EFFECT OF FERMENTATION AND MICROWAVE HEATING ON SURVIVORSHIP AND INFECTION ABILITY OF TRICHINELLA SPIRALIS LARVAE IN FERMENTED PORK (NAHM)

KITTIPONG VUBSUNTHEAR

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIRMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (PUBLIC HEALTH)
MAJOR IN INFECTIOUS DISEASES AND EPIDEMIOLOGY
FACULTY OF GRADUATE STUDIES
2008

Thesis

Entitled

EFFECT OF FERMENTATION AND MICROWAVE HEATING ON SURVIVORSHIP AND INFECTION ABILITY OF TRICHINELLA SPIRALIS LARVAE IN FERMENTED PORK

…….....

Mr. Kittipong Vubsunthear
Candidate

…….....

Assoc.Prof. Wongdyan Pandii,
Dr.P.H.
Major-Advisor

…….....

Lect. Supawadee Boonchuen,
Ph.D.
Co-Advisor

……

Asst. Prof. Prayute Buddhirakkul,
M.Sc.
Co-Advisor

……

Assoc. Prof. Prasong Temcharoen,
M.Sc. Parasitology
M.Sc. Microbiology
Co-Advisor

…….....

Assoc.Prof. Chalit Komalamisra,
Ph.D.
Co-Advisor

…….....

Prof. Banchong Mahaisavariya,

M.D.

Dean

Faculty of Graduate studies

…….....

Assoc.Prof. Chakrit Hirunpetcharat,
Ph.D.
Chair
Master of Science (Public Health)
Major in Infectious Diseases and Epidemiology
Faculty of Public Health

Thesis
Entitled

EFFECT OF FERMENTATION AND MICROWAVE HEATING ON SURVIVORSHIP AND INFECTION ABILITY OF TRICHINELLA SPIRALIS LARVAE IN FERMENTED PORK

was submitted to the Faculty of Graduate Studies, Mahidol University
For the degree of Master of Science (Public Health)
Major in Infectious Diseases and Epidemiology
on
May 30, 2008

…….
Mr. Kittipong Vubsunthear
Candidate

……
Assoc.Prof. Jirasak Rojanapremsuk, Dr.P.H.
Chair

……
Assoc.Prof. Wongdyan Pandii, Dr.P.H.
Member

…….
Lect. Supawadee Boonchuen, Ph.D.
Member

…….
Assoc.Prof. Chalit Komalamisra, Ph.D.
Member

……
Assoc. Prof. Prasong Temcharoen, M.Sc. Parasitology
M.Sc. Microbiology
Member

……
Asst. Prof. Prayute Buddhirakkul, M.Sc.
Member

……
Prof. Banchong Mahaisavariya, M.D.
Dean
Faculty of Graduate Studies
Mahidol University

……
Assoc.Prof. Phitaya Charupoonphol, M.D., Dip. Thai Board of Epidemiology
Dean
Faculty of Public Health
Mahidol University

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and deep appreciation to my Major – Advisor, Associate Professor Dr. Wongdyan Pandii, Department of Parasitology, Faculty of Public Health, Mahidol University for her precious advice, guidance, encouragement and constructive comments which enable me to carry at this thesis successfully.

I am equally grateful to my co – advisor, Associate Professor Dr. Chalit Komalamisra, Department of Helminthology, Faculty of Tropical Medicine, Associate Professor Prasong Temcharoen, Assistant Professor Prayute Buddhirakkul and Lect. Dr. Supawadee Boonchuen, Department of Parasitology, Faculty of Public Health, Mahidol University for their kind valuable suggestion concerning experimental techniques during the course of this study.

I would like to express my deepest grateful to the Head of Department, the Professor, the Lecturer, the Laboratory Assistant and the official at Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, who helped me provide the laboratory, information support which has enabled to complete my thesis.

I also sincere gratitude to external examiner, Associate Professor Dr. Jirasak Rojanapremsuk for his helpful guidance to my study.

Finally, I would like to express my utmost gratitude to my beloved family for their great care, good understandings and encouragement throughout my study.

Mr. Kittipong Vubsunthear

EFFECT OF FERMENTATION AND MICROWAVE HEATING ON SURVIVORSHIP AND INFECTION ABILITY OF TRICHINELLA SPIRALIS LARVAE IN FERMENTED PORK (NAHM)

KITTIPONG VUBSUNTHEAR 4737306 PHPH/M

M.Sc.(PUBLIC HEALTH) MAJOR IN INFECTIOUS DISEASES AND EPIDEMIOLOGY

THESIS ADVISORS : WONGDYAN PANDII, Dr.P.H., CHALIT KOMALAMISRA, Ph.D., PRASONG TEMCHAROEN, M.Sc., SUPAWADEE BOONCHUEN, Ph.D., PRAYUTE BUDDHIRAKKUL, M.Sc.

ABSTRACT

Trichinellosis is a zoonosis caused by parasitic nematodes of the genus *Trichinella* through eating uncooked pork. This study aims to study salt and garlic concentrations, periods of time for fermentation, and microwave heating on the survivorship and infection ability of *T. spiralis* larvae in mice. The pork NAHM was divided into two and samples of 2,400 grams. *T. spiralis* larvae were then mixed in the pork, at 2,400 larvae per sample. The following concentration by weight were mixed with the pork : salt (3% and 7%); garlic (12%); and rice (10%). After mixing the ingredients, each sample was divided in half. The fermentation was done for 3 and 7 days far each group. Each group was then divided again into two groups. One group was cooked by microwave heating at 3 levels (for 1, 2 and 3 minutes), and the other was not microwaved. Afterwards, *T. spiralis* larvae from all experimental groups were fed into mice (50 larvae/mice). In this study, it was founded that 0.85 NaCl and garlic could not destroy *T. spiralis* larvae since they were still infected mice. At the salt concentration at 3 and 7% and fermented for 3 and 7 days found that, for the fermented duration at 3 days, 4 % of inactive *T. spiralis* larvae was collected and they were inabled to infect mouse. Whereas the fermented for 7 days sample, *T. spiralis* larvae were not collected. For the sample which were heated by microwave for 1, 2 and 3 minutes, *T. spiralis* larvae were collected at 4.66, 6 and 0 % respectively and all were not infect mice. In conclusion, the results of this study could not be concluded because the *T. spiralis* larvae could not be collected. Anyway, the study found that salt concentration at 7 % and 2 minutes of microwave heating are able to destroy *T. spiralis* larvae.

KEY WORDS : TRICHINELLA SPIRALIS/ NAHM/ FERMENTATION

53 pp.

อิทธิพลของการหมัก และความร้อนจากไมโครเวฟมีผลต่อการรอดชีพ และความสามารถในการติดเชื้อของตัวอ่อน TRICHINELLA SPIRALIS ในแหนม (EFFECTS OF FERMENTATION AND MICROWAVE HEATING ON SURVIVORSHIP AND INFECTION ABILITY OF TRICHINELLA SPIRALIS LARVAE IN FERMENTED PORK (NAHM)

กิตติพงค์ วับสันเทียะ 4737306 PHPH/M

วท.ม. (สาธารณสุขศาสตร์) สาขาวิชาเอกโรคติดเชื้อและวิทยาการระบาด

คณะกรรมการควบคุมวิทยานิพนธ์ : วงเดือน ปั้นดี, ส.ด. ชลิต โกมลมิศร์, Ph.D. ประสงค์ เต็มเจริญ, M.Sc. สุภาวดี บุญชื่น, Ph.D. ประยุทธ์ พุทธิรักษ์กุล, M.Sc.

บทคัดย่อ

Trichinellosis เป็นโรคติดต่อที่เกิดจากพยาธิตัวกลมในกลุ่มพยาธิ *T. spiralis* โดยการบริโภคเนื้อหมูที่ปรุงไม่สุก ซึ่งในการศึกษาเชิงทดลองครั้งนี้ เพื่อศึกษาความเข้มข้นของเกลือ และกระเทียม ในระยะเวลาการหมัก และความร้อนของไมโครเวฟ มีผลต่อการรอดชีพ และความสามารถในการติดเชื้อของตัวอ่อน *T. spiralis* โดยมีกระบวนการหมักเนื้อหมู (NAHM) โดยการใช้เนื้อหมู 4,800 กรัม แบ่งเป็น 2 ตัวอย่าง ตัวอย่างละ 2,400 กรัมผสมตัวอ่อน *T. spiralis* โดยใช้ความเข้มข้นของเกลือ 3 และ 7 %ต่อน้ำหนัก และผสมข้าวสุก และกระเทียม ใช้เวลาการหมัก 3 และ 7 วัน หลังจากนั้นนำไปผ่านไมโครเวฟ 3 ระดับ (1, 2 และ 3 นาที) แล้วนำตัวอ่อน *T. spiralis* ที่ได้มาทดสอบการรอดชีพ และความสามารถในการติดเชื้อในหนู

ผลการทดลองพบว่า ในกลุ่มควบคุม ตัวอ่อน *T. spiralis* ในกระเทียม และ 0.85 % โซเดียมคลอไรค์ ไม่สามารถทำลายตัวอ่อน *T. spiralis* ได้ และมีสามารถในการติดเชื้อในหนูได้ทั้งหมด ที่ความเข้มข้นของเกลือ 3 % หมัก 3 และ 7 วัน พบว่า ในการหมัก 3 วัน ตรวจพบตัวอ่อน *T. spiralis* 4 % และไม่สามารถติดเชื้อในหนู ขณะที่ในระยะเวลาการหมัก 7 วัน ตรวจไม่พบตัวอ่อน *T. spiralis* และนำแหนมผ่านไมโครเวฟในระยะเวลา 1, 2 และ 3 นาที ตรวจพบตัวอ่อน *T. spiralis* 4.66, 6 และ 0 % ตามลำดับ และไม่สามารถติดเชื้อในหนู ส่วนความเข้มข้นเกลือ 7 % ระยะเวลาการหมัก 3 และ 7 วัน ตรวจไม่พบตัวอ่อน *T. spiralis*

สรุปผล ในการศึกษาครั้งนี้ ในกระบวนการทำแหนมโดยใช้ตัวอ่อน *T. spiralis* ผสมในเนื้อหมู ข้าว เกลือ และกระเทียม ในระยะเวลาการหมัก 3 และ 7 วัน และผ่านความร้อนไมโครเวฟ 1, 2 และ 3 นาที และทดสอบการรอดชีพ และความสามารถในการติดเชื้อในหนู พบว่ายังไม่สามารถแปลผลได้เนื่องจากหลังจากผ่านขั้นตอนดังกล่าวแล้วไม่สามารถเก็บรวบรวมเชื้อจากตัวอย่างได้ อย่างไรก็ดีจากการทดลองครั้งนี้พบว่าการใช้ความเข้มข้นของเกลือ 7 % ใช้เวลาหมักมากกว่า 3 วัน และใช้ความร้อนไมโครเวฟที่ระดับสูงสุดมากกว่า 2 นาที สามารถทำลายตัวอ่อนของ *T. spiralis* ได้

53 หน้า

CONTENTS

CONTENTS (Continued)

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

CHAPTER I
INTRODUCTION

1.1 Defining of the Research Problems

Trichinellosis is a zoonosis caused by parasitic nematodes of the genus *Trichinella*. The infection has a worldwide occurrence and although most species of *Trichinella* are found in mammals, one species is also known to infect birds and a new genotype has recently been detected in crocodiles in Africa (1). The main sources of human infection are pork and pork products, game meat and horse meat. With regard to the taxonomy of *Trichinella*, knowledge has increased greatly in the past 30 years and, to date, seven species have been identified (*Trichinella spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni, T. papuae* and *T. pseudospiralis*) and additional genotypes have been identified (2). Trichinellosis continues to be a public health concern throughout the world (1). Specifically, it has been estimated that 10 millions people worldwide could be infected and in the past 10 years an increase in the occurrence of infection has been reported among domestic pigs and wildlife, with a consequent increase among humans (3).

In Thailand, northern and northeastern parts are the important endemic areas which *T. spiralis* is the most prevalence in the northeast. Trichinellosis, an infection by a nematode *T. spiralis*, is not an uncommon disease in Thailand. The Division of Epidemiology, Ministry of Public Health reported that from 1982 to 1997 there were 3,894 human infections with 13 deaths (4,5). There was an epidemic of trichinellosis in Ban Thum Lod , Pang Ma Pha precint, Mae Hong Son Province in April 1998 (5). In 1988, Trichinellosis was endemic and about 351 cases were infected in the northern of Thailand. However, the geographical pattern of *T. spiralis* infection is not uniform (6). Human acquires *T. spiralis* larvae infection through the consumption of raw or undercooked infected pig. Approximately 1 month after infection, *T. spiralis* becomes adult worm. The life cycle of the parasite begins with the enteral phase of infection

when a person or an animal eats contaminated meat containing first stage muscle larvae. Digestive juices from the stomach dissolve the capsule-like cyst and release the larvae which pass into the small intestine, where they invade the columnar epithelium. Shortly thereafter, the larvae molt four times, mature to adult and mate. Female worms can produce 500-1,500 newborn larvae during a life span, before expulsion by the host immune system. The migratory phase of infection begins when these newborn larvae are passed into tissue, enter lymphatics and then enter the general circulation at the thoracic duct. These larvae are widely distributed in tissue by the circulation and eventually make their way through the capillaries into the muscle fibers, which initiate the muscle phase of infection. Once in the muscle fibers, they encyst, undergo development, become infection within 15 days and remain for months to years (7).

The registry showed that primary trichinellosis incidence in Mae Hong Son province in the northern of Thailand was very high, in addition 1998 incidence rate of 0.57 per 100,000 person-years in men and women respectively (6). Although most published descriptions of social habits regarding consumption of pork are anecdotal and careful sociological investigation is needed. A raw or undercooked pork is the primary vector of infection. They are contaminated by unwashed utensils, hands and food preparation process.

By these reason, the researcher is interested in the effect of fermentation process and microwave heating of NAHM on survivorship and infection ability of *T. spiralis* larvae in order to reduce contamination of *T. spiralis* larvae, by using suitable process and can apply for cooking. Hence the studies, it is considered to cut the cycle of *T. spiralis* infection from raw pork to people and to result in reducing the risk of *T. spiralis* infection in Thailand.

1.2 Research objectives

1.2.1 General objective

To study the salt and garlic concentration, period of time for fermentation and microwave heating on survivorship and infection ability of *T. spiralis* larvae in mice.

1.2.2 Specific objectives

1.2.2.1 To study survivorship and the infection ability of *T. spiralis* larvae in fermented pork (NAHM) with 3 and 7% of salt concentration (w/w), respectively.

1.2.2.2 To study survivorship and the infection ability of *T. spiralis* larvae in fermented pork (NAHM) 3 and 7 days fermentation.

1.2.2.3 To study survivorship and the infection ability of *T. spiralis* larvae in fermented pork (NAHM) under high level microwave heating for 0, 1, 2 and 3 minutes.

1.3 Research Hypotheses

1.3.1 Salt concentration affects to the survivorship and the infection ability of *T. spiralis* larvae in fermentation pork (NAHM).

1.3.2 Duration of fermentation results in different effect on survivorship and the infection ability of *T. spiralis* larvae in fermentation pork (NAHM).

1.3.3 Microwave heating time results in different effect on survivorship and the infection ability of *T. spiralis* larvae in fermentation pork (NAHM).

1.4 Scope of the study

In this research, *T. spiralis* larvae are collected from laboratory infected mice.

All mice used were *Mus musculus* strain and free from *T. spiralis* .

1.5 Limitation

The research was conducted in the laboratory scale which the ingredients, duration, and other variables could be controlled.

1.6 Definition of terms

Fermented pork "NAHM" : Thai food products which is produced by fermentation of mixed pork, garlic, cooked rice and salt.

Salt concentration: The concentration of salt was mixed in fermented pork "NAHM". In this study, 3 and 7% salt were selected and used in the fermentation process.

Mice : All mice are domesticated in laboratory and free from *T. spiralis* larvae.

Larvae free : Without *T. spiralis* larvae or infection in mice.

Microwave heating : To use high level heating.

Exposure times : Exposure times in microwave heating at 1, 2 and 3 minutes, respectively.

Survivorship : *T. spiralis* larvae which still survive in mice after are exposed to any intervention, active, inactive larvae.

Infection ability : The ability of infecting or state of being infection of *T. spiralis* larvae in mice.

Infectivity : *T. spiralis* larvae were found or not found in mice.

***T. spiralis* larvae** : Non-encapsulated larvae after pepsin digestion.

Garlic : Garlic (*Allium sativum* Linn.)used in this study, *T. spiralis* larvae and NAHM mixed with 12% of garlic (w/w).

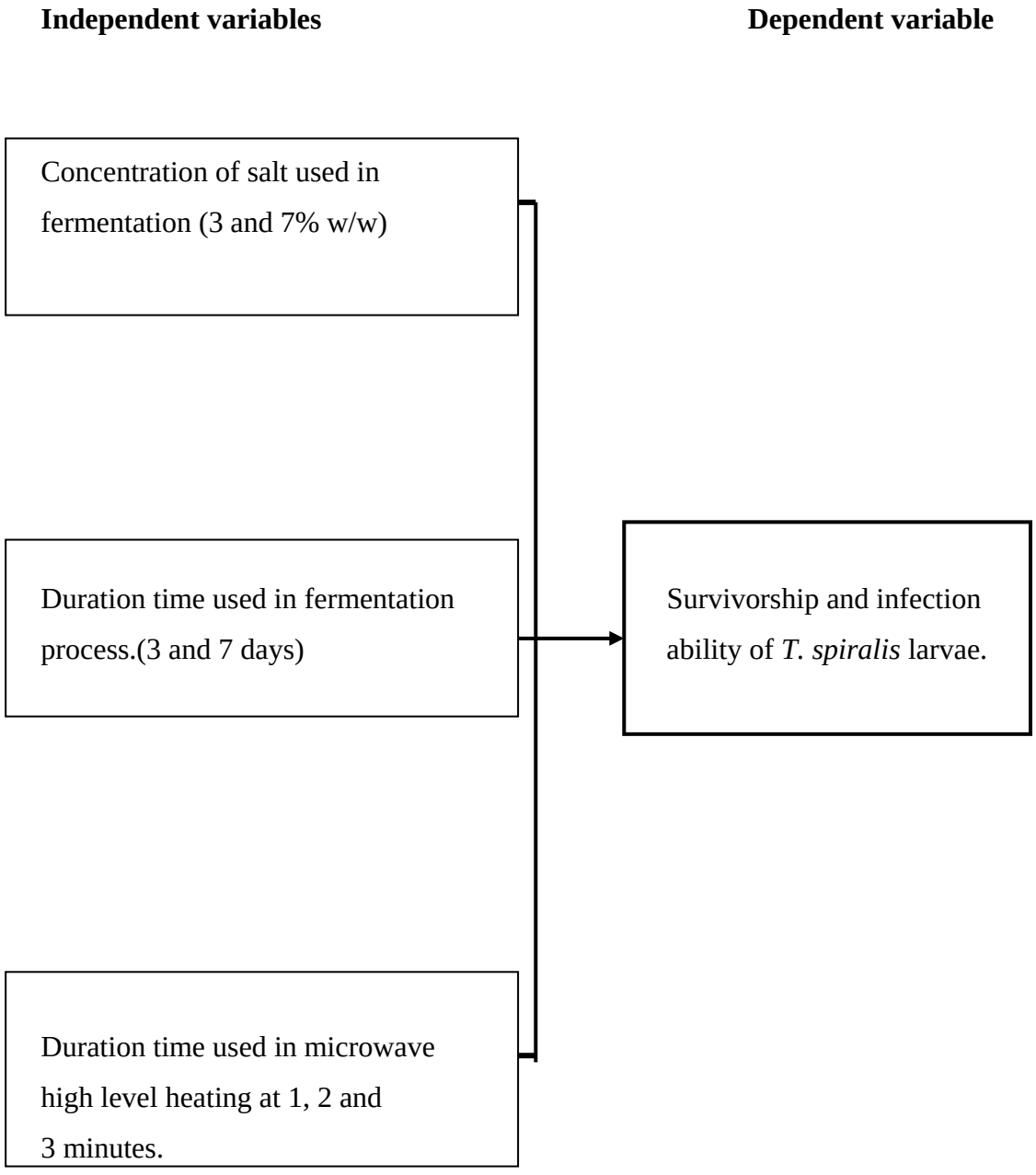

Figure 1 Conceptual framework

CHAPTER II
LITERATURE REVIEWED

Trichinellosis is a zoonosis caused by parasitic nematodes of the genus *Trichinella*. The infection has a worldwide occurrence and although most species of *Trichinella* are found in mammals. One species is also known to infect birds and a new genotype has recently been detected in crocodiles in Africa (1). The main sources of human infection are pork and pork products, game meat and horse meat. With regard to the taxonomy of *Trichinella,* knowledge has increased greatly in the past 30 years and, to date, seven species have been identified (*Trichinella spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni, T. papuae* and *T. pseudospiralis*) and additional genotypes have been identified (2). Trichinellosis continues to be a public health concern throughout the world (1).

Historical review of trichinosis in Thailand

The first outbreak of trichinosis in Thailand was in 1962 and involved 56 patients resulting in 11 deaths in the Mae Sariang District, Mae Hong Son Province. Meat from pigs was the source of outbreak (8). The highest annual number of hospital recorded on trichinosis cases was 557 in 1983. This figure was considered an underestimation of the actual number of cases involved in the outbreaks (9-14). In April 1973, an outbreak of trichinosis occurred in the Mae Sruay District, Chiang Rai Province. Thirty-one persons were involved, ranging from 9 to 72 years, and one adult female died (15). In 1980, trichinosis was reported in the Pluak Dang District of Rayong Province, the infection being caused by the consumption of wild squirrel (16, 17). An epidemic of trichinosis involving 177 patients and 13 deaths occurred in April 1981 in Kok-Ta-Back Village, Nong-Pai District, Petchabun Province, and reported the fourteenth outbreak of human trichinosis in Thailand (18-20). Khamboonruang (21) reported 118 discrete outbreaks of the disease involving 5,400 patients and 95 deaths. In the south of Thailand, an outbreak of trichinosis affecting 59 individuals

resulting in one death occurred in Chumporn Province during 1994-1995. This was the first report of an epidemic of human infection caused by *T. pseudospiralis* (22). The number of outbreaks had tended to increase in recent years. The annual epidemiological surveillance report dated 15 November 2005 recorded 7,392 patients and 97 deaths.

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 (23).

Parasitic infection is still a public health problem from the past to present, and its distribution is all around the country. It is result to loss of economy and development in the country because of reducing the efficiency of working people and increasing the treatment expense (24).

Classification of *Trichinella* genus in Thailand

Seven species belonging to the *Trichinella* genus, five with encapsulated larvae and two with non-encapsulated larvae in host muscles and three additional genotypes, have been described to date: *T. spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni, T. pseudospiralis,* and *T. papuae*. In Southeast Asia, *T. spiralis* and *T. pseudospiralis* have been documented in domestic animals and/or humans in Cambodia, Indonesia (Bali and Sumatra), Lao PDR, Malaysia, Myanmar and Thailand (25-29). In Thailand, the causative agent of most outbreaks of trichinosis has been identified as *T. spiralis* (8). Meanwhile, there was report on human infection by *T. pseudospiralis* (30). An outbreak of trichinosis affecting 59 individuals, of whom one died, occurred in south Thailand during 1994-1995. After that, there were no reports of other species of *Trichinella* in this country in either humans or animals (31). Until recently, *T. spiralis* and *T. pseudospiralis* were the only human- infecting species in Thailand.

Table 1. *Trichinella* taxonomy and distribution

Species	Genotype	Host	Distribution
T. spiralis	T1	Mammals	Cosmopolitan
T. nativa	T2	Mammals	Arctic and subarctic regions of America, Europe and Asia
	T6	Mammals	Arctic and subarctic regions of America
T. britovi	T3	Mammals	Temperate areas of Europe and Asia, Northern and Westhern Africa
	T8	Mammals	South Africa and Namibia
T. pseudospiralis	T4	Mammals and birds	Cosmopolitan
T. murrelli	T5	Mammals	Temperate areas of North America
	T9	Mammals	Japan
T. nelsoni	T7	Mammals	Eastern and Southern Africa
T. papuae	T10	Mammals and reptiles	Papua New Guinea
T. zimbabwensis	T11	Mammals and reptiles	Africa South of the Sahara

Source: http://www.iss.it/site/*Trichinella*/scripts/tbl1.asp (31).

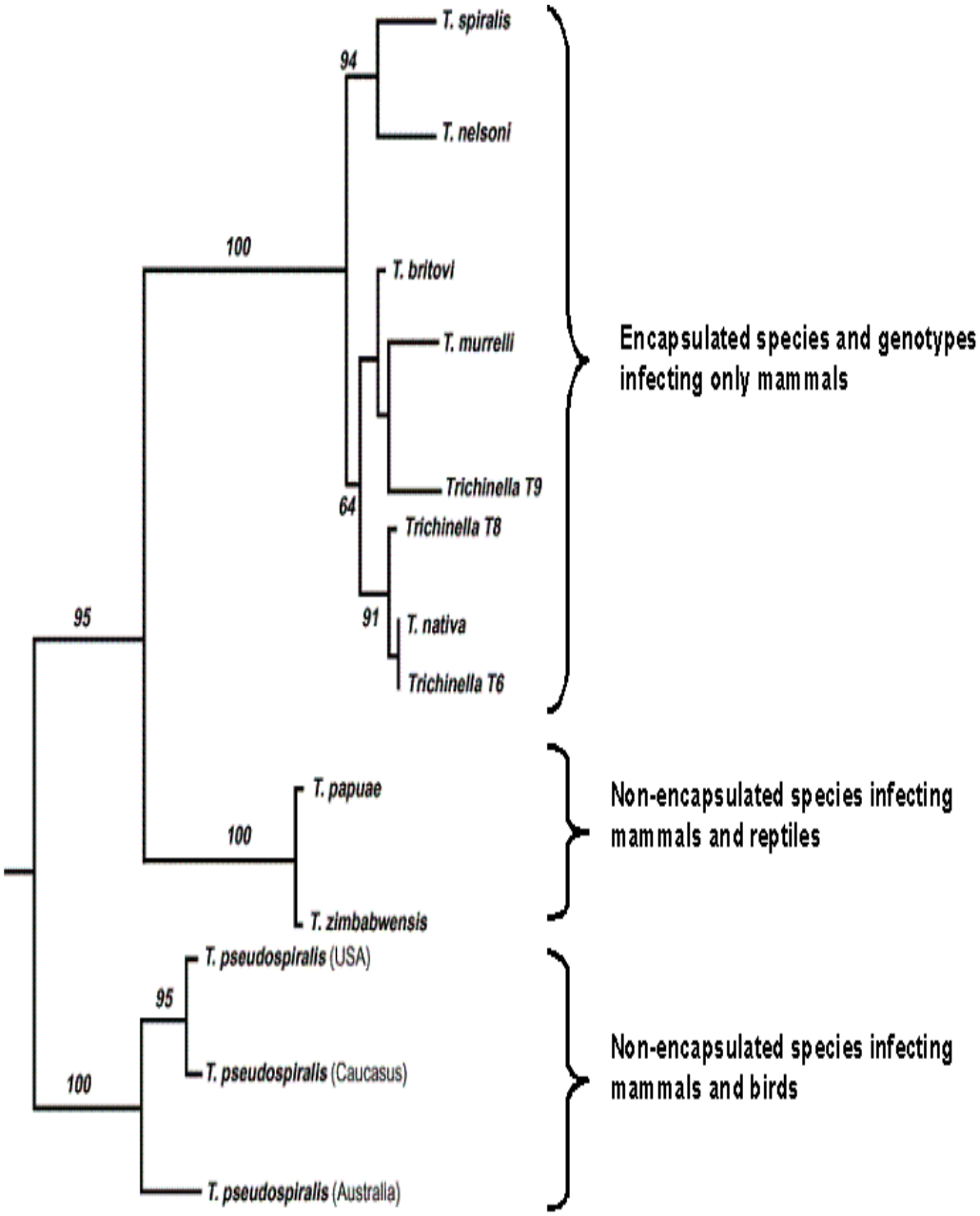

Figure 2. *Trichinella* taxonomy.

Source: http://www.iss.it/site/*Trichinella*/scripts/fig.asp (32).

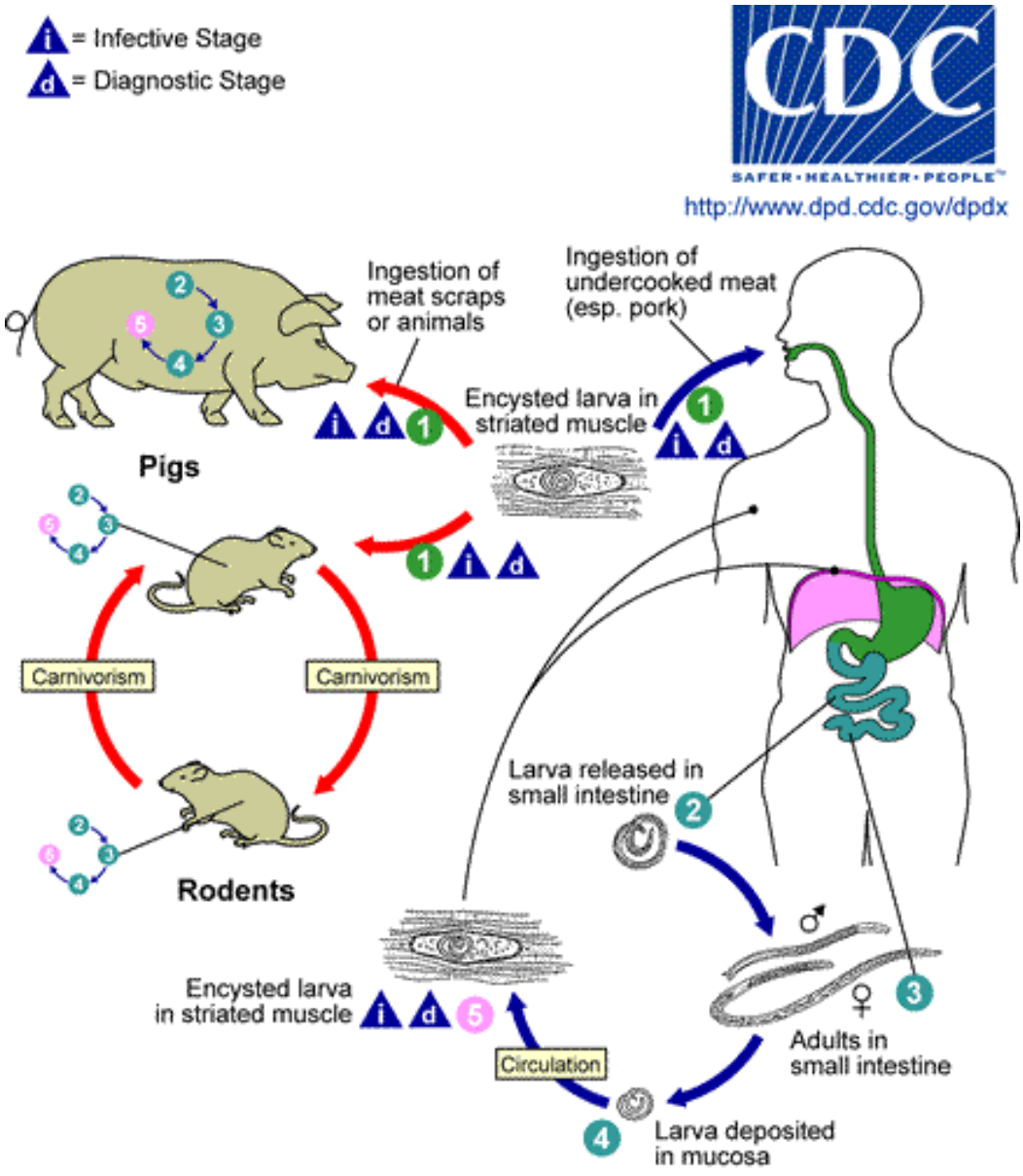

Figure 3. Life cycle of *Trichinella spiralis*

Source: www.dpd.cdc.gove/dpdx (33).

Life cycle

Trichinellosis is acquired by ingesting meat containing cysts (encysted larvae) (1) of *Trichinella*. After exposure to gastric acid and pepsin, the larvae are released (2) from the cysts and invade the small bowel mucosa where they develop into adult worms (3) (female 2.2 mm in length, male 1.2 mm; life span in the small bowel: 4 weeks). After 1 week, the females release larvae (4) that migrate to the striated muscles where they encyst (5). *Trichinella pseudospiralis*, however, does encyst. Encystment is completed in 4 to 5 weeks and the encysted larvae may remain viable for several years. Ingestion of the encysted larvae perpetuates the cycle. Rats and rodents are primarily responsible for maintaining the endemicity of this infection. Carnivorous/omnivorous animals, such as pigs or bears, feed on infected rodents or meat from other animals. Different animal hosts are implicated in the life cycle of the different species of *Trichinella*. Humans are accidentally infected when eating improperly processed meat of these carnivorous animals (or eating food contaminated with such meat) (33).

Transmission and epidemiology

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 (8). 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 (34-36). 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 increased to 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, Ministry of Public Health. Since then, about 130 outbreaks have been reported totaling 7,392 patients and 97 deaths (37).

Since 2002, the distribution of human trichinosis cases by age groups has been considered by the annual epidemiological surveillance reports according to the

hospital based. The youngest patient was about 1 year old. It was uncommon to see patients in the 10-14 and 65+ age groups, but most patients were in the age 35-44 groups, morbidity rate was 0.04 per 100,000 of people. Infection occurred in men more frequently than women at the ratio of 1.7-2:1 (29).

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 was responsible for 96.4% of all cases reported from 1962 to 2000 (13). The annual epidemiological surveillance reports from 2002 to 2005 found consistently high number of cases (289, 126, 30 and 60 respectively) in the north region. The figures 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 were 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 year (23).

Most outbreaks occurred in the north region, including 60.84% of all cases reported from 1962 to 2005. These results were reported, the north region was responsible for 96.4% of all cases (13). 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 (37).

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 (38,39).

All trichinosis cases gave a history of having consumption raw pork in the form of “lahb” and “nahm,” favorite dishes of north Thailand (15). Lahb is made from chopped raw pork mixed with lemon juice, roasted rice powder, finely cut red onion and parsley. Nahm is also made from chopped raw pork mixed with salt, garlic and chili, tightly wrapped in banana leaves for a few days for fermentation (13), Some Thai dishes are proven as viable *T. spiralis* larvae sources due to cooking procedures (40). According to the report in 1981, *T. spiralis* was found in 1.67% of 421 dogs in Tarae District, Sakonnakon Province**.** Raw dog meat was a source of infection in Kaeng Khlo District, Chaiyaphum Province (41).

Parasitological diagnosis

A muscle biopsy (2 to 4 mm), in which the piece of tissue is pressed between two slides and viewed under the microscope, will usually reveal larvae in heavy infections and is thus the most direct measure of the presence of infection. If the diagnosis is attempted before larvae begin to coil, then there is the risk of confusing the worm with fragments of muscle tissue. Alternatively, digesting a finely minced portion of the biopsy alternatively, digesting a finely minced portion of the biopsy material in 1% HCl–1% pepsin for 1 h at 37 ºC will release the larvae from their nurse cells and make them more easily observed under the microscope (42,43). 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. 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 hematoxylin and-eosin-stained sections because the pattern of striation disappears by the fourth to fifth day after infection (44). 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 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 antibodies have yet to become positive. Although detection of circulating antigen might be a useful confirmatory test (45), circulating antigen is not detected in every patient and its detection is therefore of limited value to the clinician. Detection is therefore of limited value to the clinician.

DNA-based tests have also been reported (46). 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 (42) and remain positive for years (47). 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. 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 (48).

Treatment

When the life of the patient is threatened by overwhelming infection, intensive care treatment with all available supportive therapies is mandated (i.e., fluid replacement, steroids, and treatment for shock, toxemia, and circulatory and cardiac failure) (49). Specific treatment for the parasite with various benzimidazoles (mebendazole or albendazole) is also necessary. Immunosuppression due to steroids, although often a life-saving procedure, prolongs the life of the adult parasites as well and results in further production of newborn larvae if unchecked. As already mentioned, patients may harbor adults shedding newborn larvae for several weeks during the acute phase of infection. Mebendazole (200 mg/day for 5 days) or albendazole (400 mg/day for 3 days) should be given to adults (except pregnant women), as well as to children (5 mg per kg of body weight per day for 4 days) (48). Prednisolone at 40 to 60 mg/day alleviates the fever and the side effects of inflammation due to the cell damage that results from larval penetration into the tissues. These symptoms usually disappear within days after the initial dose is given. Prolonged treatment with steroids is not recommended, although symptoms may recur when treatment is suspended. Long-term sequelae must be treated symptomatically as they arise (49).

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* (50). 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 copiousness within the barnyard community of pigs may be yet another means by which naive animals are infected (48).

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 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 (48).

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 can control infection at the level of the abattoir (42).

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%) (51). Such inspection programs are in place in most European countries but have somehow escaped the mandate of the U.S. Department of Agriculture (42).

Thorough freezing of all pork products prior to cooking ensures the death of the larvae, while cooking meat at 137 ºF (58 ºC) 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 (52). Freezing muscle tissue from game animals 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. can remain infective after several days at freezing temperatures even after they have been isolated from their host muscle tissue (53).

Home-raised and direct-from-farm swine and pork from foreign sources are unaffected by regulations and standards followed by the U.S. commercial pork production industry that have helped to reduce *Trichinella* prevalence in commercial pork. Outbreaks of trichinellosis associated with noncommercial sources of pork have been reported previously (54,55) and continue to be reported. Trichinellosis cases associated with noncommercial pork now outnumber those cases associated with U.S. commercial pork, reflecting a changing risk pattern in pork consumption.

The emergence of wild game meat as the most common source of trichinellosis and the continued occurrence of trichinellosis among consumers of pork obtained from small farms or other countries suggest that educational messages concerning the risks for eating meat cooked improperly, especially from noncommercial sources, are not reaching persons at risk for trichinellosis. USDA recommends that consumers of fresh pork cook the meat to an internal temperature of 160°F (71°C) (56). *T. spiralis* larvae in pork are killed at lower temperatures (e.g., 140°F [60°C] for 2 minutes or 131°F [55°C]) for 6 minutes (57); however, USDA has recommended a higher temperature to allow for different cooking methods that might result in uneven temperature distributions throughout the meat (e.g., microwave cooking) (58). *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes*, other foodborne microorganisms that also can be found in pork, are destroyed by thorough cooking to an internal temperature of 160°F (71°C) (56).

Freezing kills *T. spiralis* larvae in pork. Pork <6 inches thick can be made safe if frozen to -20°F (-29°C) for 6 days, -10°F (-23°C) for 10 days, or 5°F (-17°C) for 20 days (59). However, freezing might not kill other species and types of *Trichinella* found in wild game. Infective *Trichinella* spp. larvae have been found in frozen bear meat grizzly bear meat frozen at -4°-20.3°F (-20°- -6.5°C) for 27 months (60) and polar bear meat frozen at -0.4°F (-18°C) for approximately 24 months (61,62). Infective *Trichinella* spp. larvae also have been found in other wild carnivore meat frozen at 5°F (-17°C): marten frozen for 5 months, wolverine frozen for 6 months, and arctic fox frozen for 14 months (63). In addition, viable *Trichinella* spp. larvae were found in black bear meat that had been processed into ham and jerky by dry curing with a commercial salt mixture at a USDA-licensed establishment by using procedures similar to those used to prepare pork for human consumption. However, no viable

Trichinella spp. larvae were found in ground bear meat preparations (e.g., sausage, pepperoni, or salami) that had been processed according to standards mandated by USDA for processing pork (60).

The dramatic decline of trichinellosis in the United States reflects changes in industrial practices, increased government regulations, and increased public awareness. As a result, the epidemiology of the disease has changed. Because of the successful reduction in *Trichinella* prevalence among swine in the U.S. commercial pork industry, the majority of cases of human trichinellosis are now associated with wild game meat, noncommercial pork, and foreign pork. Persons at risk need a greater understanding of the changing risks for trichinellosis, of the methods for safe meat preparation, and of the limitations of those methods in certain circumstances, if further reduction in the incidence of this disease is to be achieved in the United States (63).

Microwaves

Microwaves are very short waves of electromagnetic energy that travel at the speed of light (186,282 miles per second). Microwaves used in microwave ovens are in the same family of frequencies as the signals used in radio and television broadcasting.

The theory of electromagnetic energy can be illustrated by what happens when a pebble is tossed into a quiet pond. The pebble striking the still surface causes the water to move up and down in the form of ripples, or waves, that radiate in ever-widening circles over the surface of the pond. These waves, which move up and down at right angles to the direction they are traveling, are called transverse waves. Microwaves are examples of transverse waves.

The disturbance resulting from the pebble landing in the water is transmitted through the water in the form of ripples or waves. The water serves merely as a medium through which the disturbance travels. In this sense, these ripples are more like sound waves, which also need a medium to travel through, normally using the molecules that exist in the air or water. That is why, for example, thundering rocket engines that would deafen the ears under normal circumstances, would be inaudible in the quiet vacuum of space.

On the other hand, electromagnetic forms of energy, such as microwaves, radar waves, radio and TV waves, travel millions of miles through the emptiness of space without the need of any material medium through which to travel. This is because, simply put, electromagnetic waves are, in themselves, stored energy in motion.

A phenomenal force

Electromagnetic radiation begins with a phenomenon that occurs when electric current flows through a conductor, such as a copper wire. The motion of the electrons through the wire produces a field of energy that surrounds the wire and floats just off its surface. This floating zone or cloud of energy is actually made up of two different fields of energy, one electric and one magnetic. The electric and magnetic waves that combine to form an electromagnetic wave travel at right angles to each other and to the direction of motion. If the current flowing through the wire is made to oscillate at a very rapid rate, the floating electromagnetic field will break free and be launched into space. Then, at the speed of light, the energy will radiate outward in a pulsating pattern, much like the waves in the pond. It is theorized that these waves are made up of tiny packets of radiant energy called photons. Streams of photons, each carrying energy and momentum, travel in waves like an undulating string of cars on a speeding roller coaster.

Is microwave radiation the same as radioactive radiation

No. There is a very important difference. As illustrated by the frequency spectrum on the right, microwaves used in microwave ovens, similar to microwaves used in radar equipment, and telephone, television and radio communication, are in the *non-ionizing* range of electromagnetic radiation. Non-ionizing radiation is very different from *Ionizing radiation.* Ionizing radiation is extraordinarily high in frequency (millions of trillions of cycles per second). It is, therefore, extremely powerful and penetrating. Even at low levels, ionizing radiation can damage the cells of living tissue. In fact, these dangerous rays, have enough energy and intensity to actually change (ionize) the molecular structure of matter. In sufficient doses, ionizing radiation can even cause genetic mutations. As shown on the frequency spectrum, the

ionizing range of frequencies includes X-rays, gamma rays, and cosmic rays. Ionizing radiation is the sort of radiation, we associate with radioactive substances like uranium, radium, and the fall-out from atomic and thermonuclear explosions (64).

Microwave ovens

Microwave ovens cook food by friction of the molecules of food induced by the microwave energy - specifically at 2,450 MHz produced by a magnetron. As a corollary, the energy produced at 2,450 MHz or 2.4 GHz frequency, (which can be as high as 500 W typically or more) can result in hash noise for some sensitive equipment like cordless phones or video/camera senders which operate at 2.4 GHz. So if your 2.4 GHz phone conversation breaks into a cacophony, look no further than your microwave oven for the source of interference (65).

Microwave power level

Microwave oven has 5 power levels. To choose the power level for cooking, follow the advice given in the recipe section. Generally the following recommendations apply.

High used for fast cooking or reheating soup, casseroles, canned food, hot beverages, vegetables, fish, etc.

Medium high used for fast cooking of dense foods such as roast joints, meat loaf and plated meal, also for sensitive dishes such as cheese sauce and sponge cakes. At this reduced setting, the sauce will not boil over and food will cook evenly without over cooking at the side.

Medium for dense foods, which require a long cooking time when cooked conventionally, beef dish, it is advisable to use this power setting to ensure the meat will be tender.

Medium low to defrost, select this power setting, to ensure that the dish defrosts evenly.

Low. For gentle defrosting, cream gateaux or pasty

Cooking with Microwaves

Microwaves are produced inside the oven by an electron tube called a magnetron. The microwaves bounce back and forth within the metal interior until they are absorbed by food. Microwaves cause the water molecules in food to vibrate, producing heat that cooks the food. That's why foods high in water content, like fresh vegetables, can be cooked more quickly than other foods. The microwave energy is changed to heat as soon as it is absorbed by food. Thus, it can not make food radioactive or "contaminated."

Although heat is produced directly in the food, microwave ovens do not cook food from the "inside out." When thick foods like roasts are cooked, the outer layers are heated and cooked primarily by microwaves while the inside is cooked mainly by the slower conduction of heat from the hot outer layers.

Microwave cooking can be more energy efficient than conventional cooking because foods cook faster and the energy heats only the food, not the oven compartment. Microwave cooking does not reduce the nutritional value of foods any more than conventional cooking. In fact, foods cooked in a microwave oven may keep more of their vitamins and minerals, because microwave ovens can cook more quickly and without adding water.

Glass, paper, ceramic, or plastic containers are used in microwave cooking because the microwaves pass through them. Although such containers can not be heated by microwaves, they can become hot from the heat of the food cooking inside. Some plastic containers should not be used in a microwave oven, they can be melted by the heat of the food inside. Generally, metal pans or aluminum foil should also not be used in a microwave oven, as the microwaves are reflected off these materials causing the food to cook unevenly and possibly damaging the oven. The instructions that come with each microwave oven indicate the kinds of containers to use. They also cover how to test containers to see whether or not they can be used in microwave ovens.

Food and drug administration (FDA) recommends that microwave ovens not be used in home canning. It is believed that neither microwave ovens nor conventional ovens produce or maintain temperatures high enough to kill the harmful bacteria that occur in some foods while canning.

Microwave oven safety standard

All microwave ovens made after October 1971 are covered by a radiation safety standard enforced by the food and drug administration (FDA). The standard limits the amount of microwaves that can leak from an oven throughout its lifetime. The limit is 5 milliwatts of microwave radiation per square centimeter at approximately 2 inches from the oven surface. This is far below the level known to harm people. Furthermore, as you move away from an oven, the level of any leaking microwave radiation that might be reaching you decreases dramatically. For example, someone standing 20 inches from an oven would receive approximately one-hundredth of the amount of microwaves received at 2 inches.

The standard also requires all ovens to have two independent interlock systems that stop the production of microwaves the moment the latch is released or the door opened. In addition, a monitoring system stops oven operation in case one or both of the interlock systems fail. The noise that many ovens continue to make after the door is open is usually the fan. The noise does not mean that microwaves are being produced. There is no residual radiation remaining after microwave production has stopped. In this regard a microwave oven is much like an electric light that stops glowing when it is turned off.

All ovens made since October 1971 must have a label stating that they meet the safety standard. In addition, FDA requires that all ovens made after October 1975 have a label explaining precautions for use. This requirement may be dropped if the manufacturer has proven that the oven will not exceed the allowable leakage limit even if used under the conditions cautioned against on the label.

To make sure the standard is met, FDA tests microwave ovens in commercial establishments, dealer and distributor premises, manufacturing plants, and its own laboratories. FDA also evaluates manufacturers' radiation testing and quality control programs. When FDA finds a radiation safety problem in a certain model or make of oven, it requires the manufacturer to correct all defective ovens at no cost to the consumer.

Although FDA believes the standard assures that microwave ovens do not present any radiation hazard, the agency continues to reassess its adequacy as new information becomes available (66).

Techniques for parasitological examination of intermediate hosts (Pepsin digestion, Baermann's apparatus)

Digestion method

Preparation of *T. spiralis* larvae

Third – stage larvae (Tsl_3) *T. spiralis* collection (pepsin digestion).

- Tsl_3 infected mice is sacrificed using chloroform or anaesthetic ether.
- Head, foot and tail of mice are removed.
- Skinning and cut to open the abdomen and thorax.
- The viscera are removed.
- The remained body of mice is chopped as fine as possible.
- The chopped mice is transferred to beaker.
- Small amount of 1% acid – pepsin (1% HCl – 1% pepsin) solution is added and the mixture is blent by using blender.
- The acid – pepsin solution is refilled and the preparation is incubated in a 37 $^{\circ}$C water bath for 1 hr.
- After incubating, the preparation is added into Baermann's apparatus and it is left at room temp for half an hour.
- The sediment containing Tsl_3 is removed into petri – dish and is washed with normal saline.
- The Tsl_3 is collected and counted.

T. spiralis larvae are isolated from mice by pepsin digestion. After the mice flesh are digested in a solution of 1% pepsin A and 1% HCL for 2 hours at 37ºC in shaking water bath. Following digestion, the suspension was filtered and then sediment in conical flasks for 30 minutes. The sediment is washed three times with normal saline to prevent larvae from rupturing and lysis.

Related researches

Food-borne nematode, a worldwide problem, causes mainly trichinellosis. The several types of nematode also use minnows as the intermediate host. Traditional ingestion of undercooked and uncooked pork preparations are a major factor in pathogen acquisition.

Hu X. and Mallikarjunan K. (2002) (67) report mathematical modeling of heat transfer of microwave heated transfer of microwave. According to their studies, fish gel. Fish gel was formed into a cylinder shape and heated from temperature of 75 ºC using power levels of 60, 70 and 80% for 36, 33 and 24 seconds. Fiber-optic probes measured the temperature at geometric center, surface, and bottom of the cylinder gel and the headspace. A 2-D cylindrical geometry model was established and a finite element method was applied to solve the equations. The model could predicted the heating profile within the temperature range of 8 ºC for different compositions of fish gel. Cold spots were observed at the bottom of the cylinder. There was a potential to use formulated fish gel to mimic seafood products for better understanding of microwave processing mechanisms.

Jirina H, et al., (2000) (68) studied the temperature profile in dough product during microwave heating with and without susceptors was followed in experiments with certain types of food samples. A household microwave oven (650 W), susceptors from commercial packages for microwave popcorn, samples of two commercial pizza products and two types of dough were used in the experiment together with Luxtron temperature measurement system. The temperatures reached at the end of heating on the bottom surface of sample varied between 103 and 115 ºC at the heating without susceptor, and between 110 and 115 ºC at the heating with susceptor. Not only the susceptor but also the parameters of the heated sample (the moisture content, height/weight, the initial temperature) influenced the increase of the temperature on the bottom surface of the samples. The highest temperatures were found at the end of the heating of samples from dough with a lower content of moisture. The linear correlation between the temperature at the bottom of the sample and the logarithm of the time of heating was proved only with the heating of sample from one type of dough. The application of susceptor in the microwave heating alters not only the product temperature in the places of contact with susceptor but so to a certain content in other places of the product. The change in the shape of the vertical temperature profile in the heated sample was found in the experiments with susceptor heating. For the optimal result of the heating with susceptor, the optimization on of certain product parameters (namely the moisture content and the dimensions) have to be made.

Sripotongnak S. (1996) (69) determined the effect of microwave heating on the inhibition of microbial growth. An attempt had been made in studying the efficiency of microwave in destruction of *Staphylococcus aureus, Bacillus cereus, Salmonella typhimurmum and Escherichia coli* in ready to eat food such as various curries, vegetable soup and dish of fried vegetable . Each pure microorganism was inoculated in each food types before freeze in the refrigerator for 24 hrs. Later microorganism counting was performed by pour plate techingue. The initial organisms were 10^7 cells/gram then put each into the microwave. The exposure time for destruction cells was 1, 2, 3 and 4 min in the microwave of 2,450 MHz. 600 watts as medium high.

After various times, the viable cell count had been detected by pour plate technique. The result showed that efficiency of microwaves in the destruction of the pathogenic microorganisms in food depended on food types and microorganisms types and the exposure time for destruction cells. All type of microorganisms in various curries was more destroyed than the microorganisms onto vegetables in the same period. The contamination of microorganisms in various foods, the most destroyed was *E. coli*. In dish of fried vegetables the lowest destroyed was *S. aureus*. In conclusion, the exposure period for destroy all microorganisms by microwave in various food was 2 min for food safety.

Limsuwan S. et al., (1994) (70) Reported a clinical study on trichinosis in Changwat Phayao, Thailand. An epidermic of trichinosis occurred in Northern Thailand. The source meat was a 150 kgs hilltribe pig. A clinical investigation was conducted using indirect IgG ELISA as a criteria for diagnosis. 52 suspected cases who had eaten the trichinous pork and developed relevant symptoms were hospitalized. 49 of them gave positive ELISA within 64 days after infection. The most common clinical features were myalgia (100%), fever (93.88%) and facial edema (87.71%). Diarrhea was found in approximately one half of the patients (55.10%). Skin rashes of various types were unexpectedly high (40.82%)

CHAPTER III

MATERIALS AND METHODS

Experimental design

This study was a true experimental research with factorial design type 2 x 2 x 3 x 3. The fermented pork consisted of salt, garlic and rice. The preparation of NAHM product, were 4,800 grams pork which was divided in half into two samples. Then T. spiralis larvae were mixed in pork, at 2,400 larvae per sample. Added the salt to make the concentration at 3 and 7% by weight an then garlic and rice were added at fix 12 and 10% by weight, respectively. After mixing these ingredients, each sample was divided in half, at 1,200 grams per sample. The fermentation was done for the period of 3 and 7 days separately.

To study the effect of microwave heating was divided into four groups. Three groups were cooked by microwave heating each sample at 3 levels, 1, 2 and 3 minutes respectively, and another one at control group which was not passed microwave heating. Afterward, T. spiralis larvae from NAHM of all experimental groups were harvested and test for the survivorship and infectivity.

Laboratory

The experiment was done at the Department parasitology, Faculty of Public Health and Department of Helminthology, Faculty of Tropical medicine, Mahidol University.

Duration of study

September 2007 – January 2008

Materials and equipments

- Tissue blender
- Beaker 200, 500 and 1,000 ml
- Pasteur pipettes
- Stereomicroscope
- Petridish
- Scissors
- Forceps
- Sedimentation flasks (conical flasks)
- Slides and cover glasses (22 x 22 mm.)
- Glasses plate (15 x 12 cm.)
- Syring 3 ml. and polyethylene tube
- Blades
- Auto pipette
- Balance
- Baermann's apparatus
- Glass bowl
- Microwave oven (SHARP) R – 215 Specific, Electricity Power 800 W, Wave 2,450 MH.

Experimental animals

Both sexes of mices strain, age between 3 and 4 weeks were used as experimental host of *T. spiralis*. These animals were bred and supplied by the National Laboratory Animal Center, Mahidol University. Mice were fed with a commercial laboratory diet. After infection with *T. spiralis* larvae were isolated from NAHM, these animals were divided and housed in groups of five mice each. A total of 60 mice were used during the course of the study.

Chemical Substance

- Pepsin A (BDH)
- 1% HCL
- NaCl

- Ether
- Sodium hydroxide

Experimental Process

1. Preparation of *T. spiralis* larvae

1.1 Third – stage larvae (Tsl3) of *T. spiralis* collection (pepsin digestion).

- Tsl3 infected mice was sacrificed using chloroform or anaesthetic ether.
- Head, foot and tail of mice was removed.
- Skinning and cut to open the abdomen and thorax.
- The viscera was removed.
- The remained body of mice was chopped as fine as possible
- The chopped mice was transferred to beaker.
- Small amount of 1% acid – pepsin (1% HCl – 1% pepsin) solution was added and the mixture was blent by using blender.
- The acid – pepsin solution was refilled and the preparation is incubated in a 37 °C water bath for 1 hr.
- After incubating, the preparation was added into Baermann's apparatus and it was left at room temp. for half an hour.
- The sediment containing Tsl3 was removed into Petri – dish and was washed with normal saline.
- The Tsl3 was collected and counted for 200 larvae per glass block.

T. spiralis Larvae were isolated from mice by pepsin digestion. After the mice flesh were digested in a solution of 1% pepsin A and 1% HCL for 2 hours at 37ºC in shaking water bath. Following digestion, the suspension was filtered and then sedimented in conical flasks for 30 minutes. The sediment was washed three times with normal saline to prevent larvae from rupturing and lysis.

2. Mixing of *T. spiralis* larvae into pork

T. spiralis larvae suspension of 4,800 larvae were mixed into 4,800 gm. pork sample.

3. Production of NAHM mixing with *T. spiralis* larvae

The proportions of the ingredients were approximately 4,800 g of pork mixing with *T. spiralis* larvae were separated and mixed with 3 and 7% salt concentration and each sample were added which 10% (w/w) rice and 12% (w/w) garlic respectively. All ingredients were mixed in a glass bowl. The fermentation were done for each salt concentration at 3 and 7 days, respectively.

4. Microwave heating

NAHM was divided into 2 groups by a salt concentration and a period of time. The first group was cooked by the high level of microwave heating and the other not passed. There were 0, 1, 2 and 3 minutes microwave heating time. After that the whole NAHM was minced by pepsin digestion methods. The sediment was washed three times with normal saline to prevent the larvae from rupturing and lysis

5. Collection of *T. spiralis* larvae and observing for larvae survivorship.

Larvae of *T.* spiralis were isolated from NAHM by sedimentation. After filtered in the Baermann's apparatus and sedimented in conical flasks for 30 minutes. The sediment was washed three times with normal saline to prevent the larvae from rupturing and lysis. After that they were divided into 2 groups by salt concentration and a period of time for using in microwave heating experiment.

6. Infectivity of *T. spiralis* larvae in mice.

Two sexes of forty eight of mice, age 3-4 weeks, from the National Laboratory Animal Center, Mahidol University, were used in the experiments. They are divided into two groups, 24 animals per group and 12 animals for control group. The first group fed with NAHM from the high level microwave heating and another group was fed with no pass. The mice feeding equipment consist of polyethelene tube 3", needle 21 G. and syringe 2 cc. After making unconscious mice by ether, *T.*

spiralis larvae in 0.85% NaCl were fed into mice (50 larvae/mice) using polyethelene tube insert into alimentary canal.

After mice were infected with *T. spiralis* larvae for 3-4 weeks. They were sacrificed. The diaphragm and body muscle of mice were examined under a stereomicroscope for *T. spiralis* larvae.

Control group

Group1. Control group (*T. spiralis* larvae in 0.85% NaCl)

T. spiralis larvae were collected from infected mice by pepsin digestion.

T. spiralis larvae were mixed with 0.85% NaCl and left there at the room temperature for 3 and 7 days.

T. spiralis larvae were fed into mice (50 larvae/mice).

Group2. Garlic group

T. spiralis larvae were mixed with 12% garlic by weight and left there at the room temperature for 3 and 7 days.

T. spiralis larvae were collected from garlic by filter.

3. *T. spiralis* larvae were fed into mice (50 larvae/mice).

Quality Control

- Only one performed all of the experiments in the whole research work
- Tools used in the experiment were passed accuracy and reliable testing.
- The mice (*Mus musculus*) used in this research were free from *T. spiralis*.
- The experimenter is trained in *T. spiralis* larvae counting technique; to count the number of *T. spiralis* larvae.

Data Analysis

1. Descriptive statistics percentage means S.D.

2. Statistical analysis ANOVA and find difference by using comparison method.

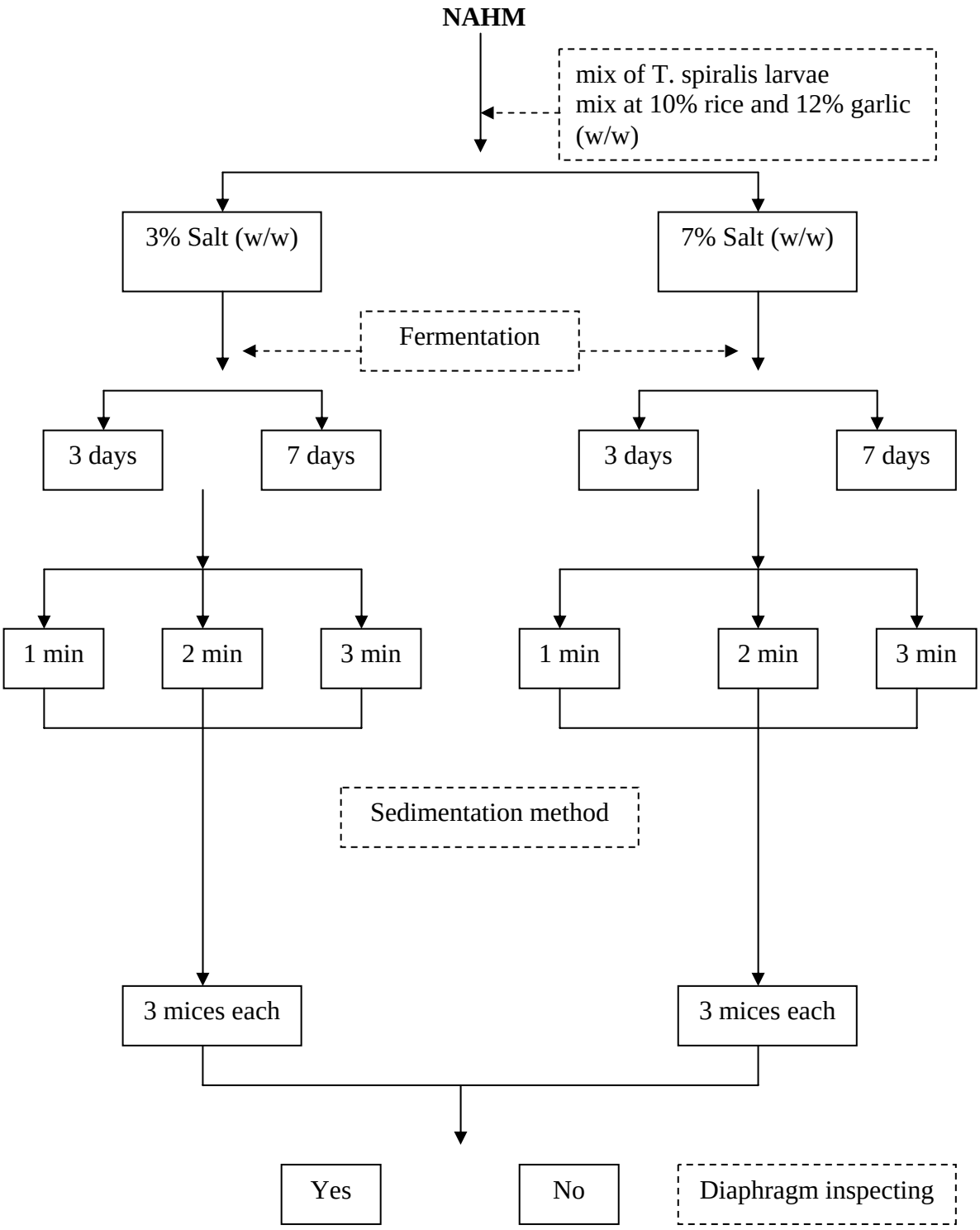

Figure 4 Experimental diagram for fermentation (NAHM) and Microwave heating on survivorship and infection of mice by larvae of T. spiralis

CHAPTER IV

RESULTS

In these studies, salt and garlic concentration, period of time for fermentation and microwave heating on survivorship and infection ability of *T. spiralis* larvae in mice were investigated.

This study was a true experimental research with factorial design type 2 x 2 x 3 x 3. The fermented pork consisted of salt, garlic and rice. The pork was divided into two samples in half, at 2,400 grams per sample. Then *T. spiralis* larvae were mixed in pork, at 2,400 larvae per sample. The salt was concentrated at 3 and 7% by weight, garlic and rice verve fixed at 12 and 10% by weight, respectively. After mixing an ingredient, each sample was divided in half, at 1,200 grams sample each. The duration of fermentation was at 3 and 7 days of each group.

Experimental result of average temperature NAHM cooked by microwave heating at duration of time for 1, 2, and 3 minutes respectively, were 51.6, 74.3 and 80.6 °C respectively.(as shown in Table 2)

Table 2 Effect of microwave heating on the temperature to NAHM.

Exposed time (minutes)	Mean temperature (°C)	S.D
1	51.6	9.2
2	74.3	5.1
3	80.6	3.7

Group controls of larvae *T. spiralis* mix with 0.85%NaCl, and larvae *T .spiralis* mix with 12% garlic by weight, duration of time at 3 and 7 days and the infection ability of larvae of *T. spiralis*.

In the control group; *T. spiralis* larvae mixed with 0.85% NaCl, it was found that, after 3 days of fermentation 6.6 and 43.33 larvae were active and inactive, respectively. After 7 days of fermentation 3.3 and 46.6 larvae were active and inactive, respectively. The infectivity of these larvae in mice vere 100%. The details was shown in Table 3.

Table 3 Mean and standard deviations of *T. spiralis* larvae mixed with 0.85%NaCl duration of time at 3 and 7 days and the infection ability of *T. spiralis* larvae (2 replicates).

Exposed time	Active			In active			Infection ability (%)	
(Days)	Mean	S.D	%	Mean	S.D	%	Infection	Non-infection
3	6.6	0.5	33.3	43.3	0.5	66.6	100	-
7	3.3	1.5	66.6	46.6	1.5	33.3	100	-

For *T. spiralis* larvae mixed with 12% garlic, it was found that, after 3days of fermentation, and 50 larvae were active and inactive, respectively. After 7 days of fermentation, and 50 larvae were active and inactive, respectively. The infectivity of these larvae in mice were 100%. The details was shown in Table 4.

Table 4 Mean and standard deviations of larvae *T.spiralis* mix with 12% duration of time at 3 and 7 days and the infection ability of larvae of *T.spiralis* (2 replicates).

Exposed time (Days)	Active Mean	S.D	%	In active Mean	S.D	%	Infection ability (%) Infection	Non-infection
3	0.0	0.0	0	50.0	50.0	100	100	-
7	0.0	0.0	0	50.0	50.0	100	100	-

For larvae of *T.spiralis* in fermentation (NAHM) by 3% of salt concentration (w/w). Duration of time used for fermentation for 3 and 7 days, and high level microwave heating for 1, 2, and 3 minutes, respectively. The results of percentiles at 3 days by through at 0 minute were active and inactive for larvae *T.spiralis*. The results of mean at active at 3 days were 0 larvae and inactive at 3 days were 2 larvae, and at 7 days by through at 0 minute were 0 larvae and inactive at 7 days were 0 larvae respectively, The results of mean at 3 days by through at 1, 2 and 3 minutes were 2, 2 and 3 larvae respectively, at 7 days were not discover. By through infection ability of mice's at 100 % at 3 days at 0 minute, but at 7 days were not infection ability (as show in Table 5).

Table 5. Number larvae of *T.spiralis* in fermentation (NAHM) by 3% of salt concentration (w/w). Duration of time used for fermentation for 3 and 7 days, and high level microwave heating for 1, 2, and 3 minutes (2 replicates).

Day	Expose time (minutes)							
	0		1		2		3	
(n=50)	Active	In active	Active	In active	Active	In active	Active	In active
3	-	2	-	2	-	2	-	3
7	-	-	-	-	-	-	-	-

For larvae of *T.spiralis* in fermentation (NAHM) by 7% of salt concentration (w/w). Duration of time used for fermentation for 3 and 7 days, and high level microwave heating for 1, 2, and 3 minutes, respectively. The examined not discover *T.spiralis* lavae.

CHAPTER V
DISCUSSION

The results of the studies showed that the preconditioning temperature of NAHM after cooked by microwave for 1, 2 and 3 minutes at 51.6, 74.3 and 80.6°C respectively could kill *T. spiralis* larvae. According to the studies by Gramble HR. (2006) (71), *T. spiralis* was kill in 47, 6 and < 1 minutes at 52 (125.6°F), 55 (131°F) and 60°C (140°F), respectively. Although this was considerably higher than temperatures at which trichinae were killed (about 55°C or 131°F), it allowed for different methods of cooking which did not always result in even distribution of temperature throughout the meat. It should be noted that heating to 77 °C (171°F) or 82°C (180°F) was not completely effective when cooking was performed using microwaves (71).

From the studies, *T. spiralis* larvae mixed with 12% garlic (w/w) and fermented for 3 and 7 days, it were found that the larvae were not killed and they had ability to infect mice. The experiment result from studies conducted by Anthony JP, Fyfe L and Smith H. (2005) (72). 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 *Leishmania*, 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. Abu EI Ezz NM. (2005) (73) reported the results of the studies on prophylactic and therapeutic effect of two oils either prior to infection or post infection in rats. Each rat in either case was orally administered with *Nigella sativa* oil or *Allium cepa* oil in a dose 5 mg/kg body weight/day for 2 weeks. Assessment of results was by: adult worm count in the

intestine on 7th and 20th day post infection. Larval count in the muscles on the 60th day post infection. Index of reproductive capacity and detection of antibodies against *T. spiralis* larvae were done by using ELISA. The results showed that, *N. sativa* oil as prophylactic treatment prior to *T. spiralis* infection was more effective than *A. cepa* oil on both adult worms and muscle larval count. While, *A. cepa* oil was showed more effectiveness than *N. sativa* on decline number of adult worms and muscle larvae when used as therapeutic treatment post infection. The level of antibody was recorded early in the groups that treated with *N. sativa* oil. In conclusion, *N. sativa* and *A. cepa* oils had anthelmintic effect in the rats infected with *T. spiralis* and increased the production of antibodies generated during life cycle of this parasite. From the experiment for larvae of *T. spiralis* of NAHM by microwave heating duration of time for 1, 2, and 3 minutes respectively, had ability to kill *T. spiralis* in 47, 6 and <1 minutes at 52°C (125.6°F), 55°C (131°F) and 60°C (140°F), respectively. It should be noted that heating to 77°C (171°F) or 82° C (180° F) was not completely effective when cooking was performed by using microwaves (71). From the experiment, salt concentration, period of time for fermentation under the conditions of the study, preparing Genoa Salami with salt concentration as low as 2% did not appear to effect the destruction of *Trichinella* larvae (74).

CHAPTER VI
CONCLUSION AND RECOMMENDATION

The studies were conducted, according to the process of doing NAHM, by using *T. spiralis* larvae and pork mixed with rice, salt and garlic and left for fermentation for 3 and 7 days. After that they were uncooked and cooked for 1, 2 and 3 minutes in microwave.

From the sample, *T. spiralis* larvae were observed for their survivorship and ability to infect mice. They were found that microwave heating at these 3 periods could kill *T. spiralis* in NAHM.

Recommendations from research finding

1. Although the method of sedimentation could collect the whole larvae in the sample. However the small or limited number of *T. spiralis* larvae used in preparing NAHM caused the less number of larvae collected.

2. *T. spiralis* larvae were non-encapulated. Some of them might be digested by acid between the fermentation. This situation caused the loss of some *T. spiralis* larvae.

3. The active and in active larvae were observed for their survivorship and all larvae were infected into the same mice for their ability of infection. In these studies, if the active and inactive larvae were infection into the separate mice. The results of infection could be compared for their ability of infectivity.

Suggestions for future researches

1. *T. spiralis* larvae in raw meat of mice should be used in preparation of NAHM. These, they would be encapsulate larvae and more resistant to be destroyed by acid between fermentation. They might endure to high temperature when were cooked in microwave. And, they would also be high ability for infection.

2. The efficiency of *Allium cepa*, at different concentration and fermentation pried, in killing *T. spiralis* larvae capsulate be studies.

REFERENCES

1. Pozio E. New patterns of *Trichinella* infection. Vet. Parasitol 2001;98:133–48.
2. Pozio E. Factors affecting the flow among domestic, synanthropic and sylvatic cycles of *Trichinella*. Vet. Parasitol 2000;93:241-62.
3. Dupouy-Camet J, Kociecka W, Bruschi F, Bolas-Fernandez F, Pozio E. Opinion on the diagnosis and treatment of human trichinellosis. Expert Opin. Pharamacother 2002;3(8):1117–30.
4. Division of Epidemiology, Office of the Permanent Secretary for Public Health, Ministy of Public Health. Ann Epidemiol Surv Rep 1982-1998 (Thai).
5. Nontasut P, Chullawichit S, Vichit A. A Follow-up Study on Trichinellosis in Mae Hong Son Province, Thailand. J Trop Med Parasitolgy 1999;22(1):32-3.
6. Division of Epidemiology, Office of the Permanent Secretary for Public Health, Ministy of Public Health. Ann Epidemiol Surv Rep 2006; 124–6(Thai).
7. Mitreva M, Jasmer, D.P. Biology and genome of *Trichinella spiralis*. WormBook 2006;3–4.
8. Boonthanom P, Nawarat A. The outbreaks of trichinosis at Amphur Mae Sarialng. Bull Pub Health 1963;33:301-8.
9. Morakote N, Khamboonruang C, Siriprasert V, Suphawitayanukul S, Marcanantachoti S, Thamasonthi W. The value of enzyme-linked immunosorbent assay (ELISA) for diagnosis of human trichinosis. Trop Med Parasitol 1991;42:172-4.
10. Morakote N, Sukhavat K, Khamboonruang C, Siriprasert V, Suphawitayanukul S, Thamasonthi W. Persistence of IgG, IgM, and IgE antibodies in human trichinosis. Trop Med Parasitol 1992;43:167-9
11. Limsuwan S, Siriprasert V. A clinical study on trichinosis in Changwat Phayao, Thailand. Southeast Asian J Trop Med Public Health 1994;25:305-8
12. Chumkasian P. Trichinosis in Thailand. Annual Epidemiological Surveillance

Report, Bangkok, Office of permanent secretary, MOPH, Thailand, 1999.

13. Takahashi Y, Mingyuan L, Waikagul J. Epidemiology of trichinellosis in Asia and the Pacific Rim. Vet Parasitol 2000;93:227-39.
14. Ittiprasert W, Butraporn P, Kitikoon V, Klongkamnuankarn K, Pholsena K, Vanisaveth V, Sakolvaree Y, Chongsa-nguan M, Tapchaisri P, Mahakunkijcharoen Y, Kurazono H, Hayashi H, Chaicumpa W. Differential diagnosis of schistosomiasis mekongi and trichinellosis in human. Parasitol Int 2000;49:209-18.
15. Khamboonruang C, Nateewatana N. Trichinosis: A recent outbreak in Northern Thailand. Southeast Asian J Trop Med Public Health 1975 6 74-8.
16. Dissamarn R, Chai-ananda P. The present position of trichinosis in thailand. Bull Off Int Epizoot 1965;63:217-9.
17. Wiwatanaworapant T. Trichinosis: a case report. Siriraj Hosp Gaz 1980; 32: 220-2.
18. Chalermchaikit T, Nawarat A, Muangyai M, Brahmasa R, Chumkasian P. Epidemiological surveillance of trichinosis outbreak in Petchaboon province. Thai J Vet Med 1982;12:1-23.
19. Oonsombat P, Attanatho V, Trishnananda M. Human trichinosis: the fourteenth outbreak in Thailand. Siriraj Hospital Gazette1982;34:69-72.
20. Pinanong M, Tongma S, Hongphinyo V. Human trichinosis : an outbreak at Petchaboon province. Royal Thai Air Force Med Gaz 1985;31:87-94.
21. Khamboonruang C. The present status of trichinellosis in Thailand. Southeast Asian J Trop Med Public Health 1991; 22 Suppl:312-5.
22. Chotmongkol V, Intapan PM, Koonmee S, Kularbkaew C, Aungaree T. Case report: acquired progressive muscular hypertrophy and trichinosis. Am J Trop Med Hyg 2005;72:649-50.
23. Kaewpitoon N, Jatesadapattaya S, Philasri C, Leksomnoon R, Maneenin C, Sirilaph S, Pengsaa P. Trichinosis : Epidemiology in Thailand 2006 ; 12(40):6440–5.
24. Porndamnernpong S. Reduction of *Ascaris suum* eggs in lettuce with different washing agent. [M.Sc.Thesis in Public Health]. Bangkok:Faculty of Graduate Studies, Mahidol University 2002.

25. Geller ER, Malykhina AN, Silakova LN, Timonov EV. Morpho-physiologic criteria for the taxonomic independence of the species *Trichinella pseudospiralis* Garkavi, 1972. Parazitologiia 1977;11:113-6.

26. Pozio E, La Rosa G, Murrell KD, Lichtenfels JR. Taxonomic revision of the genus Trichinella. J Parasitol 1992;78:654-9.

27. Pozio E, La Rosa G, Yamaguchi T, Saito S. *Trichinella britovi* from Japan. J Parasitol 1996;82:847-9.

28. Pozio E, La Rosa G. *Trichinella murrellin*. sp: etiological agent of sylvatic trichinellosis in temperate areas of North America. J Parasitol 2000;86:134-9.

29. Charkrit S. Study on clinical manifestations of trichinosis in Payao province. Com Dis J 1998;24:242-7.

30. Jongwutiwes S, Chantachum N, Kraivichian P, Siriyasatien P, Putaporntip C, Tamburrini A, La Rosa G, Sreesunpasirikul C, Yingyourd P, Pozio E. First outbreak of human trichinellosis caused by *Trichinella pseudospiralis*. Clin Infect Dis 1998;26:111-5.

31. *Trichinella* systematics and distribution. [serial on line] 2002 February [cited 2008 February 11]; Available from: URL: http://www.iss.it/site/Trichinella/scripts/tbl1.asp.

32. *Trichinella* systematics and distribution. [serial on line] 2002 February [cited 2008 February 11]; Available from: URL: http://www.iss.it/site/Trichinella/scripts/tbl1.asp.

33. Trichinellosis. Parasites and Health. [serial on line] 2002 February [cited 2008 February 11]; Available from: URL: http://www.dpd.cdc.gov/dpdx/HTML/Trichinellosis.htm .

34. Doege TC, Thienprasit P, Headington JT, Pongprot B, Tarawanich S. Trichinosis and raw bear meat in Thailand. Lancet 1969;1:459-61.

35. Suriyanon V, Klunklin K. Human trichinosis: analysis of cases during the tenth outbreak in North Thailand. Southeast Asian J Trop Med Public Health 1972;3:390-6.

36. Dissamarn R, Indrakamhang P. Trichinosis in Thailand during 1962-1983. Int J Zoonoses 1985;12:257-66.

37. Division of Epidemiology, Office of the Permanent Secretary for Public Health, Ministy of Public Health. Ann Epidemiol Surv Rep 1996-2005 (Thai).

38. Doege TC, Thienprasit P, Headington JT, Pongprot B, Tarawanich S. Trichinosis and raw bear meat in Thailand. Lancet 1969 ;1:459-61.

39. Suriyanon V, Klunklin K. Human trichinosis: analysis of cases during the tenth outbreak in North Thailand. Southeast Asian J Trop Med Public Health 1972;3:390-6.

40. Dissamarn R, Indrakamhang P. Trichinosis in Thailand during 1962-1983. Int J Zoonoses 1985;12:257-66.

41. Srikitjakarn L, Korakovit M, Toboran P, Srutaravong V, Bettermann G, Lingelback W. *Trichinella spiralis* in dog meat for human consumption in Sakon Nakon province. Thai Vet Med Assoc 1981;32:271-7.

42. Dordevic, M. Detection of *Trichinella* by various methods in Yugoslavia. Southeast Asian J. Trop. Med. Public Health 1991;22(Suppl.):326–8.

43. Murrell, D., and F. Bruschi. Clinical trichinellosis, *In* T. Sun (ed.), Progress in clinical parasitology. CRC Press, Boca Raton, Fla 1994;p.117–50.

44. Purkerson, M., and D. Despommier. Fine structure of the muscle phase of *Trichinella spiralis* in the mouse, *In* C. Kim (ed.), Trichinellosis. Intext Publishers, New York 1974; p.7–23.

45. Dzbenski, T. D., E. Bitkowski, and W. Plonka. Detection of circulating antigen in acute infections with *Trichinella spiralis*: diagnostic significance of findings. Zentralbl. Bakteriol 1994;281:519–25.

46. Pozio, E., G. La Rosa, P. Rossi, and K. D. Murrell. Biological characterizationof trichinella isolates from various host species and geographical regions. J. Parasitol 1992;78:647–53.

47. Froscher, W., F. Gullotta, M. Saathoff, and W. Tackmann. Chronic trichinosis. Clinical bioptic, serological and electron myographic observations. Eur. Neurol 1988;28:221–6.

48. Capo V, Despommier. Clinical aspects of infection with *Trichinella* spp. Clinical Microbiology Reviews 1996;9(1):47-54.

49. Pawlowski, Z. S. Clinical aspects in man, *In* W. C. Campbell(ed.), *Trichinella* and trichinosis. Plenum Press, New York 1983;p.367–402.

50. Pozio, E., P. Varese, M. A. Morales, G. P. Croppoi, D. Pellicia, and F. Bruschi. Comparison of human trichinellosis caused by *T. spiralis* and *T. britovi* . Am. J. Trop. Med. Hyg 1993;48:568–75.

51. Bailey, T. M., and P. M. Schantz. Trends in the incidence and transmission patterns of trichinosis in humans in the United States: comparisons of the periods 1975–1981 and 1982–1986. Rev. Infect. Dis 1990;12:5–11.

52. Leight, J. C. Public health aspects (with special reference to the United States), *In* W. C. Campbell (ed.), *Trichinella* and trichinosis Plenum Press, New York 1983;p. 501–28.

53. Pozio, E., and G. La Rosa. General introduction and epidemiology of trichinellosis. Southeast Asian J. Trop. Med. Public Health 1991;22(Suppl.):291–4.

54. McAuley JB, Michelson MK, Schantz PM. Trichinellosis surveillance, United States, 1987-1990. In: CDC Surveillance Summaries (December). MMWR 1991;40(No. SS-3):35-42.

55. McAuley JB, Michelson MK, Hightower AW, Engeran S, Wintermeyer LA, Schantz PM. A trichinosis outbreak among Southeast Asian refugees. Am J Epidemiol 1992;135:1404-10.

56. Food Safety and Inspection Service, United States Department of Agriculture. Safety of fresh pork...from farm to table. Washington, DC: Food Safety an Inspection Service, United States Department of Agriculture, [serial on line] 2002 September [cited 2008 February 11]; Available from: URL: http://www.fsis.usda.gov/OA/pubs/pork.htm.

57. Kotula AW, Murrell KD, Acosta-Stein L, Lamb L, Douglass L. *Trichinella spiralis*: effect of high temperature on infectivity in pork. Exp Parasitol 1983;56:15-9.

58. Gamble HR. Parasites associated with pork and pork products. Rev Sci Tech 1997;16:496-506.

59. Moorhead A, Grunenwald PE, Dietz VJ, Schantz PM. Trichinellosis in the United States, 1991-1996: declining but not gone. Am J Trop Med Hyg 1999;60:66-9.

60. Worley DE, Seesee FM, Espinosa RH, Sterner MC. Survival of sylvatic *Trichinella spiralis* isolates in frozen tissue and processed meat products. J Am Vet Med Assoc 1986;189:1047-9.

61. Kjos-Hanssen B. *Trichinella* isolates from polar bears in Svalbard. Freeze resistance and infectivity in rats and swine. Nord Vet Med 1984;36:57-61.

62. Chadee K, Dick TA. Designation and freezing resistance of isolates of *Trichinella spiralis* from wild carnivores. J Wildl Dis 1982;18:169-73.

63. Sharon L. Adriana S. Peter M. Schantz. Trichinellosis Surveillance-United States, 1997-2001 2003; 52(SS06):1-8 [cited 2008 February 11]; Available from: URL: http://www.cdc.gov/mmwr/preview/mmwrhtml/ss520al.htm.

64. The complete Microwave Oven Service Hand book. [serial on line] 1989-2008. [cited 2008 February 11]; Available from: URL: http://www.gallawa.com/microtech/mwave.html.

65. Microwave Oven Safety Information. [serial on line] 2000-2005 [cited 2008 February 11]; Available from: URL: http://www.bestnetcraft.com/links/microwave.html.

66. Microwave Oven Radiation [serial on line] 2008. [cited 2008 February 11]; Available from: URL: http://www.fda.gov/cdrh/consumer/microwave.html.

67. Hu X, Mallikarjunan K. Abstract : Mathematical modeling of heat transfer of microwave heated fish gel. American Society of Agricultural Engineers 2002. [cited 2008 February 11]; Michigan Available from: URL: www. Asae.org/technical library.html.

68. Jirina Hare, Karel H. Temperature Profiles in Dough Products during Microwave Heating with Susceptors. Czech J Food Sci 2002;20(4):151-60.

69. Sripotongnak S. Effect of microwave heating on the inhibition of microbial groeth : Thai Food. [M.Sc. Thesis in Public Health]. Bangkok:Faculty of Graduate Studies, Mahidol University, 1996.

70. Limsuwan S, Siriprasert V. A clinical study on Trichinosis in Changwat Phayao, Thailand. An epidemic of trichinosis occurred in Northern Thailand. Southeast Asian J Trop Med Pubic Health 1994;25(2):306-8.

71. Gamble HR. *Trichinae*: pork facts-food quality and safety. Beltsville, MD: United States Department of Agriculture Animal and Plant Health Inspection Service, 2001. [cited 2008 February 11]; Available from: URL: http://www.aphis.usda.gov/vs/trichinae/docs/fact_sheet.htm

72. Anthony JP, Fyfe L, Smith H. Plant active components - a resource for antiparasitic agents? Trends Parasitol. 2005 Oct;21(10):462-8.

73. Abu El Ezz NM. Effect of *Nigella sativa* and *Allium cepa* oils on *Trichinella spiralis* in experimentally infected rats. J Egypt Soc Parasitol. 2005;35(2):511-23.

74. H J Smith, S Messier, and F Tittiger Destruction of *Trichinella spiralis* spiralis during the preparation of the "dry cured" pork products proscuitto, proscuittini and Genoa salami. Can J Vet Res. 1989;53(1): 80–3.

APPENDIX

APPENDIX A

Preparation of *Trichinella spiralis*

T. spiralis larvae were isolated from mice by pepsin digestion. After the mice flesh were digested in a solution of 1% pepsin A and 1% HCL for 2 hours at 37ºC in shaking water bath. Following digestion, the suspension was filtered and then sedimented in conical flasks for 30 minutes. The sediment was washed three times with normal saline to prevent of larvae from rupturing and lysis. *T. spiralis* larvae were examined and counted at Department of Helminthology, Faculty of Tropical Medicine, Mahidol University.

APPENDIX B

Recording Form

Table B - 1 : Recording form

Effect of microwave heating on the temperature to NAHM.

Sample(NAHM)	**1(min)**			**2(min)**			**3(min)**		
	1	2	3	1	2	3	1	2	3
NAHM (1/80g.)									
Total									

Table B - 2 : Recording form

Mean and standard deviations of the infection ability of *T. spiralis* larvae mixed with 0.85% NaCl for 3 and 7 days.
(2 replicates).

Day	**Active**			**In active**			**infection**					
							Yes			**No**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
3												
7												

Table B - 3 : Recording form

Mean and standard deviations of the infection ability of larvae of *T. spiralis* in 12% garlic for 3 and 7 days. (2 replicates).

Day	Active			In active			infection					
							Yes			No		
	1	2	3	1	2	3	1	2	3	1	2	3
3												
7												

Table B – 3 : Recording form

Number larvae of *T. spiralis* in NAHM at by 3 and 7% of salt concentration (w/w). Duration of time used for fermentation for 3 and 7 days, and high level microwave heating for 1, 2, and 3 minutes (2 replicates).

Sample	0(min)		Infection		Microwave						Infection	
					1(min)		2(min)		3(min)			
	Active	In active	Yes	No	active	In active	active	In active	active	In active	Yes	No
1												
2												
3												

APPENDIX C

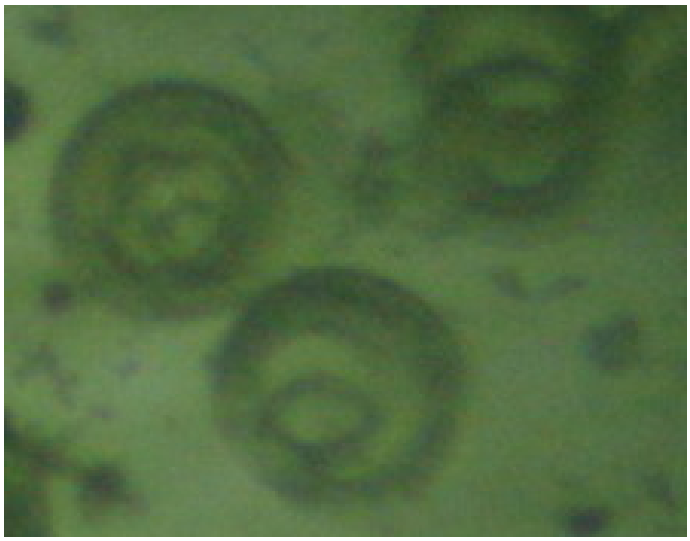

Figure 5 : *Trichinelle spiralis* adult digested from laboratory infected mice.

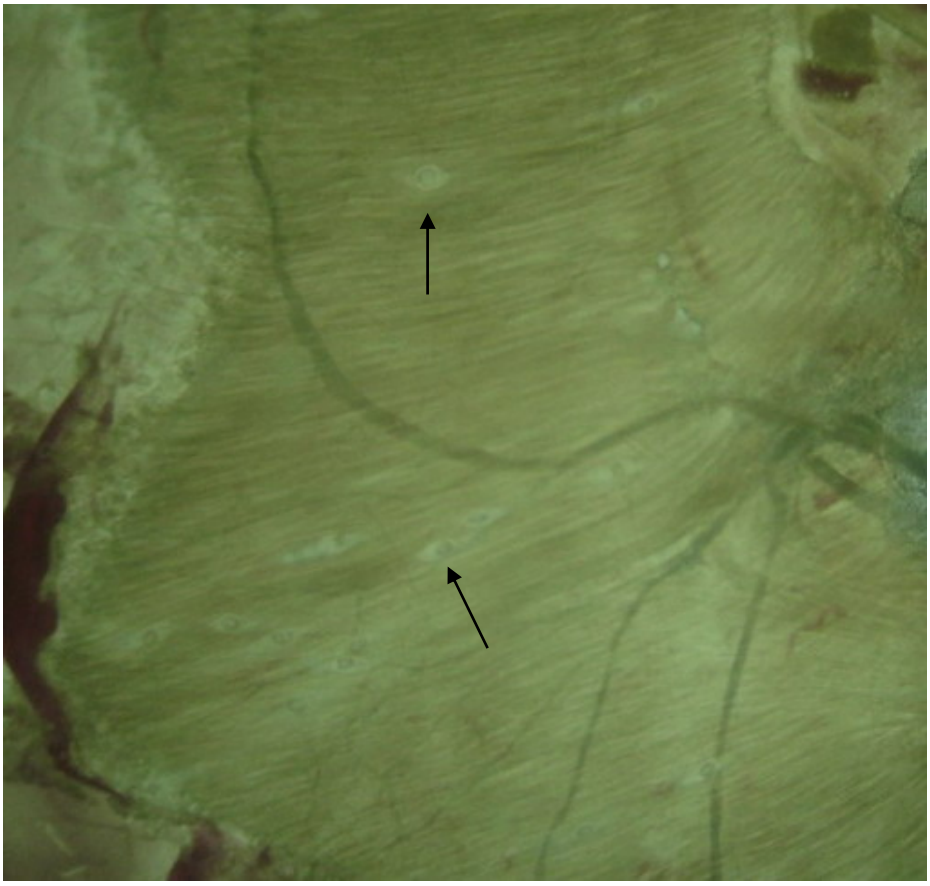

Figure 6 : *Trichinelle spiralis* larvae in mice diaphragm.

BIOGRAPHY

NAME	Mr. Kittipong Vubsunthear
DATE OF BIRTH	OCTOBER 26, 1974
PLACE OF BIRTH	Nakhonratchasima, Thailand
INSTITUATION ATTENDED	Mahidol University, 2000 - 2003
	Bachelor of Public Health
	Mahidol University, 2004 – 2007
	Master of Science (Public Health)
	Major in Infectious Diseases and Epidemiology
POSITION & OFFICE	
Position	Public Health Technician
Office	Sungneon Hospital
	Nakhonratchasima, Thailand
	Tel. 08-7608-5217, 08-1266-1145