," or raw spiced meat, is made of finely minced meat mixed with spices and dry chilies. The dish usually served along with vegetables, is promptly eaten as soon as the ingredients have been added. "Naem," similar to what Americans call "spam," is made from freshly ground pork, garlic, cooked rice, salt, sodium glutamate and potassium

nitrate, thoroughly mixed and tightly wrapped in a plastic wrapper or banana leaf. It is commonly consumed after being left to ferment at room temperature for 2 to 5 days. From 18,765 samples of "Nam" taken between 1962 to 1973 from various provinces of northern Thailand and analyzed for *T. spiralis* larvae, 23 samples were found to contain larvae, ranging from 0.5-20 larvae per gram of "Naem" (8).

The first outbreak of trichinellosis in Thailand was recorded in Mae Sariang district of Mae Hong Son Province (1). The district is located about 800 kilometers northwest of Bangkok along the Thai-Myanmar border. The villagers had pooled labor, or "long kag" in Thai, to help thatch the village school. After finishing their work they celebrated by illegally slaughtering a pig and using its meat to prepare a dish of "lab" to be served during the party celebration. Two weeks later those villagers (36 male and 20 females) fell sick and 11 patients died. The epidemiology of the infection was investigated. It was found that the pig was a hill tribe pig. Hill tribe pigs are domestic pigs, allowed to free forage in the mountain. They may become feral and some of them have crossbred with wild boars. Since trichinellosis was first recognized in 1962, there have been outbreaks of the disease almost every year. Almost all outbreaks have occurred in the northern province of Thailand (9). Histologic findings from muscle biopsy demonstrated a nurse cell-larva complex. Treatment with albendazole resulted in a very favorable outcome. Trichinosis remains a major public health problem in Thailand, often associated with rural people celebrating local and traditional festivals, such as the northern Thai New Year and wedding ceremonies, at which raw and/or under-cooked wild animals are eaten (2).

Epidemiology of Trichinellosis in Thailand

Trichinosis is more common in temperate regions than in tropical regions. The epidemiology of trichinosis was first reported in 1962 in patients who consumed pig meat (1). The second outbreak was in 1963 at Prao District, Chiang Mai Province. Since then, outbreaks have occurred each year, mostly in the northern part of Thailand where people have eaten raw or under-cooked pork and/or wild animals. The annual epidemiological surveillance reports indicated that trichinosis cases increased from 61 in 1997 to 351 in 1998. In 1999 and 2000, the number of reported cases decreased to 16 and 128 respectively. No cases were recorded in 2001, hospital based or by the Bureau of Epidemiology, that clearly showed a human trichinosis case this

year, but then 289, 126 and 212 occurred in 2002, 2003 and 2004 respectively. In 2005, 75 cases were reported by the Bureau of Epidemiology, Department of Disease Control, and Ministry of Public Health. Since then, about 130 outbreaks have been reported totaling 7392 patients and 97 deaths. Since 2002, the distribution of human trichinosis cases by age groups has been considered by the annual epidemiological surveillance reports which data were the hospital based. The youngest patient was about 1 year old. Charkrit (10) reported a patient of the same age. It is not uncommon to see patients in the 10-14 and 65+ age groups, but most patients are in the age 35-44 groups, morbidity rate was 0.04 per 100 000 of people. Infection occurs in men more frequently than women at the ratio of 1.7-2:1 (calculated from trichinosis cases of 2002-2005). This result was similar to the 1.2-2.6:1 found by Charkrit (10). The epidemiological surveillance reports of trichinosis have been conducted almost every year and data investigation reveals that the outbreaks have occurred predominantly in rural areas. The north part of Thailand is responsible for 96.4% of all cases reported from 1962 to 2000 (2). The annual epidemiological surveillance reports from 2002 to 2005 found consistently high numbers of cases (289, 126, 30 and 60 respectively) in the north region. For 2004 reported 124 in the northeast, the first time that a region other than the north has had the highest number of cases. Only small numbers of trichinosis cases were recorded in the central and south regions in 2005. In 2005, 75 trichinosis cases were reported, the highest number occurring in October, August, March and September, 36, 16, 7 and 5 respectively. The cases were reported in Chiang Rai, Nan, Chiang Mai, Si Sa ket, Nakon Phanom, Kalasin, Nakhon Ratchasima, Nakhon Nayok and Surat Thani, all provinces located in different parts of Thailand. The main age group was 35-44 years and the youngest patient was 1.Most outbreaks occurred in the north region, including 60.84% of all cases reported from 1962 to 2005. The most severely affected areas in the north region were the highland provinces of Chiang Rai, Nan, Chiang Mai, Mae Hong Son and Payao. The numbers of cases in other parts of Thailand were very few. In the central region, Uthai Thani, Karnchanaburi, Nakhon Pathom and Nakhon Nayok provinces reported 0.28% of the total number of cases. Chumporn, Songkla and Surat Thani were the only three provinces of the south region in which cases of trichinosis were observed, these accounting for only 0.28% of cases. The northeast of Thailand was responsible for the

highest number of cases in 2004 and the second highest (38.5%) in 2005. Provinces involved were Nong Bua Lam Phu, Buri Ram, Kalasin, Sakol Nakorn, Sri Saket, Nong Kai, Khon Kaen, Nakhon Phanom and Nakhon Ratchasima. These results showed trichinosis as a serious problem, particularly in the north and northeast regions of Thailand (2).

Figure 1 Reported provinces for trichinellosis in Thailand from 1962-2005
Source : Kaewpitoon N, Kaewpitoon S, Philasri C, Leksomboon R. (2006)

2. *Trichinella spiralis*

Trichinellosis has threatened human health for thousands of years. The earliest reported infection was in an Egyptian man who lived around 1200 BC. The infection was detected from his mummified body (11). *Trichinella* includes two species;

T. spiralis and *T. pseudospiralis*. The former shows the following subspecies: *T. spiralis spiralis, T. spiralis native, T. spiralis nelsoni* (12).

2.1 The taxonomy

On the basis of results from ribosomal deoxyribonucleic acid (DNA) sequences, the present higher-level classification of *Nematoda* will need revision into two classes, *Secernentea* and *Adenophorea* (13).

2.2 Morphology

The adults look like pieces of thread, being 2.2-3 mm long in the female and 1.2-1.5 mm long in the male, a short muscular part of the esophagus is followed by an array of stichocytes, through which the thin esophageal duct is running. Then, the intestine follows, opening at the anus in the female and cloaca in the male. In the male, a pair of conical papillae necessary for copulation exists at the caudal end, but there are no spicules. Both mail and female worms have a set of genital organs. In the female, the ovary is located in the posterior part of the body, followed by the seminal receptacle and long uterus, the latter half of which is filled with hatched larvae. The larva is discharged by ovoviviparity out of the vulva located around one-fourth the body length from the anterior end. In the male, the testis is located in the posterior part of the body, followed by the vas deferens running forward and turning near the posterior end of the esophagus, the seminal vesicle, and the ejaculatory duct, which forms the cloaca together with the intestine (14).

2.3 Life cycle

The developmental history of this worm is unique. A single animal acts as the final and intermediate hosts, and the worm itself never appears outside of the host. Furthermore, two individual animals are required to complete its life cycle, which can be of either the same or different species. In which adults are parasitic in the small intestine of an animal (intestinal trichinella), while larvae are in striated muscles of the same host (muscle trichinella) for example, when a rat eats swine muscle, the meat is digested and a larva 1 mm long is freed to invade the intestinal mucosa. It grows there rapidly through four molts, and on the second day it becomes mature and starts copulation. The male worm dies

soon after that, while the female worm survives and starts giving larvae by ovoviviparity on around the sixth day.

It dies after completion of giving 500-1,000 larvae over a period of 4-6 weeks. The larvae 0.1 mm long individually enter the lymphatic and blood vessels in the intestinal walls. After going through the heart and the pulmonary circulation, they are distributed all over the body by the greater circulation. Only those which come into striated muscles start further development their, while those carried to other places again enter the greater circulation to be redistributed repeatedly. Larvae that reach striated muscles invade muscular fibers to be encysted. With the development of the larva, the cyst wall is thickened to form a cyst of characteristic spindle form. The cyst wall is made by the host and as large 0.5×0.25 mm, its major axis being in parallel with the muscle fibers. Usually, each cyst has one coiled larva, which is 0.8-1 mm long, and calcification begins from six to nine months Larvae normally survive for several years, and possibly for 25-30 years. Larvae tend to be parasitic in those striated muscles which are always in motion such as the diaphragm, intercostals muscles, abdominal muscles, the biceps brachial muscle, the gastrocnemius muscle, muscles of the larynx, the masseter, and the tongue (14).

2.4 Clinical pathology

Pathogenesis of trichinellosis and its clinical expression are complex due to two *Trichinella* generation, the adult form and larvae with their two respective biotopes the small intestine and muscles and several pathogenetic mechanism being involved.

2.4.1 Vasculitis

One of the basic pathomechanisms involved at the acute stage of trichinellosis is though an attachment of circulation antigen-antibody complexes at the surface of mast cells. With the involvement of IgE antibodies, immediate type hypersensitivity mediators are released from the mast cells. These include histamine, serotonin, slow reacting substance of anaphylaxis (SRS-A), platelet activating factor (PAF) and eosinophil chemotactic factor of anaphylaxis (ECHF). The release mediators also include vasoactive amine, particular bradykinin, responsible for the

main capillaries, so inducing passage of fluid, electrolytes and plasma proteins as well as passage of morphotic blood elements to the surrounding tissues. Vasculitis is the leading pathogenic effect at the acute stage of the disease and involves multiple organs, but the extent to which it affects intestinal tissues is not clear (12).

2.4.2 Eosinophil response

Eosinophilia is a very early sign of the disease, appearing in the fist of intestinal invasion and being included by the adult form of *Trichinella* (15). The augmented level of eosinophilic granulocytes is thought to be induced by mast cell mediators but the involvement of complement, in particular its components C5, C6, C7, or C5a is also important. Thus, eosinophils are associated with the protective mechanism against Trichinella invasion. They are thought to be attracted to sites of the parasite invasion and to the mediators released there, forming the first line of defence against the parasite.

Independent of the basic role of the immune phenomena, the circulating larvae and their passage into tissue cause pathomorphological, metabolic and functional lesion in multiple organs including the intestine. All these may lead to clinical symptoms of the acute stage of trichinellosis such as fever, periorbital oedema, haemorrhage of conjunctivae, nail beds and internal organs, muscular pain and intestinal dysfunction (12).

2.4.3 Clinical findings

T. spiralis outbreaks occur most frequently within a community or among family members. Since there are so many variables modifying the clinical picture of *Trichinella* spp. trichinellosis, single individuals acquiring the infection challenge clinicians who are unaware of the life cycle and epidemiology of *T. spiralis*. The symptoms mimic those of many other diseases. The duration of the incubation period is related to the number of larvae ingested, which, in turn, usually determines the severity of disease. Patients also vary in their symptoms according to time elapsed from the ingestion of infected meat and the infecting species of *Trichinella*. In addition, host immunity, age, sex, and general health of the infected individual are important factors in the outcome of disease. As can be readily seen, there are

symptoms that highly correlate with the stage of the infection (i.e., enteral phase) and are related to the presence of the parasite's infective form and those that correlate with the parenteral phase (e.g., inflammatory and allergic responses due to tissue invasion by larvae) (16).

2.4.5 Enteral Phase

Most individuals who become infected in an outbreak after eating contaminated meat are asymptomatic, as are the majority of patients in single sporadic cases who, at most, experience mild transient diarrhea and nausea. related to the penetration of the intestinal mucosa. Thus, the first week of the enteral phase in patients with moderate to severe infection is associated with upper abdominal pain, diarrhea or constipation, vomiting, malaise, and low-grade fever, all of which can vary in severity and last only a few days. This clinical presentation is characteristic of many enteral disorders (e.g., food poisoning or uncomplicated indigestion), and thus it is easily misdiagnosed. Patients usually do not seek medical advice at this time in their infection and request medical attention only when the nature of the symptoms changes with the onset of the parenteral (systemic) phase (16).

2.4.5 Parenteral Phase

During weeks 2 through 6 after infection, the enteral phase is still present, but symptoms that correlate with intestinal disease abate. At this time, signs and symptoms due to the migratory stage, the newborn larva, develop. In mild infection resulting from the ingestion of low numbers of larvae in muscle, symptoms related to the migratory and parenteral phases are usually the first to be clinically detected since these patients experience no symptoms during the enteral phase. Mild to moderate infection can produce the following signs and symptoms: diffuse myalgia in 30 to 100% of patients; a paralysis-like state (10 to 35%); periorbital and/or facial edema (15 to 90%); conjunctivitis (55%); fever (30 to 90%); headache (75%); skin rash (15 to 65%); difficulties in swallowing (35%) (16) or in opening the mouth; insomnia; weight loss; peripheral nerve sensations; hot flashes; coryza; hoarseness (5 to 20%); bronchitis (5 to 40%); splinter hemorrhages of the nail beds and/or the retinae; visual disturbances; and paralysis of the ocular muscles. These data were

derived from the numerous clinical outbreaks cited above. All signs and symptoms are either directly or indirectly due to the indiscriminate penetration of tissues by the migrating newborn larva. After the second week of the parenteral phase of infection, most patients have developed specific serum antibodies against the secreted antigens of the larvae in muscle (17). Patients with severe infection are often the index cases of an epidemic. They are the first to be diagnosed because of their presentation with the classical signs and symptoms of the disease. Their symptoms are more prominent than are those of patients suffering from milder or moderate infection. High fever and elevated levels of circulating eosinophils (30 to 60% or more), severe muscle pain, skin rash, and headaches, as well as swelling of eyelids, face, or extremities, are characteristic of these cases. Patients may develop neurologic manifestations that rarely appear before the end of the second week of infection and provoke distress. Headache, vertigo and tinnitus, deafness, aphasia, convulsions, and abnormalities related to peripheral reflexes, among others, are frequent complaints or signs found in severely infected individuals. Generally, patients are alert but apathetic, and prolonged insomnia affects their behavior, causing them to become irritable. Other neurologic symptoms such as meningitis, encephalitis, and/or hemiplegia may develop in relation to diffuse damage of brain tissue due to occlusion of arteries or to granulomatous inflammation. These symptoms need to be treated immediately with steroids, since they are due to inflammation resulting from tissue damage caused by large numbers of migrating newborn larvae. In moderate to severe infection, symptoms due to invasion of muscle cells (e.g., weakness, pain, paralysis, and photophobia) increase during the third week, and edema of the face, eyelids, hands, and feet becomes a prominent feature. The patient’s breathing is often difficult and shallow. Dysphagia and hoarseness are also frequent consequences of clinical infection. Thrombosis of arterioles may occur, probably as a result of the hypercoagulability associated with eosinophilia. During this time, clinical signs and symptoms can lead to a misdiagnosis because trichinellosis is often confused with angioneurotic edema, serum sickness, septicemia, periarteritis nodosa, allergic reactions to food or drugs, coronary thrombosis, typhoid fever, infection by a *Toxocara* sp., autoimmune diseases, eosinophilic syndrome, and recently, chronic fatigue syndrome. Poliomyelitis, meningitis, encephalitis, cerebral hemorrhage, multiple neuritis, pneumonia,

bronchopneumonia, pleurisy, spastic bronchitis, glomerulonephritis, and ‘‘ciguatera,’’ a condition induced by eating certain tropical saltwater fishes found mostly in the Caribbean and Indo-Pacific regions, should also be included in the differential diagnosis. Endocarditis, myocarditis, and even cardiac failure in fatal cases are attributed to the damaging effects of the migratory phase of the infection. All symptoms associated with acute illness progressively diminish at the onset of convalescence (i.e., between weeks 5 to 6 after ingestion of infected meat), but dyspnea, edema of the extremities, and bronchitis persist to the sixth to eighth week. Infectious first-stage larvae remain in their nurse cells for months to years following recovery, but after some time a portion of them die. This process may be more rapid for non-*T. spiralis* infections. Dead nurse cell-parasite complexes become highly calcified, but whether or not the calcification process results in the death of the worm is still not resolved by experimental evidence (16)

2.5 Laboratory findings

Leukocytosis (12,500 to 18,000 cells per mm^3) is common early, with a predominance of circulating eosinophils of about 1,400 to 8,700 eosinophils per mm^3. Thus, eosinophilia is the earliest and most characteristic laboratory finding of trichinellosis (18) and is correlated with the intensity of infection. Even among asymptomatic cases eosinophilia reaches modest levels (5 to 15% of leukocytes). A sudden reduction in the level of circulating eosinophils to 1% or none is an indication of severe infection and may even signal the onset of death of the patient (19). Only the adult stage of trichinella has been shown to elicit eosinophilia. Eosinophilia is maximum during the third to fourth week and usually stabilizes at this time. These cells infiltrate the infected portion of intestinal tissue and locate adjacent to the adult worms and enter into damaged muscle tissue after newborn larvae penetrate them. It is not known what role these cells play in human infection with this parasite. Another positive laboratory finding is an elevation in circulating levels of muscle enzymes (e.g., creatinine phosphokinase (CPK), 1, 6-diphosphofructoaldolase, lactate dehydrogenase aldolases, and aminotransferases. They may be elevated in 35 to 100% of infected individuals and are present in serum due to the destruction of muscle tissue by migrating newborn larvae (16).

2.6 Diagnosis

A muscle biopsy (2 to 4 mm^3), in which the piece of tissue is pressed between two slides and viewed under the microscope, will usually reveal larvae in heavy infections (18) and is thus the most direct measure of the presence of infection. If the diagnosis is attempted before larvae begin to coil (i.e., up to 2 weeks after larvae enter the muscle cell), then there is the risk of confusing the worm with fragments of muscle tissue.

Alternatively, digesting a finely minced portion of the biopsy material in 1% HCl–1% pepsin for 1 h at 37°C will release the larvae from their nurse cells and make them more easily observed under the microscope. Unfortunately, this method, while good for detecting older larvae that are not susceptible to the digestion procedure, is not useful in detecting young larvae that can be destroyed by this process (16).

Routine histopathological examination of the biopsy sample is another method of demonstrating the presence of muscle larvae. Even if larvae are not seen in histological sections, infected muscle cells undergo basophilic changes once they are penetrated by the newborn larvae, providing a clue to their presence. This change is easily noted on standard hematoxylinand-eosin-stained sections because the pattern of striation (i.e., actin and myosin filaments) disappears by the fourth to fifth day after infection (20). Absence of larvae in sections or changes in muscle tissue, however, do not rule out infection, since infection may be light or larvae may simply be missed because of their uneven distribution in muscle tissue. On histopathologic section, it is possible in some cases to determine whether or not the infection is recent or old by observing several characteristics of the nurse cell-parasite complex. The absence of a capsule and the presence of straight (i.e., nonspiraled) worms in the complex indicate that the infection is ongoing. A mature capsule and a coiled parasite indicate an older infection that may have been acquired sometime previous to admission to the clinical setting. Detection of circulating antigens by immunoassay techniques, although not available in most laboratories, can be useful for the diagnosis during the beginning of the parenteral phase, when standard serological tests designed to detect specific

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antibodies have yet to become positive. Although detection of circulating antigen might be a useful confirmatory test (21), circulating antigen is not detected in every patient and its detection is therefore of limited value to the clinician. DNA-based tests have also been reported (22, 23). DNA sequences amplified by PCR have been identified and are specific for *T. spiralis* and other *Trichinella* species as well. This new generation of diagnostic test is not yet available commercially. Antibody detection tests are useful adjuncts to diagnosis starting on about day 12 after infection. By 14 days, when most patients suffering from clinical symptoms seek medical assistance, immunofluorescence-based assays and enzymelinked immunosorbent assays (ELISA) for IgG antibodies may be positive (19) and remain positive for years. The sensitivity of the IgG-ELISA reaches 100% on day 50. The test remains positive for more than 2 years in 88% of infected people (24). Other immunoglobulins (e.g., IgA and IgE) behave in a similar manner, but tests to detect them have a lower sensitivity. The indirect hemagglutination test may be a useful alternative for diagnosing trichinellosis, as 95% of 60 known positive samples were positive by this test, whereas the precipitin and the bentonite flocculation tests were positive in 93.2 and 43.9% of the same samples, respectively (16, 25).

2.7 Therapy

2.7.1 General considerations

In general, treatment of trichinellosis is based on anthelmintics directed against pre-adult or adult forms of *T. spiralis*. Treatment with corticosteroids suppresses hypersensitivity symptoms which may be necessary at the acute stage of the disease. Correcting hypoproteinaemia, electrolyte imbalance and treatment of complications in the later stage of the disease may be necessary. The efficacy of human trichinellosis treatment depends on the intensity. Of the invasion, strain of *Trichinella* involved and the stage at which the treatment is started: early, acute or late stage of the disease (26). The anthelmintic treatment directed against intestinal *Trichinella* is obligatory for each invasion, wheter the invasion is symptomatic or symptomless and is independent of the disease. The treatment is useful up to the sixth week of the invasion. It aims at removing adult forms from the intestine and at preventing or reducing muscle invasion.

2.7.2 Drugs available

The drugs which may be used are: pyrantel at 10 mg per kg bodyweight for 5 days; levamisole at 2.5 mg per kg bodyweight (maximum of 150 mg per day) for 3 days; mebendazole at 5 mg per kg bodyweight for 5 days (usually 200 mg twice daily for adults). Pyrantel is not absorbed from intestinal lumen and kills exclusively intestinal forms of *Trichinella*. As a rule, it is well tolerated. Mebendazole is a partially absorbed drug and affects intestinal forms as well as the muscle larvae. Mebendazole is well tolerated by patients and only exceptionally may induce intense muscle pains, sub-febrile status or high eosinophilia. For this reason in heavy invasions corticosteroids are administered together with mebendazole to suppress an excessive reaction. Thiabendazole (25 mg/kg bodyweight for 5 days) is also effective but is no longer in use in human trichinellosis because of frequent adverse reactions. Albendazole is probably as active as mebendazole against *T. spiralis* infections, in doses 400 to 800 mg per day for 5 to 6 days in adults. As yet, albendazole has not been tested in children suffering from trichinellosis (26).

2.7.3 Preventive treatment

The use of antihelmintics, mainly mebendazole or thiabendazole in the above mentioned doses within the first two days after infection kills or sterilizes *Trichinella* adult worms in the intestine and may prevent trichinellosis (26).

2.7.4 Symptomatic treatment

Application of corticosteroids prolongs the intestinal phase of infection. Therefore corticosteroid treatment is indicated only when symptoms of intestinal trichinellosis appear in parallel with sings of the acute stage of the disease, fever, periorbital oedema, muscle pain, haemorrhage, neurological disturbances, and where suppression of hypersensitive reactions is required.

In cases of intense and long-lasting diarrhea, receiving anthelmintic treatment, concomitant supplementation of water and electrolyte as well as protein deficits is necessary (26).

2.8 Prevention and control

The consumption of raw or rare infected meat from game animals or from pigs raised in situations that favor the existence of rodent populations is the most frequent source of infection by any species of *Trichinella*. Infection of pig herds by *T. spiralis* is usually perpetrated by the animals scavenging on infected rodent populations or, less commonly, by cannibalism of sick animals. Immune pigs experiencing a second infection expel some of their worm burden soon afterwards as first-stage infective larvae, and it is therefore suspected that coprophagy within the barnyard community of pigs may be yet another means by which naive animals are infected. Feeding of raw meat scraps collected from local slaughterhouses to farm animals is illegal in the United States but no doubt occurs whenever the economic situation dictates, since steam cooking scraps is an added cost most farmers cannot easily afford. In other countries, where controls on domestic farm practices are less rigid, feeding raw pork scraps to livestock may or may not be more widespread, but in most situations, meat ‘‘scraps’’ are too valuable a source of human food to end up on the table of the pig. Less common, but with often devastating consequences, the disposal of carcasses of furbearing animals by feeding the remains to farm animals has inadvertently spread *T. spiralis* to large communities of consumers without malicious intent on the part of the farmers, who were unaware of the broad host range of this parasitic nematode (16).

Prevention at the community level depends on proper animal husbandry and on the withholding of uncooked meat in the feed of all farm animals, especially pigs. Microscopic inspection of portions of pig muscle tissue (directly or by the pooled digestion test) can control infection at the level of the abattoir. An ELISA for swine trichinellosis is now approved for the certification of pork by the U.S. Department of Agriculture. However, because there are several options available to meat packers for the certification of pork, it is difficult to convince industry that slaughterhouse testing is cost-effective because trichinellosis is such a low-prevalence disease (0.001%). Such inspection programs are in place in most European countries but have somehow escaped the mandate of the U.S. Department of Agriculture. Thorough freezing of all

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pork products prior to cooking ensures the death of the larvae, while cooking meat at 1,378 °F (588 °C) for 10 min also kills them. Microwave cooking is not 100% effective in killing larvae in large pieces of meat, such as a whole fresh ham, since there are unavoidable ‘‘cold spots’’ in the pattern of the microwave beam. Freezing muscle tissue from game animals (e.g., black bear, raccoon, or opossum) is not effective, since it is thought that the antifreeze protein molecule common to most wild animals also protects worms in their muscle tissue from ice crystal formation and even preserves the worms in carcasses until such time as the carcasses can be consumed by another animal. Some *Trichinella* spp. (i.e., *T. nativa* and T6) can remain infective after several days at freezing temperatures even after they have been isolated from their host muscle tissue (16).

Preventive measures for pork containing temperate zone strains would include refrigeration at 5 F (-15 °C) for not less than 20 days, at -10 °F for 10 days, or at -20 °F for 6 days or deep freezing (-37 °C). Smoking, salting, and drying are not effective. In 1981, the U.S. Department of Agriculture issued a news release that suggested that microwave cooking might not kill the larvae. On the basis of a number of subsequent studies, the current recommendation states that ``all parts of pork muscle tissue must be heated to a temperature not lower than 137° F (58.3 °C)". It has been recommended that an internal meat thermometer be used when one is cooking pork; the meat can be tested after being removed from the microwave oven if the oven is not equipped with an internal thermometer. Reduction in the number of cases is due primarily to regulations requiring heat treatment of garbage and low-temperature storage of the meat. Occasional outbreaks are frequently due to problems with feeding, processing, and cooking of pigs raised for home use (27, 28).

3. Source of Thai human infection

Trichinosis is a parasitic disease of mammals caused by the nematode parasite *Trichinella* spp. It has an important zoonosis with humans becoming infected by eating raw or inadequately cooked infected meat. Infection is more common in omnivores (horses, humans, pigs and rats) and carnivores (cats, dogs, and seals). Pigs and rodents seem to play the most important role in the epidemiology of the disease. The main source of infection in Thailand has been pigs, but wild boar, jackal and

black bear were also reported as sources of trichinosis (29, 30). All trichinosis cases gave a history of having consumed raw pork in the form of “Lahb” and “Naem,” favorite dishes of north Thailand (31). Lahb is made from chopped raw pork mixed with lemon juice, roasted rice powder, finely cut red onion and parsley. Nam is also made from chopped raw pork mixed with salt, garlic and chili, tightly wrapped in banana leaves for a few days for fermentation (2).

4. Naem

Naem is the popular food product to Thai people. It could be consumed uncooked or partially or fully cooked. The production is mostly at small scale, such as cottage industry, to small industry (2).

Fermented pork sausage (Naem) is traditionally made from fresh lean pork that is trimmed; minced; mixed thoroughly with salt, potassium/sodium nitrate/nitrite, cooked rice and seasonings; and packed in either banana leaves (32) or cylindrical plastic bags (33). Naem production in Thailand carries out through the fermentation with lactic acid bacteria and nitrate-reducing bacteria from air, utensils or ingredients which present in nature. It is a long process-generally the fermentation lasts 3–5 days depending on the season. When Naem is packed into cylindrical plastic bags, which exclude air, and is held in the bag during fermentation, a microenvironment is selected for microorganisms that are not only salt tolerant but can also grow in the absent of air. In these Gram-positive fermentative types of microorganisms, lactic acid bacteria are predominant (34-36).

Fermentation traditionally offers an easy and low-energy preservation method for meats that result in distinctive products that have an important part in the diet of people making them. Such fermented meats contribute both nutritional value and pleasure to meals. However, products are not the same from time to time. Indeed, the product may spoil, cause illness due to pathogenic microorganisms or their toxins, and even become lethal due to botulinum toxin production if the normal beneficial microbial flora does not multiply as usual. To prevent these problems, the use of starter cultures has become commonplace in many countries, including developing countries. One example of such fermented meat is Nam, a traditional Thai sausage. Nam is made by mixing salt (3 percent by weight) and garlic with ground lean pork.

Nitrate and nitrite salts also are added in commercial production. The mixture is then wrapped in a banana leaf or stuffed in cellulose tubing. Fermentation is at ambient temperature (about 30°C in Thailand) for 3 to 4 days, after which it remains in good condition for only 1 to 2 days without refrigeration. Since Nam is frequently eaten raw, it is important that pathogenic bacteria be killed as well as that botulinum toxin and staphylococcal enterotoxins are not produced. Since hogs are frequently infested with *Trichinella spiralis,* these larvae should not be viable. A study was conducted on Nam made with and without the addition of one of two levels of a commercially available dry starter culture preparation (37). Portions in polyethylene film bags were inoculated, sealed, and incubated at 30°C. The inoculum was *S. aureus* (a mixture of three enterotoxin-producing strains) and *E. coli* (three strains). Microbial numbers, pH, and titratable acidity were determined at intervals during the fermentation. The meat used was from two hogs that had been experimentally infected with trichinae at weaning; viable trichinae were determined at 24-hour intervals. *S. aureus* was able to multiply (10×) and remain viable only in the control inoculated samples. *E. coli* was not detected at 96 hours in the sausage made with the higher level of starter (1.5 percent by weight) and had decreased greatly in products made with the 0.75 percent level. The use of the higher level of starter preparation resulted in loss of infectivity of the trichinae larvae, although further research is necessary to confirm this effect. The addition of starter culture resulted in more rapid acid production and slightly lower end-point pH. It is important to keep in mind that natural fermentations are difficult to replicate in other settings. For example, the meat mixture for Nam is traditionally wrapped in small banana leaf packets. The leaves contribute to the surface flora of the sausage, which no doubt changes the fermentation pattern. Flora of work surfaces and of the pork itself may be different. Drying often follows fermentation of similar meat products to provide for long-term preservation. Dendeng giling, Indonesian seasoned beef that has only a traditional short fermentation period before drying, was found to have a lower pH and total gram-negative bacteria, staphylococci, and *E. coli* counts when prepared with a starter culture of *Lactobacillus plantarum* than in the traditional manner (38). Those with a starter culture dried more rapidly at 50°C and had lower water activities (2). The effectiveness of lactic acid bacteria in suppressing the multiplication of undesirable microorganisms is largely attributed to the production of

organic acid. However, additional factors include the production of bacteriocins and hydrogen peroxide. More general effects include competition for essential nutrients. To maximize the quality, reproducibility, and safety of the product, strains of bacteria are selected based largely on the qualities of self-stability and viability as used, rapid acid production, and desirable product qualities. As in the starter culture preparation used for Nam, strains of *Lactobacillus* and *Pediococcus* are the most common (39, 40). The compatibility of strains is important, which includes resistance to or lack of production of bacteriocins. In addition to tolerance to the salt and nitrite levels of the mixture, the culture must be active in the temperature range used for the fermentation. The product must have the expected palatability characteristics. No harmful compounds may be produced. These same attributes can be more efficiently arrived at through the application of the techniques of molecular biology. The success of traditional fermentations depends on the complex interaction of the food components, the natural flora of the ingredients, and the surfaces in contact with the food, atmosphere, and ambient temperature. Our knowledge of these conditions is still limited for many of the fermented meats. Alaskan outbreaks of botulism from native sea and land mammal products may have increased as plastic bags became the common container and the fermentation rate was speeded by placing the container near the stove (41). Thus, as transitions occur from traditional fermentations to new adaptations, knowledge of the basic processes becomes essential (42).

5. Lactic acid

The storage life of perishable fish and meats can be extended by acid-fermentation with added carbohydrates and salt. Rice, millet, flour and even syrup or sugar is used as carbohydrate sources. Both salt-water fish and freshwater fish are preserved by acid production from added cereals. The amount of added carbohydrate and the salt concentration primarily control the extent of acid fermentation and the keeping quality. Fermented sausages, similar to salami in Europe, are made in some Southeast Asian countries (43).

Lactic acid bacteria (LAB) are found as the dominant microorganisms in many fermented fish products (44-46). The primary role of LAB is to ferment the available carbohydrates and thereby cause a decrease in pH. The combination of low pH and

organic acids (mainly lactic acid) is the main preservation factor in fermented fish products. Generally, pH should be below 5–4.5 in order to inhibit pathogenic and spoilage bacteria (47). In addition, salt and spices (such as garlic, pepper or ginger) may add to the safety of products. Also, in some products garlic may serve as a carbohydrate source for the fermentation (48). The salt concentration may range from one to 10% (w/w) in different types and batches of fermented. This is likely to have a pronounced influence on the microbial growth and the rate of fermentation, and thereby on the sensory quality and safety of the product. It is therefore of interest to identify the optimal salt concentration, which does not inhibit the growth of the fermenting microorganisms, and in addition contributes positively to the flavour and texture of the product (49).

Functions of Lactic Acid Bacteria

The growth and activity of microorganisms play an important role in controlling the whole environment and ecosystem. The type of bacterial flora that develops in each fermenting food depends on the water activity, pH, salt concentration, temperature and the composition of the food. Lactic acid bacteria are perhaps the most widespread and desirable microorganisms in food fermentations. They convert most available carbohydrates to lactic acid, with small amounts of acetic acid, resulting in a lowering of the pH. If the fermentation is prolonged, the environment will be changed to become more suitable for yeast growth (50). *Lactobacillus penfosus* and *L. plantarum* strains that contain meso-diaminopimelic acid in the cell wall are the predominant rod-shaped LAB in fermented Thai foods. *Pediococcus petifusaceus* strains are the major coccal bacteria although *P. halophilus* strains occur in products containing high concentrations Strains of *L. sake, L. fermentum, L. brevis, L. confuses* other *Lactobacillus* spp., *Pediococcus acidilactici,* and *Leuconosnoc* spp. are the minor LAB in fermented Thai foods. *Lactobacillus pentosus, L. plantarum, L. fermenturn* and *Pediococcus pasaceus* are widely distributed, but the LAB are not generally specific for one kind of fermented product though the concentration of salt influences the flora of LAB. The lactobacilli containing meso-diaminopimelic acid in the cell wall, however, are usually found in fermented tea leaves (miang). Such bacteria include *L. pentosus, L, planfarum, L.*

vaccinosfercus and some other *Lactobacillus* spp. (51, 52). The tannin in miang may have an effect on the growth of these cultures.

Halobacterium salinarium produces proteases, which are important for fish-sauce fermentation (53). Staphylococci from fermented fish, which are coagulase-negative and haemolysin-negative (54, 55), produce a small amount of lactic acid but do not seem to play a role in the fermentation and ripening of the fermented foods. *Staphylococcus carnosus* strains were originally found in dry sausage and in a starter for the production of sausages in Germany (56). Not only can they reduce nitrate but they can also destroy peroxidases during sausage fermentation (57). Enterococci, especially *E. faecajis* and *E. faecium* strains, are common organisms in the intestinal tract of man and other animals and it is difficult to keep them out of foods (58). Their presence in fermented foods may indicate inadequate sanitary practices. In western and European countries (59, 57, 50), a variety of LAB is involved in the making of bread, cheese, fermented milk, sausage and fermented vegetables. LAB has been employed empirically in these areas for a long time; the main LAB fermentation leads to the production of lactic acid, which contributes to the preservation of the foods. Most of the meat, dairy and vegetable food fermentations in these areas are now based on the use of dried, freeze-dried or frozen starter cultures, In addition to lactic acid, some starter cultures produce antimicrobial properties (60). Bacteriocins of several LAB, including nisin and lactacin, inhibit spoilage bacteria and pathogens such as *Listeria monocyfogenes, Saphylococcus aureus, Clostridium botulinum* and *Bacillus* spp. (61). *Lactobacillus acidophilus, L. sake, L. bulgaricus, L, helveticus, L. curvatus, L. plantarum, Lactococcus lactis, Lactococcus cremoris, P. pentosaceus* and *P. acidilacfici* strains produce lactacin, sakacin, buIgaricin, helveticin, lactocin, plantaricin, nisin, diprococcin, lactostrepcin and pediocin (60, 61), Lactic acid bacteria are widely distributed and dominant in many kinds of fermented foods in Thailand. They occur naturally with other microorganisms in mixed-culture fermentations. Recently several researchers have tried to use LAB as starters in the fermentations of fermented pork and vegetables (62).

6. Garlic

Garlic (*Allium sativum*) has been used as a medicine and health-promoter for 5,000 years. It was widely used in ancient Assyria, Egypt, India, Greece and China. Garlic was used in medieval and Renaissance Europe to treat poisons, bites, edema, ulcers, toothaches, plague and smallpox (63). Albert Schweitzer used garlic in Africa to cure cholera and typhoid in the early 20th century. Garlic was widely used in Europe, especially England, to treat war wounds and dysentery during World War I (63). In modern Europe and the U.S. garlic supplements are widely used. There were at least 1,200 pharmacologic studies done on garlic by mid-1997, as well as many hundreds of studies on the chemistry of garlic (63).

There are more than 300 varieties of garlic being grown around the world, but the three major types are American, Mexican and Italian Elephant. American garlic is white-skinned and the most strongly flavored. Mexican and Italian both have mauve-colored skins and a slightly milder flavor. Elephant (also called Giant or Spanish) garlic yields cloves that are two to three times larger than common garlic. It carries the mildest flavor, and in fact, is not true garlic but a relative of the leek (64).

The chemistry of garlic is extremely complex, but research has shown that it is the unusual organosulfur compounds relatively unique to garlic that promote its broad range of lipid-lowering, antithrombotic, anti-blood coagulation, anti-hypertension, anticancer, antioxidant, and antimicrobial effects. The most well-known and widely studied garlic compound is allicin, yet ironically allicin does not exist in fresh, undamaged garlic cloves. The predominant garlic sulfur compound is alliin.1 Garlic also contains high levels of an enzyme called “allinase.”(63).

When fresh garlic cloves are crushed or chopped, or garlic powder that has been carefully dried to preserve its alliin/allinase content is added to water, allicin is produced in seconds by the action of allinase on alliin (63). Allicin and other thiosulfinates are somewhat unstable, but dilution and dissolving in water “greatly improve their stability” (63). Allicin can decompose into a broad range of compounds, including S-allylmercaptocysteine, allylmercaptan, diallyl disulfide, allylmethyl disulfide, vinyldithiins, ajoene, and possibly allylsulfinic and allylsulfonic acid (63). Cavallito and Bailey first reported in 1944 that allicin is the garlic compound chiefly responsible for the broad-spectrum antibacterial action of garlic (65). Lawson has noted that various actions of garlic, such as its cholesterol-lowering and antibacterial

effect, are primarily due to its allicin content, since removal of alliin from garlic, or inactivation of allinase by microwave cooking, eliminates these effects, while adding allicin back into garlic powders so treated restores such garlic's anticholesterol/ antibacterial activity (63).

Allicin is apparently well-absorbed. An animal study with radioactive-labeled allicin showed 79 percent absorption within 30-60 minutes after oral intake, with 65 percent excretion of radioactive allicin metabolites within 72 hours (63). Four animal and five human studies have shown that orally consumed crushed garlic and allicin-related compounds have systemic antimicrobial effects in the lungs, kidney, brain, blood and cerebrospinal fluid, further showing absorption and activity of allicin and its metabolites (63)

Allicin is the gold standard of garlic because of the centrality of allicin and its metabolites to the health benefits of garlic, many garlic supplements are standardized to yield a certain "allicin potential," for example, "allicin potential: 10,000 ppm." When garlic cloves are properly prepared and dried, the alliin and allinase activity of fresh whole garlic are preserved. When such dried garlic powder is added to water, the alliin and allinase quickly react and allicin is produced. This is how "allicin potential" is measured.3 However, the situation is completely different when such garlic supplements are swallowed. Allinase enzyme is rapidly and completely destroyed by stomach acid (66). Many garlic supplements are coated with a special coating that protects the garlic from stomach acid, but dissolves in the alkaline conditions of the small intestine, where the allicin should then theoretically be produced. Unfortunately such supplements usually don't work as designed. Lawson and Wang reported the results of testing 23 coated, U.S. garlic supplements in 2001 (66). Twenty of 23 failed to release even 15 percent of their claimed "allicin potential" when placed in simulated intestinal fluid. Lawson and Wang concluded that allicin potential is an extremely poor measure of garlic supplement activity in the human body.

The benefits of allicin

Allicin is the chief thiosulfinate (about 75 percent of total) formed when fresh raw garlic is crushed, chopped or chewed (63). Lawson reports there is "good evidence" that allicin and possibly other thiosulfinates are the main compounds essential to garlic's antimicrobial, lipid-lowering, antithrombotic,

fibrinolytic, antioxidant, anticancer and pro-immune effects (63). He notes: “This does not mean that all of the effects of garlic are due solely to the thiosulfinates, but no other compound has yet been identified with significant activity at levels present in whole or crushed garlic” (63). Perhaps allicin’s most important power in our modern age of antibiotic-resistant germs and ever-new microbial diseases (SARS, flesh-eating Strepto-coccus, West Nile encephalitis virus, AIDS, etc.) is its amazingly broad-spectrum antimicrobial activity. In their 1999 review of allicin’s antimicrobial activities, Ankri and Mirelman report on the antibacterial, antifungal, antiparasite, antiviral activity of allicin (67). They note that a broad range of bacteria, including *E. coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Pseudomonas aeruginosa, Acetobacter baumanii, Klebsiella pneumoniae, Enterococcus faecium, Mycobacterium tuberculosis, H. pylori, Salmonella*, *Clostridium* and *Shigella* are allicin-sensitive (67). Garlic extract can inhibits the growth of different species of bacteria, including *Mycobacterium tuberculosis*, the organism responsible for tuberculosis. However, very high concentrations of garlic extract were needed to slow down the growth of *M. tuberculosis*, so some experts are concerned that these levels may be toxic to people. While further research in people is needed, one animal study found that garlic oil (which is a higher concentration than the extract) also inhibited *M. tuberculosis* and reduced the tuberculosis lesions in the lungs of these animals. Some scientists speculate that a combination of garlic extract or garlic oil with anti-tuberculosis drugs may eventually prove effective against the disease. The large quantities of fresh, raw garlic may have antiparasitic properties against the roundworm, *Ascaris lumbricoides*, which is the most common type of intestinal parasite. Garlic for this purpose, however, has not yet been tested in people. Some of the bacteria listed are killed by allicin concentrations as low as 3-15 ppm (3-15 mg/ml) (67). Fortunately, friendly bacteria such as *Lactobacillus, Enterococcus* and *Pediococcus* are fairly resistant to allicin (63, 68). They also note that allicin synergizes with antibiotics, and that most bacteria are unable to develop resistance to allicin (68). They also report from their own research that various multi-drug resistant bacteria are also effectively killed by allicin, some at doses as low as 15-30 ppm (15-30 mg/ml) (67). Allicin has a powerful antifungal effect, with a minimum inhibitory concentration (MIC) against various Candida species of only 0.15 to 0.8 mcg/ml, and

is effective against other fungal species of *Cryptococcus, Trichophyton, Epidermophyton* and *Microsporum* at MIC of 1.57-6.25 mcg/ml (67). Allicin has shown antiparasite activity at 30 mcg/ml against *Entamoeba histolytica, Giardia lamblia,* and *Leshmania* (67).

7. Related Articles

Ankri (1997 p. 2286) (3) present evidence of the remarkable inhibitory effect that pure allicin has on the ability of amebic trophozoites to destroy monolayers of baby hamster kidney cells and on the inactivation of the various cysteine proteinases. In 1994 Lun, Burri, Menzinger and Kaminsky (68) to studied the of activity of diallyl trisulfide (Dasuansu) on human and animal pathogenic protozoa (*Trypanosoma* sp., *Entamoeba histolytic*a and *Giardia lamblia*) in vitro. The results indicated that the compound has potential to be used for treatment of several human and animal parasitic diseases.

The paper title "Evaluation of the antiparasitic effect of aqueous garlic (*Allium sativum*) extract in hymenolepiasis nana and giardiasis" (5) the author studied the effect of serial dilutions of crude garlic (*Allium sativum*) extract on adult *Hymenolepis nana, A. sativum* was found to be efficient, safe and shortens the duration of treatment. The possible mode of action of *A. sativum* and the correlation between the clinical and parasitological findings were discussed.

In 1990 Campos R et al (69) have shown the usefulness of garlic (*Allium sativ*um) for the treatment of ascaridiasis, trying to confirm the popular belief on this matter. Little efficacy was observed, and this fact enhances the importance of not accepting propositions not based on adequately conducted studies.

Grudzinski, Frankiewicz-Jozko and Bany (70) studied the antioxidant capacity of diallyl sulfide (DAS) in the course of experimental trichinellosis in mice. The results suggest that diallyl sulfide may be an effective antioxidant candidate and may therefore play a significant role in the defense against lipid peroxidation in trichinellosis.

Anthony, Fyfe and Smith (71) studied plant essential oils (and/or active components) can be used as alternatives or adjuncts to current antiparasitic therapies. Garlic oil has broad-spectrum activity against *Trypanosoma, Plasmodium, Giardia*

and *Leishmani*a, and *Cochlospermum planchonii* and *Croton cajucara* oils specifically inhibit *Plasmodium falciparum* and *Leishmania amazonensis*, respectively. Some plant oils have immunomodulatory effects that could modify host–parasite immunobiology, and the lipid solubility of plant oils might offer alternative, transcutaneous delivery routes. The emergence of parasites resistant to current chemotherapies highlights the importance of plant essential oils as novel antiparasitic agents.

CHAPTER III
MATERIALS AND METHODS

Experimental Design

This study was a true experimental design in the type of 3x3 factorial experiment. The first 3 (factor A) is a quantity of concentrated garlic juice which is 1 %w/v, 4 %w/v and 12 %w/v. The second 3 (factor B) is a weight of steamed sticky rice transmuted into lactic acid which is 4 gm, 10 gm and 20 gm.

Unit of Analysis

T. spiralis infected mice and non-infected ones.

Experimental Process

1. Preparation of *T. spiralis* Larvae

T. spiralis larvae used in this study were from Pakpimol Mahannop doctoral dissertation Mahidol University. Larvae were selected from mice at age of four weeks. Each mouse was infected with 100 larvae in only one infection and maintained for 4 weeks. These mice were bred and supplied by the National Laboratory Animal Center, Mahidol University.

2. Collection of *T. spiralis* Larvae from Infected Laboratory Mice

The infected laboratory mice were sacrificed 4 weeks after infection. The bellies were cut opened longitudinally. After the skin, head, tail, tarsus, metatarsus, carpus, metacarpus and all visceral organs (except diaphragm) of each mouse were removed, the remaining carcasses were chopped and digested with 1% acid pepsin-HCl proportion 1: 10 (meat 1 gm per 1% acid pepsin-HCl 10 ml) in a 37°C shaking water bath for 2 hours. The preparation was allowed to set by the modified Baermann’s technique. The active larvae were collected from the

Baermann's apparatus and washed with 0.85% normal saline. Larvae were divided into 50 larvae per experiment.

3. Preparation of Garlic and Lactic Acid from Fermented Steamed Sticky Rice

3.1 Preparation of Garlic Juice

The garlic used in this study was from Sri Sa Kate province, harvested in November to December of 2007. Garlic was peeled and blended by electric blender, and then weighed at 1 gm, 4 gm and 12 gm; after that each pile of blended garlic was added by 100 ml of water as illustrated in the experiment layout (Figure 3).

3.2 Preparation of Lactic Acid

Pork was blended by electric blender and weighed at 100 gm per group. One gram of salt was added to each group of pork and mixed. Then, lactic acid was prepared by adding the steamed sticky rice into the mixture of blended and salt, and the blended garlic and steamed sticky rice into the mixture of blended and salt, as illustrated in the experiment layout (Figure 4 and 5). Such mixed materials were fermented in the plastic bag at 30°C for 3 days and after that the fermented fluid was sieved and pH value was checked by pH paper.

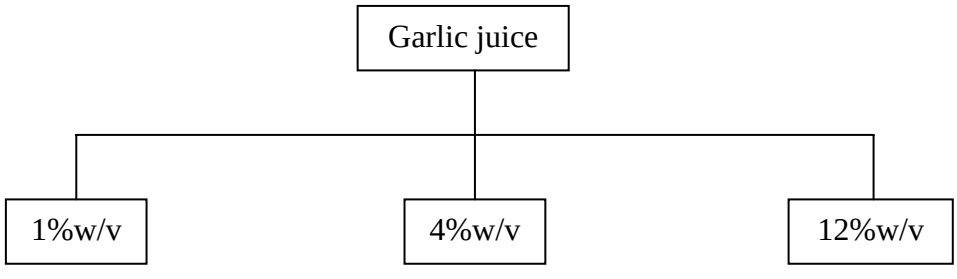

Figure 2: Experimental layout for garlic juice preparation

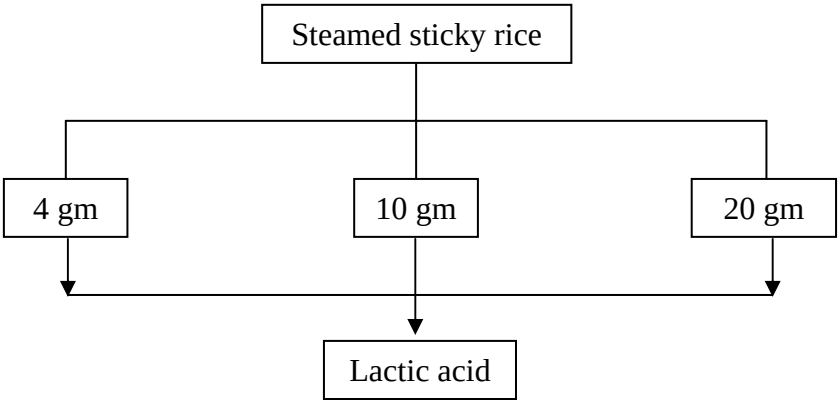

Figure 3: Experimental layout for preparation of lactic acid from fermented steamed sticky rice

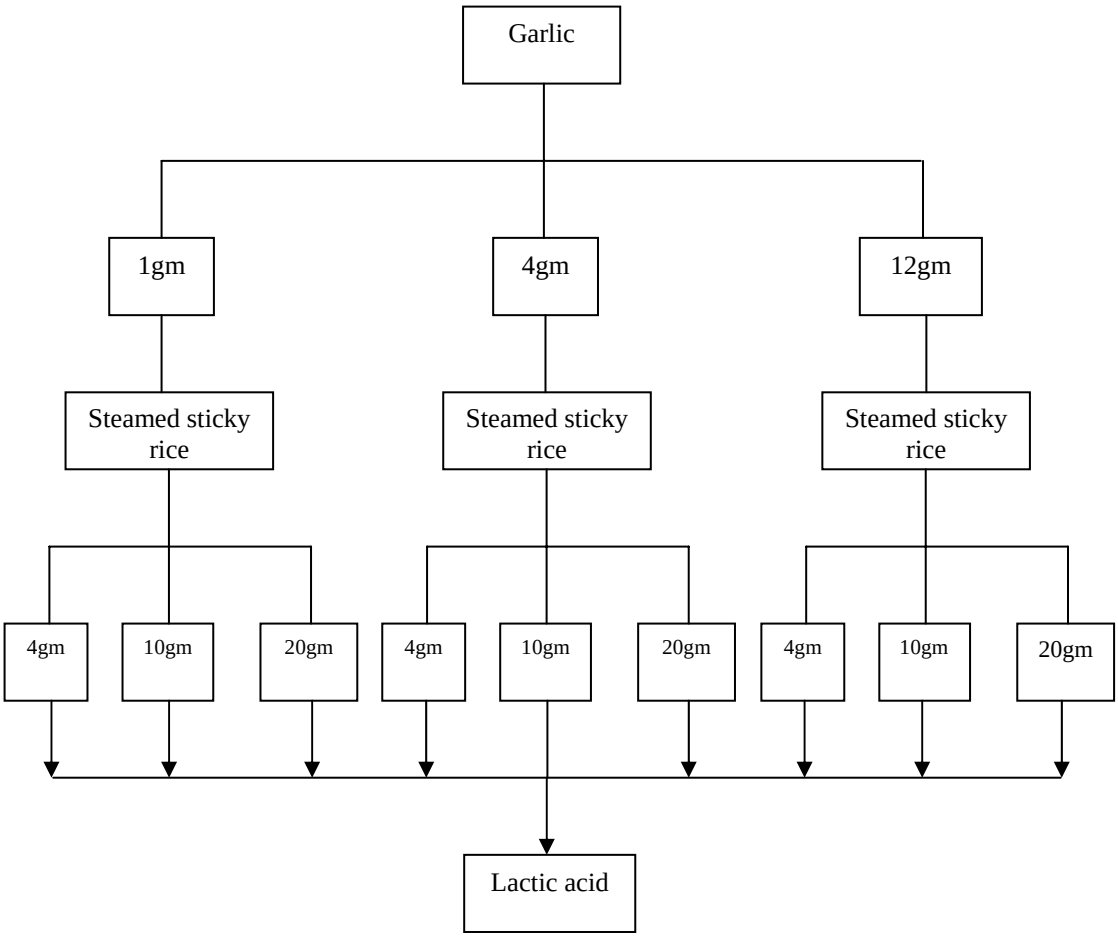

Figure 4: Experimental layout for preparation of lactic acid from fermented blended garlic combined with steamed sticky rice

4. Examination for the Effect of Garlic and Lactic Acid from Steamed Sticky Rice on Viability of *T. spiralis* Larvae

4.1 Examination for the Effect of Garlic

The larvae were moved into the glass block; each group was done in the triplicate experiment and each glass block was provided for one experiment 50 larvae were sucked into a glass block and 100 µl of garlic juice was pumped into it (According to Figure 2). Larvae movements were checked under microscope. Then, all experiments were left at 30°C for 18 hours. After examining viability of *T. spiralis* larvae, larvae movement was checked again.

4.2 Examination for the Effect of Lactic Acid from Steamed Sticky Rice

The larvae were moved into the glass block; each group was done in the triplicate experiment and each glass block was provided for one experiment. 50 larvae were sucked into a glass block. 100 µl of fermented fluid from steamed sticky rice was then pumped into it (According to Figure 3 and 4). Larvae movements were checked under microscope. Then, all experiments were left at 30°C for 18 hours. After examining viability of *T. spiralis* larvae, larvae movement was checked again.

The control group in the experiment:

Positive control: feeding 50 larvae to mice.

Negative control: mice not feeding by larvae.

Control: larvae to be examined for viability with fermented fluid from fermented blended pork and salt without garlic and steamed sticky rice and then feeding larvae to mice, its was the control of experimental.

5. Feeding of *T. spiralis* Larvae to Mice

Female mice at age of 3-4 weeks were provided by the National Laboratory Animal Center, Mahidol University for the experiment. Those mice were divided into 18 groups: 3 mice per group. *T. spiralis* larvae in 0.85% normal saline were fed into mice 50 larvae/mice by using polyethylene tube insert into alimentary

canal of mice, the larvae feeding was not provided for the mice in negative control group.

6. Examination for Infectivity of Larvae

Larvae circulated in the lymphatic system or mesenteric venules to the body tissues, primarily striated muscle. The deposition of larvae continued for approximately 4 weeks. The infected mice were operated by longitudinally through the belly. The diaphragm was removed and put on the glass block. The tissue was then covered with another glass before pressing the glass until the tissue was broken. The infection of larvae in the diaphragm was checked under microscope.

7. Statistical Analysis

- The association between the result of experiment and the study group by using χ^2-test and used Fisher's extract when the expected cell value less than 5.

CHAPTER IV
RESULTS

In this study, garlic and lactic acid from fermented steamed sticky rice were tested for viability and infectivity of *T. spiralis* larvae. The garlic were blended and added with 100 ml of water (%w/v), and the lactic acid was prepared by pork and steamed sticky rice after that fermented for 3 days. The lactic acid was tested for its sour flavor and pH was lower. Thus in this study, pH of lactic acid from fermented steamed sticky rice was checked and examined for the viability and infectivity of *T. spiralis* on mice.

pH of Lactic Acid in Fermented Steamed Sticky Rice

The lactic acid from fermented steamed sticky rice was prepared for the experiment by blending pork, salt, steamed sticky rice and garlic before fermenting all blended materials for 3 days. After that, pH value was measured by using pH paper. The pH of the control group was high (pH 9). The lactic acid and sticky rice were prepared for every experiment: steamed sticky rice for 4 gm, 10 gm and 20 gm. The pH in the experiment from steamed sticky rice at 4 gm was the highest and steamed sticky rice at 10 gm was the second highest, and the lowest was steamed sticky rice at 20 gm (pH 6, 5.5 and 5 respectively). The details were shown in Table 1.

Isolation of *Trichinella spiralis* from Infected Laboratory Mice

T. spiralis larvae were provided from the doctoral dissertation research conducted by Pakpimol Mahannop of Mahidol University. The larvae were collected for mice at age of 4 weeks: each mouse was infected with 100 larvae per mouse that were maintained for 4 weeks. After that the isolation of *T. spiralis* larvae, 2,550 larvae were divided into 17 experiment groups: 50 larvae per experiment and each group was subdivided into 3 experiments or totaling 51 experiments. The larvae in diaphragm were coiled in capsule (Figure 5) and then mice were chopped and digested in 1% acid

pepsin-HCl at 37°C for 2 hours. After that the digested larvae were collected by Baermann technique and washed by 0.85% normal saline. Larvae had some movement.

Table 1: pH of lactic acid in fermented steamed sticky rice for each experiment.

Experiment	pH
Control	9
Steamed sticky rice (4 gm)	6
Steamed sticky rice (10 gm)	5.5
Steamed sticky rice (20 gm)	5
Garlic (1 gm) + Steamed sticky rice (4 gm)	6
Garlic (1 gm) + Steamed sticky rice (10 gm)	5.5
Garlic (1 gm) + Steamed sticky rice (20 gm)	5
Garlic (4 gm) + Steamed sticky rice (4 gm)	6
Garlic (4 gm) + Steamed sticky rice (10 gm)	5.5
Garlic (4 gm) + Steamed sticky rice (20 gm)	5
Garlic (12 gm) + Steamed sticky rice (4 gm)	5.5
Garlic (12 gm) + Steamed sticky rice (10 gm)	5
Garlic (12 gm) + Steamed sticky rice (20 gm)	5

Examination for viability and infectivity of *T. spiralis* larvae

After the digested larvae were collected by Baermann technique, larvae had some movement. But when examining viability with garlic juice and lactic acid from fermented steamed sticky rice for 18 hours, larvae in all experiments were motiveless, they were coiled (Figure 6). After that viability and infectivity in mice fed by larvae were examined: one moue for one experiment mouse and that mouse has been maintained for 30 days. Infectivity on diaphragm was then checked. The results showed that there was an infection of *T. spiralis* larvae in diaphragm of the control group. Infection was found in 3 mice from the total of 3 mice. For the steamed sticky rice group, infection was found in 8 of 9 mice. For the garlic combined with steamed sticky rice group, infection was found in 21 of 30 mice. But the infection was not

found in other groups with *T. spiralis* larvae in diaphragm such as garlic juice group. The details were shown in Table 2.

Table 2: Numbers of infected and non-infected mice with *T. spiralis* larvae in diaphragm.

Experiment	Number of mice in experiment	
	Total mice	Infectious mice
Positive control	3	3
Control	3	3
Garlic (1 %w/v)	3	0
Garlic (4 %w/v)	3	0
Garlic (12 %w/v)	3	0
Steamed sticky rice (4 gm)	3	3
Steamed sticky rice (10 gm)	3	3
Steamed sticky rice (20 gm)	3	2
Garlic (1 gm) + Steamed sticky rice (4 gm)	3	1
Garlic (1 gm) + Steamed sticky rice (10 gm)	3	3
Garlic (1 gm) + Steamed sticky rice (20 gm)	3	0
Garlic (4 gm) + Steamed sticky rice (4 gm)	3	3
Garlic (4 gm) + Steamed sticky rice (10 gm)	3	2
Garlic (4 gm) + Steamed sticky rice (20 gm)	3	2
Garlic (12 gm) + Steamed sticky rice (4 gm)	3	2
Garlic (12 gm) + Steamed sticky rice (10 gm)	3	3
Garlic (12 gm) + Steamed sticky rice (20 gm)	3	2

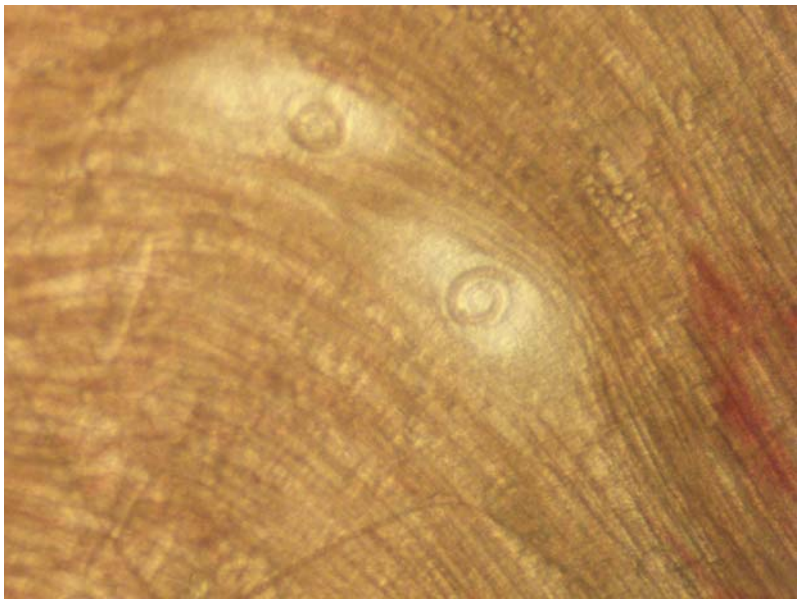

Figure 5 *Trichinella spiralis*'s larvae in mice's diaphragm

Figure 6 Movement of larvae after soaked in garlic juice and lactic acid from fermented steamed sticky rice for 18 hours

Table 3: Number of mice recovers *T. spiralis* larvae in diaphragm

Group	Total mice	Infected mice	% of infection
Control	3	3	100
Garlic	9	0	0.0
Steamed sticky rice	9	8	88.9
Garlic + Steamed sticky rice	30	21	70

The numbers of infected mice were analyzed by completely raised program. The positive control group and the negative control group were not analyzed because they were groups of quality control mice in this experiment.

Table 4: The association between the control group and the experiment group

Group	Infected mice	P-value
Control and Garlic		0.005
Control and Steamed sticky rice		0.750
Control and Garlic combined steamed sticky rice		0.328

- Association of the control group and the garlic group

Comparing the relationship of infectivity of *T. spiralis* larvae between the control group and the garlic group, there was a significance at $\alpha = 0.05$ (p-value = 0.0045), which meant that garlic could inhibit larvae infection when compared with the control group.

- Association of the control group and the steamed sticky rice group

Comparing the relationship of infectivity of *T. spiralis* larvae between the control group and the steamed sticky rice group; there was no significance at $\alpha = 0.05$, which meant that steamed sticky rice could not inhibit larvae infection when compared with control group.

- Association of the control group and the garlic combined steamed sticky rice group

Comparing the relationship of infectivity of *T. spiralis* larvae between the control group and the garlic combined steamed sticky rice group; there was no significance at $\alpha = 0.05$. This meant that garlic combined with steamed sticky rice could not inhibit larvae infection when compared with the control group.

CHAPTER V
DISCUSSION

Human infection is initiated from the ingestion of raw or poorly cooked pork, bear, walrus or horse meat or meat of other mammals (carnivores and omnivores) containing viable, infective larvae. The tissue is then digested in the stomach. The encysted larvae then invade into the intestinal mucosa, develop through four larval stages, become mature and mate by the second day. By the sixth day of infection, the female worms begin to deposit motile larvae, which are carried by the intestinal lymphatic system or mesenteric venules to the body tissues, primarily striated muscle. Deposition of larvae continues for approximately 4 weeks: each female larva produces up to 1,500 larvae in the non-immune host (28, 29).

Preventive measures for pork containing temperate zone strains would include refrigeration at 5°F (-15°C) for not less than 20 days, at -10°F for 10 days, or at -20°F for 6 days or deep freezing (-37°C). Smoking, salting, and drying are not effective. In 1981, the U.S. Department of Agriculture issued a news release suggesting that microwave cooking might not kill the larvae. On the basis of various subsequent studies, the current recommendation states that “all parts of pork muscle tissue must be heated to a temperature not lower than 137°F (58.3°C)” (28, 29).

All trichinellosis cases gave a history of having raw pork in the form of “lahb” and “naem”, favorite dishes at the north of Thailand (2). Lahb is made from chopped raw pork mixed with lemon juice, roasted rice powder, finely cut red onion and parsley. Naem is also made from chopped raw pork mixed with salt, garlic and steamed sticky rice before being fermented for a few days. Some Thai dishes are proven as viable *T. spiralis* larvae sources due to cooking procedures, especially Naem that may be eaten in the raw or undercooked form. The materials of Naem included the blended pork, salt, garlic and steamed sticky rice. The role of salt in the fermentation process is to control the microbiological spoilage, contamination and to supply good taste; garlic has a good smell and controls the microbiological spoilage too. Garlic also

possesses the ability of inhibiting the growth of parasites in the intestines, including amoebas which cause dysentery. It should be noted that amoebic dysentery is a potentially serious condition which requires the assistance of a trained physician. Garlic has also been used in the traditional medicine in many parts of the world to treat pinworms, an annoying but generally harmless intestinal parasite (6). Steamed sticky rice was the carbohydrate source of lactic acid bacteria transmuted into lactose and then transmuted into lactic acid. Naem has a sour taste and inhibits growth of microorganism too. This study included 3 different quantities of garlic and lactic acid from fermented steamed sticky rice and its effect on viability and infectivity of *Thichinella spiralis* in mice. Amplified larvae in mice as collected by Baermann technique are examined to find out viability and infectivity. After we collected larvae, they were motile and active. When examining viability in garlic juice and lactic acid from fermented steamed sticky rice and the movement of the larvae was checked for 5 minutes, they were motile and active, and then they were kept at 30°C for 18 hours. Larvae did not move, and were found to be coiled. When examining infectivity in mice, the garlic group was not infected, but other groups became infected; therefore, the larvae died when they were examined for viability and infectivity in the garlic group, but they survived when examining for viability and infectivity in steamed sticky rice group and garlic combined with steamed sticky rice groups because larvae could be infected in mice. Although larvae did not have any movement, they might be shocked when they were in the concentration of garlic and fermented fluid, and the reflex of larvae cause it to be coiled, but larvae did not die and could infect mice.

When viability was examined on garlic juice and lactic acid from fermented steamed sticky rice and put in 30°C for 18 hours, larvae rolled and exhibited no movement; the positive control group was the same. The concentration of garlic juice and lactic acid from fermented steamed sticky rice made larvae suffer and shocked, this was a reflex of larvae to concentration or to become momentarily inactive. Then, they were fed to mice the result showed that, in the garlic group, infected larvae in mice were not found, so garlic could inhibit viability of larvae. For the groups of fermented fluid from steamed sticky rice and fluid fermented from steamed sticky rice combined with garlic, the infection was found in mice, so those two groups may not inhibit viability of larvae.

Larvae infection were inhibited by garlic. This correlated with Soffar S.A and Mokhtar G.M.(5), Grudzinski IP, Frankiewicz-Jozko A and Bany J. (70). A flavor component found in garlic *(Allium sativum)* played a role in the inhibition of chemically active compounds. One of its major components, Allicin, is known that it contains antibacterial and antifungal activity for a long time. Diallyl trisulfide is a chemically stable final transformation product of Allicin which was synthesized. But, in this study, garlic was mixed with blended pork, salt and steamed sticky rice, it did not inhibit infectivity of larvae, perhaps because the concentrate of components in the garlic were diluted and absorbed into the pork, and during a long fermentation time, such components evaporated.

Garlic used in this study was from Sri Sa Ket province, harvested in November to December. The types were American garlic; it was white-skinned and the most strongly flavored (65), with a small clove size and many cloves.

Lactic acid from fermented steamed sticky rice did not inhibit infectivity of larvae and the reason was a lowering concentrate, but no research presented effects of lactic acid on the parasite. The future projects will study the effect of lactic acid on the parasite.

In this study, the pH of fermented steamed sticky rice was 6.0-5.0 but the pHs of lactic acid in commercial Naem were 5.5-4.0. The pHs in experimental conditions were higher than in that of commercially-produced Naem, because the weight of steamed sticky rice was lower. In 20 gm steamed sticky rice with pH 5.0 in the experiment, nearly the pH of commercially-produced Naem whereas 10 gm and 4 gm of steamed sticky rice had pHs of 5.5 and 6.0 respectively. The result showed that when the weight of steamed sticky rice was increase, the pH of fermented Naem was decrease but when the weight of steamed sticky rice was decrease, the pH was increase. Because steamed sticky rice was the carbohydrate source of lactic acid bacteria (LAB), LAB was the main mechanism of fermentation and it converted most available carbohydrate to lactic acid, resulting in a lowering of the pH. But in the control group, fermented without steamed sticky rice, pH was high (pH 9.0).

A positive control group and a negative control group were used in this study. The positive control group was the group with controlled active larvae, and the negative control group was the group with controlled infected mice. In this study,

larvae were active, and mice were cleaned and not infected with *T. spiralis* before being used in this study.

Larvae were examined for viability fed to mice that have been maintained for 5 days, when viability was checked at the intestinal area of mice. Larvae grew into adult worms after 5 days and they moved to intestinal area. The result showed that, in the garlic groups, the adult worms were not found in the intestinal area. However, for the lactic acid from fermented teamed sticky rice groups and lactic acid from the fermented steamed sticky rice combined with garlic groups, the adult worms were found. Thus, the result confirmed the effect of garlic to inhibited viability of *T. spiralis* larvae.

CHAPTER VI
CONCLUSION

Steamed sticky rice was the carbohydrate source of lactic acid bacteria (LAB); it converted most carbohydrates into lactic acid. In this study, we prepared some fluid fermented from blended pork, salt, steamed sticky rice and garlic for 3 days. Each experiment had a different factor in each group. Factors in this study were steamed sticky rice and garlic. The result showed that when the weight of steamed sticky rice was increase, pH was decrease (pH 6-5). For the control group without steamed sticky rice, pH was high (pH > 7)

In the isolation of *T. spiralis* larvae by Baermann technique, larvae were active and had some movement when observing viability on garlic juice, lactic acid from fermented steamed sticky rice and lactic acid from fermented steamed sticky rice combined with garlic, which were kept at 30°C for 18 hours. The larvae became coiled and had no movement. The ability of larvae was also observed and infection on mice's diaphragm was checked. The result showed that there was no infection of larvae in the garlic groups, so garlic could inhibit viability and infectivity of *T. spiralis* larvae. The result showed that there was an infection of larvae in lactic acid from fermented steamed sticky rice groups and lactic acid from fermented steamed sticky rice combined with garlic groups. These results showed that these groups did not inhibit viability and infectivity of *T. spiralis* larvae.

Suggestions from Research Finding

1. *T. spiralis* larvae used in this study were not natural larvae (encapsulated).

2. *T. spiralis* larvae used in this study were non-encapsulated. When larvae were soaked in garlic juice for examining viability, the chemical in garlic might be affected directly to larvae. This situation caused the infected of larvae was not found. Thus garlic juice was not found infected *T. spiralis* larvae in mice.

3. Although the method of sedimentation by Baermann technique could collect the active larvae when kept at 30°C for 18 hours, the larvae became coiled and inactive because the were excysted that made them shocked and inactive.

Recommendation for Future Research

1. To study encysted larvae for examined the effected of garlic and lactic acid from fermented steamed sticky rice on viability and infectivity of encysted *T. spiralis* larvae.

2. Examined the effected of flesh garlic and other mixture in natural of Naem on viability and infectivity of *T. spiralis* larvae.

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APPENDIX

Numbers of infected and non-infected mice with *T. spiralis* larvae

Table 5: Numbers of infected and non-infected mice with *T. spiralis* larvae

Group	Treatment	Replications number	Infectious mice	Non infectious mice
1	Control	1	+	-
		2	+	-
		3	+	-
2	Garlic 1%w/v	1	-	+
		2	-	+
		3	-	+
3	Garlic 4%w/v	1	-	+
		2	-	+
		3	-	+
4	Garlic 12%w/v	1	-	+
		2	-	+
		3	-	+
5	Steamed sticky	1	+	-
	rice 4 gm	2	+	-
		3	+	-
6	Steamed sticky	1	+	-
	rice 10 gm	2	+	-
		3	+	-
7	Steamed sticky	1	+	-
	rice 20 gm	2	+	-
		3	-	+
8	Garlic 1 gm +	1	+	-
	Steamed sticky	2	-	+
	rice 4 gm	3	-	+
9	Garlic 1 gm +	1	+	-
	Steamed sticky	2	+	-
	rice 10 gm	3	+	-

Table 5. Numbers of infected and non-infected mice with *T. spiralis* larvae (Continues)

Group of experiment	Experiment	No. experiment	Mice were infected larvae	Mice were not infected larvae
10	Garlic 1 gm +	1	-	+
	Steamed sticky	2	-	+
	rice 20 gm	3	-	+
11	Garlic 4 gm +	1	+	-
	Steamed sticky	2	+	-
	rice 4 gm	3	+	-
12	Garlic 4 gm +	1	+	-
	Steamed sticky	2	+	-
	rice 10 gm	3	-	+
13	Garlic 4 gm +	1	+	-
	Steamed sticky	2	-	+
	rice 20 gm	3	+	-
14	Garlic 12 gm +	1	-	+
	Steamed sticky	2	+	-
	rice 4 gm	3	+	-
15	Garlic 12 gm +	1	+	-
	Steamed sticky	2	+	-
	rice 10 gm	3	+	-
16	Garlic 12 gm +	1	+	-
	Steamed sticky	2	-	+
	rice 20 gm	3	+	-
17	Positive control	1	+	-
		2	+	-
		3	+	-
18	Negative	1	-	+
	control	2	-	+
		3	-	+

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