

Mahidol University Faculty of Tropical Medicine

## SATELLITE SYMPODIUM : MEAT HYGENE IN ASIAN COUNTRIES



The outbreaks of *Trichinella papuae* infection in BanRai District, Thailand: Experience of successful control of the disease

### Teera Kusolsuk : M.D., D.T.M.& H.

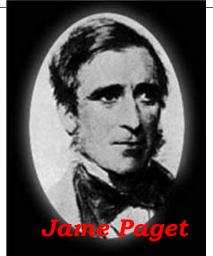
Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand teera.kus@mahidol.edu

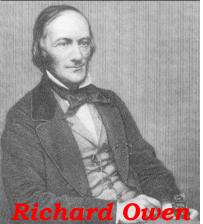
Thawatchai Ketboonlue, Wiroj Tubtim, Artit Janklang, Chowalit Khumjui, Pakkaniskarn Praditsuwan, Yukifumi Nawa, Paron Dekumyoy

# Outline

### **o** History of Trichinellosis

- Epidemiology of Trichinellosis in Thailand
- Briefly two outbreaks of Trichinellosis papuae in BanRai District, Thailand
- Experience of control the disease
- The follow up patients and villagers in 10 years after two outbreaks





ON THE FRICHINA SPIRALIS

OBSERVATIONS

BY WILLIAM TURNER, M.B. Lond., BENIOR DEMONSTRATOR OF ANATOMY IN THE UNIVERSITY OF EDINBURGH. (REPRINTED FROM THE EDINEURON MEDICAL JOURNAL, SEPTEMBER 1880.)

# History

XXXV. Description of a Microscopic Entozoon infesting the Muscles of the Human Body. By RICHARD OWEN, Esq., F.R.S. & Z.S., Assistant Conservator of the Museum of the Royal College of Surgeons in London.

Communicated February 24, 1835.

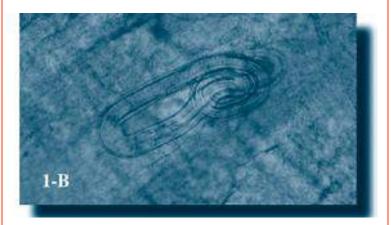
- 1835: First identified under the microscope by Paget and Owen
- 1846: The parasite was first reported in the United states
- 1860: Rudolf Virchow and Leukart had described the complete life cycle of *Trichinella spirals*
- 1865: It was shown that *Trichinella* is a significant human pathogen capable of causing severe disease

## Trichinella species : 2 clades

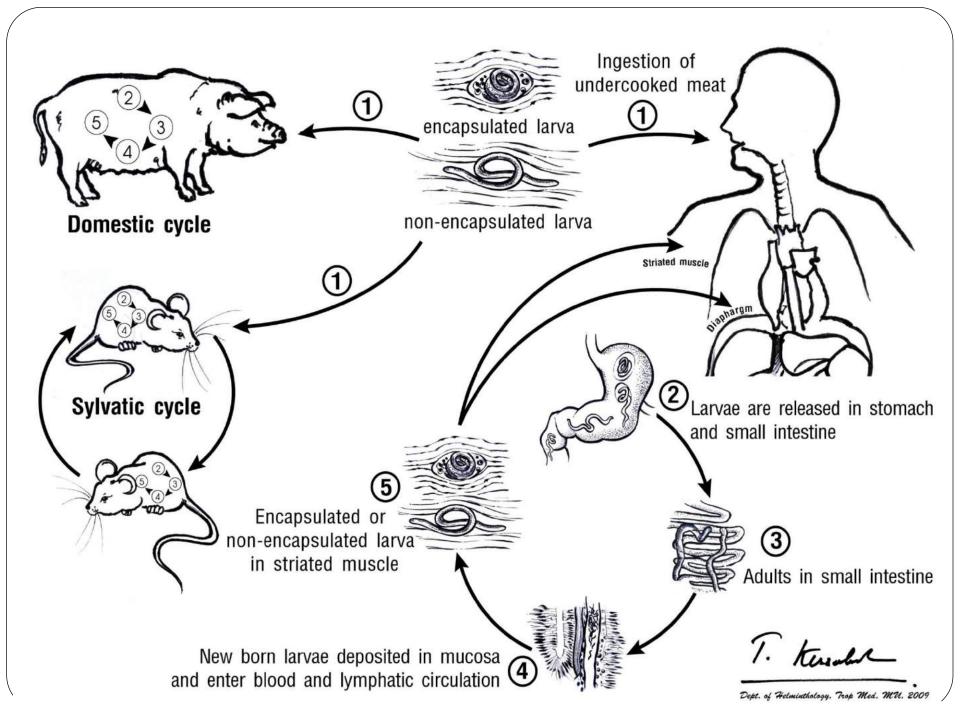
- Encapsulated
- \* T.spiralis
- \* T.nativa
- \* T.britovi
- \* T.murrelli
- \* T.nelsoni



- Nonencapsulated
- T.pseudospiralis
- ► T.papuae
- T.zimbabwensis



Species	Distribution Major Hosts		Reported from human			
T. spiralis	Cosmopolitan	Domestic pigs, wild mammals	Yes			
T. britovi	Eurasia/Africa	Wild mammals	Yes			
T. murrelli	North America	Wild mammals	Yes			
T. nativa	Arctic/subarctic, Palaearctic	Bears, foxes	Yes			
T. nelsoni	Equatorial Africa	Hyenas, felids	Yes			
T. pseudospiralis *	Cosmopolitan	Wild mammals, birds	Yes			
T. papuae *	Papua New Guinea, Thailand	Pigs, crocodiles	Yes			
T. zimbabwensis *	East and South Africa	Crocodiles, lizards, lions	No			
encapsu	lated group	* non-encapsulated group				



# Outline

• History of Trichinellosis

### Epidemiology of Trichinellosis in Thailand

- Briefly two outbreaks of Trichinellosis papuae in BanRai District, Thailand
- Experience of control the disease
- The follow up patients and villagers

## Epidemiology of Trichinellosis in Thailand

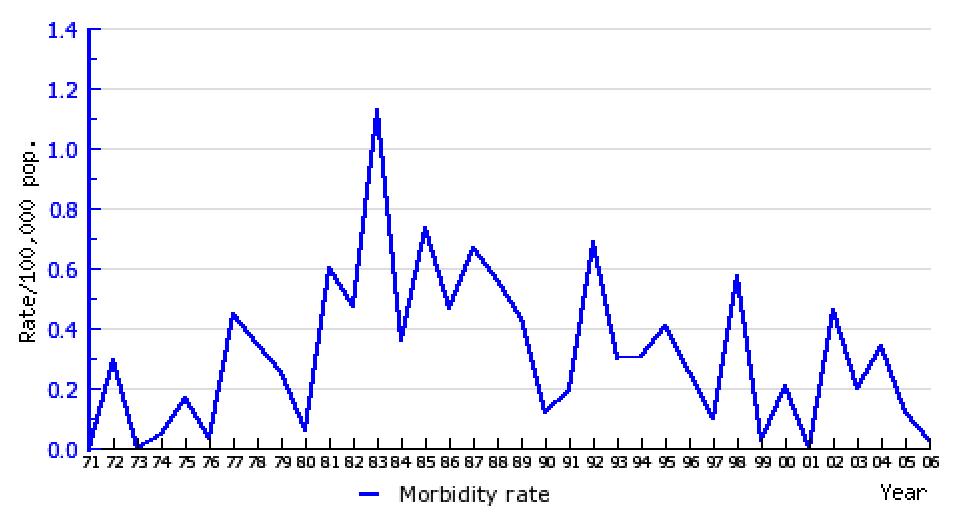
- In Thailand, the year 1962, the first outbreak of Trichinellosis is recorded in Mae Sariang District, Mae Hong Son Province
- In 1962-1983; 2,792 patients were infected by trichinellosis and 85 cases were died
- □ All most outbreaks in the Northern Thailand, such as CheingRai Province, Nan Province etc., the causative agent of most outbreaks has been identified as *T. spiralis*
- In 1994, an outbreak occurred in the Ta-Sae District, Chumporn Province, Southern Thailand, 59 patients were infected and one case died and later on can identified as *Trichinella pseudospiralis*. (Jongwutiwes S.; Clin Infect Dis. 1998)

## The epidemiology of trichinellosis in Thailand

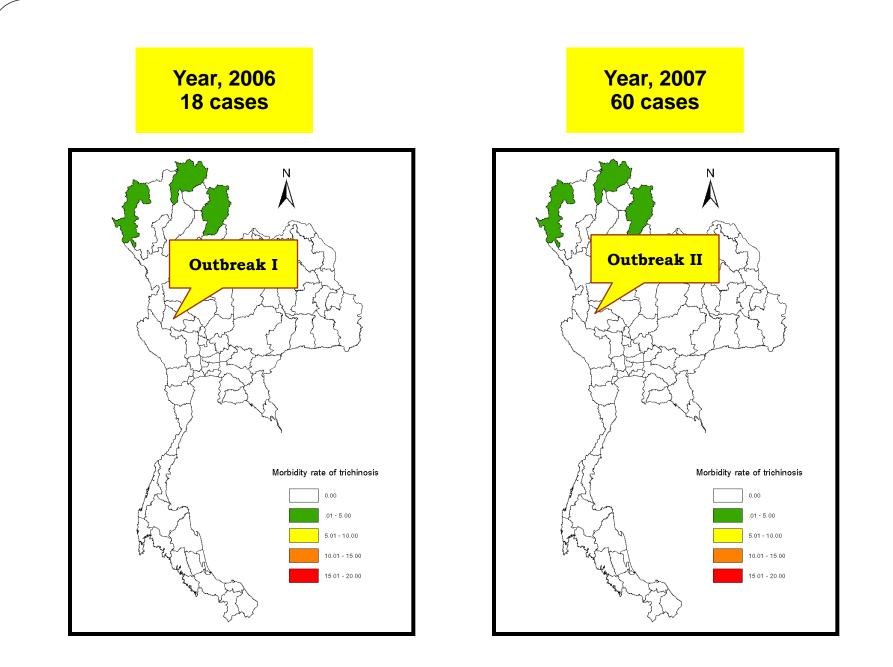
Year	No. of patients	No. of death	Year	No. of patients	No. of death
1986	247	0	1997	61	1
1987	356	0	1998	351	0
1988	307	3	1999	16	0
1989	242	0	2000	128	0
1990	67	0	2001	0	0
1991	109	0	2002	289	0
1992	397	1	2003	126	0
1993	174	1	2004	164	0
1994	183	1	2005	133	0
1995	242	0	2006	18	0
1996	153	0	2007	60	0

Source; Bureau of Epidemiology, Department of Diseases Control, Ministry of Public Health, Thailand.

#### Morbidity rate(per 100,000 population) of Trichinosis cases from report 506 (1971-2006)



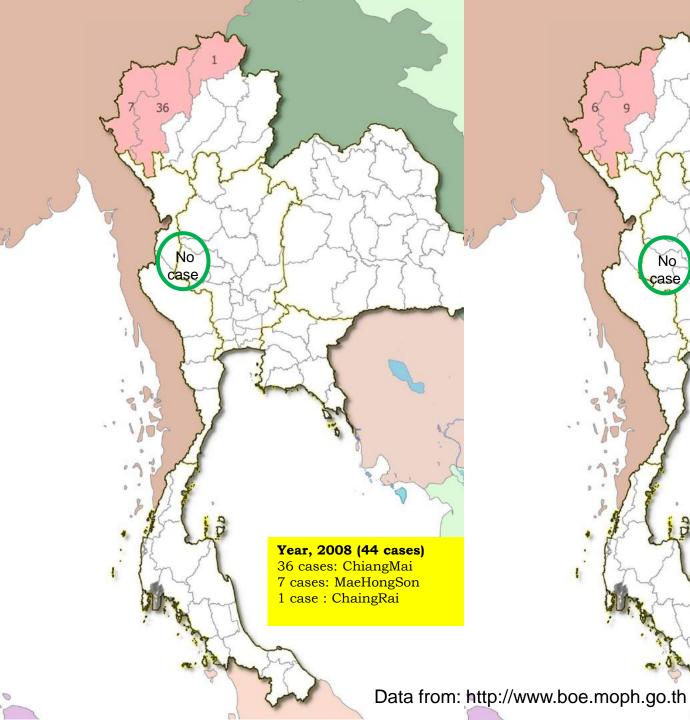
Source; Bureau of Epidemiology, Department of Diseases Control, Ministry of Public Health, Thailand.



# Trichinellosis surveillance in Thailand during year, 2008 -2018

# (Ten years after two outbreaks of *T. papuae* in BanRai District)

Data from: http://www.boe.moph.go.th

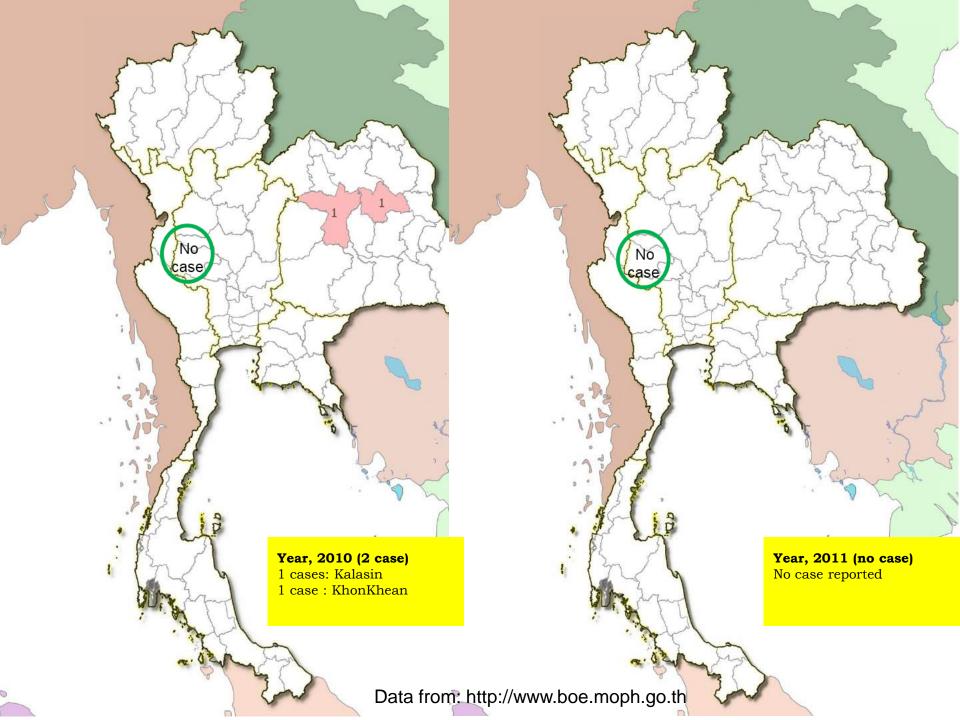


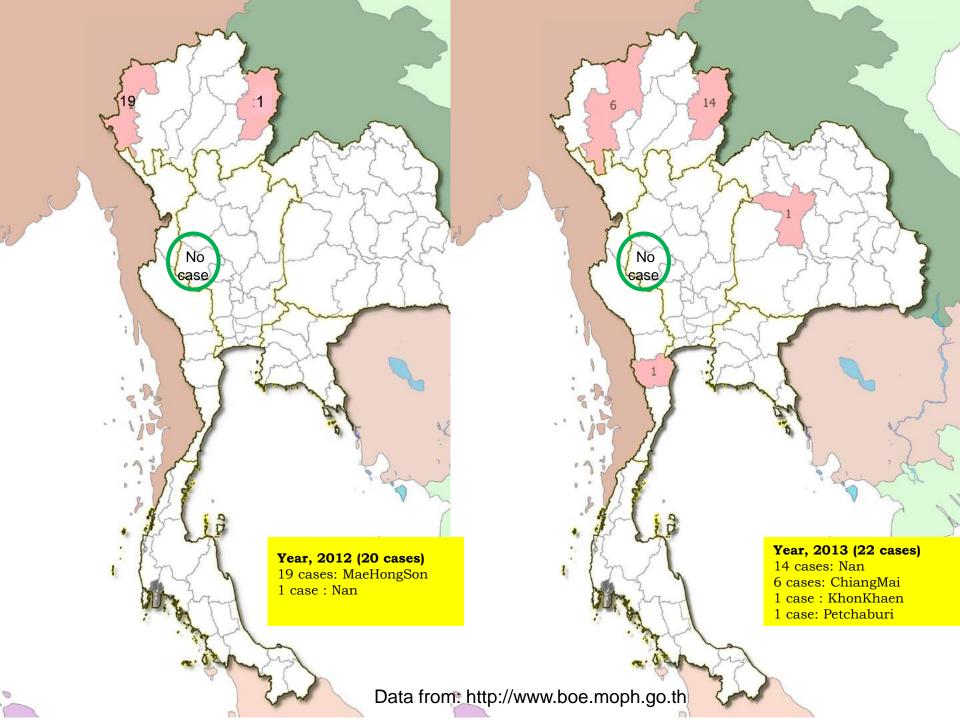
Year, 2009 (40 cases) 25 cases: Nan 9 cases: ChiangMai 6 case : MaeHongSon

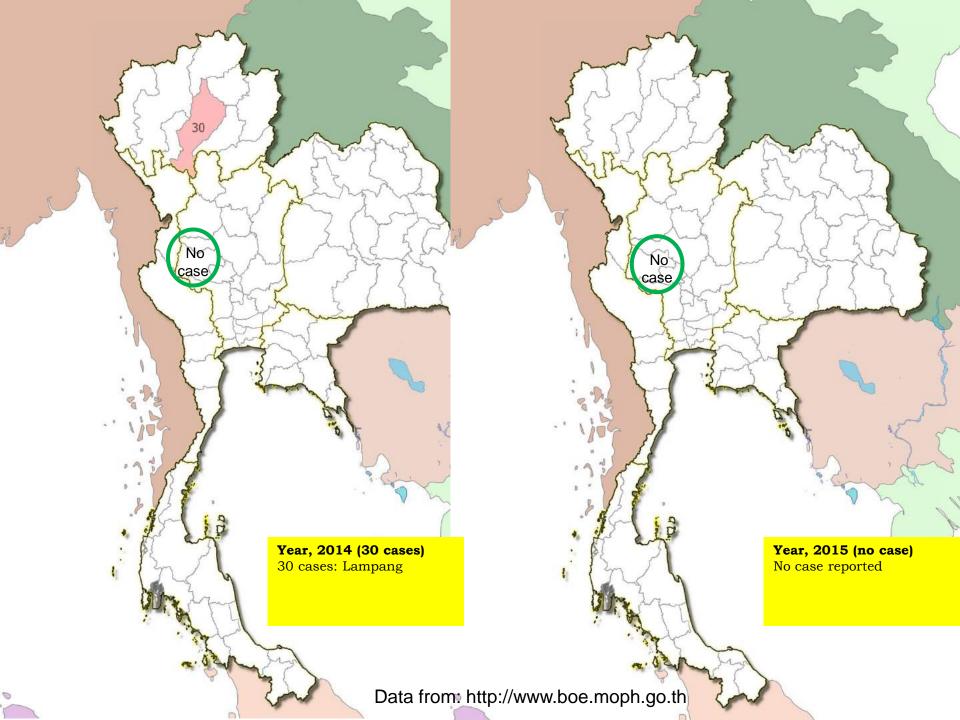
25

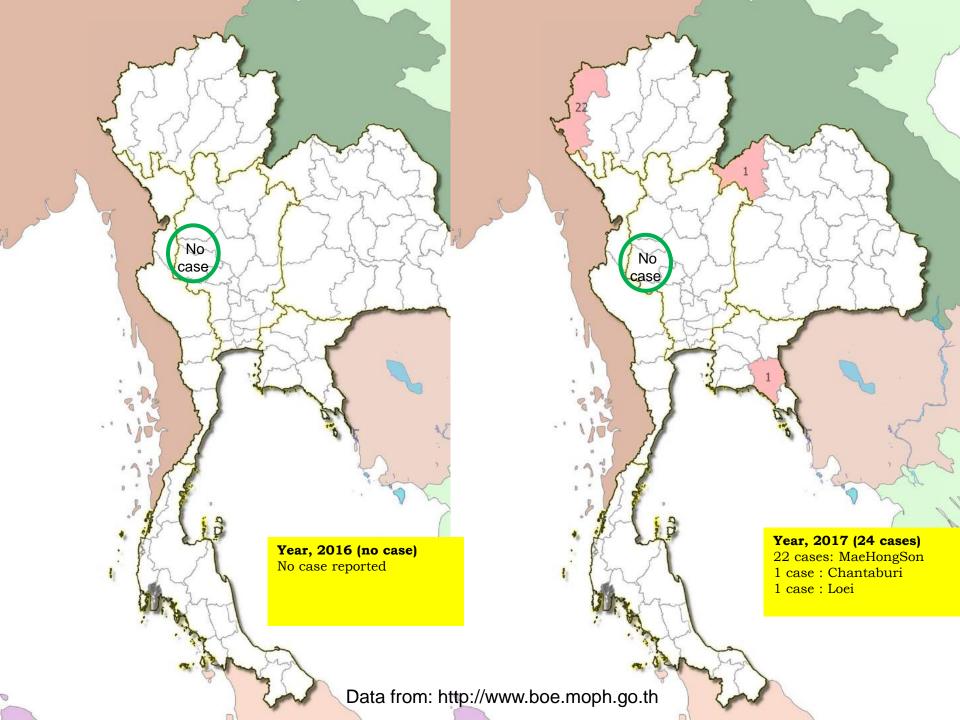
No

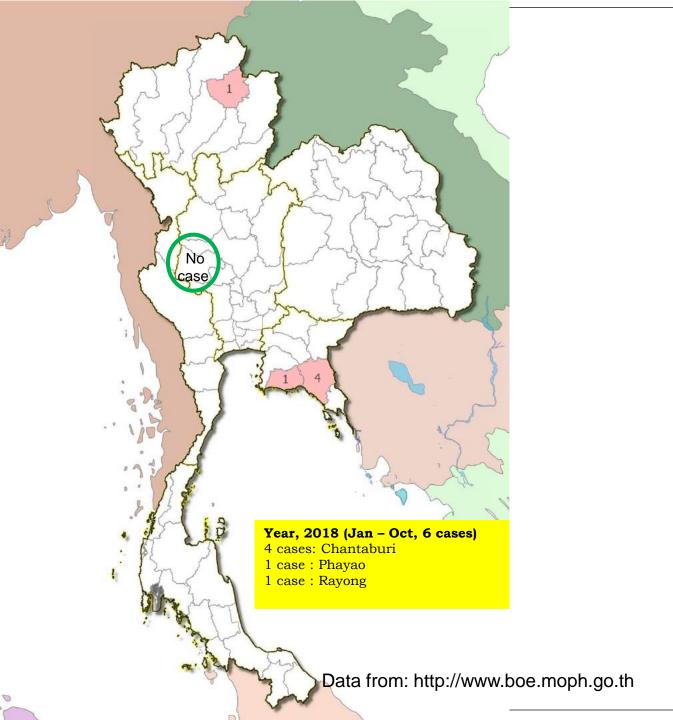
case



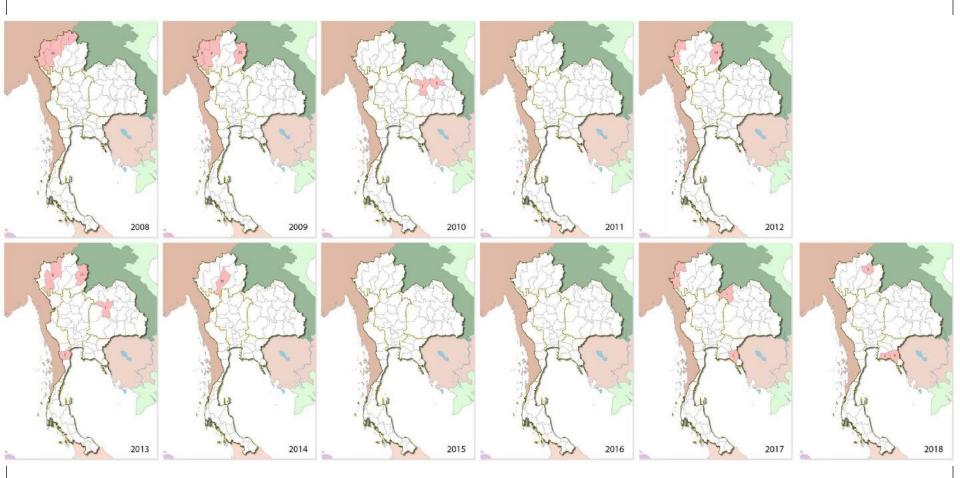




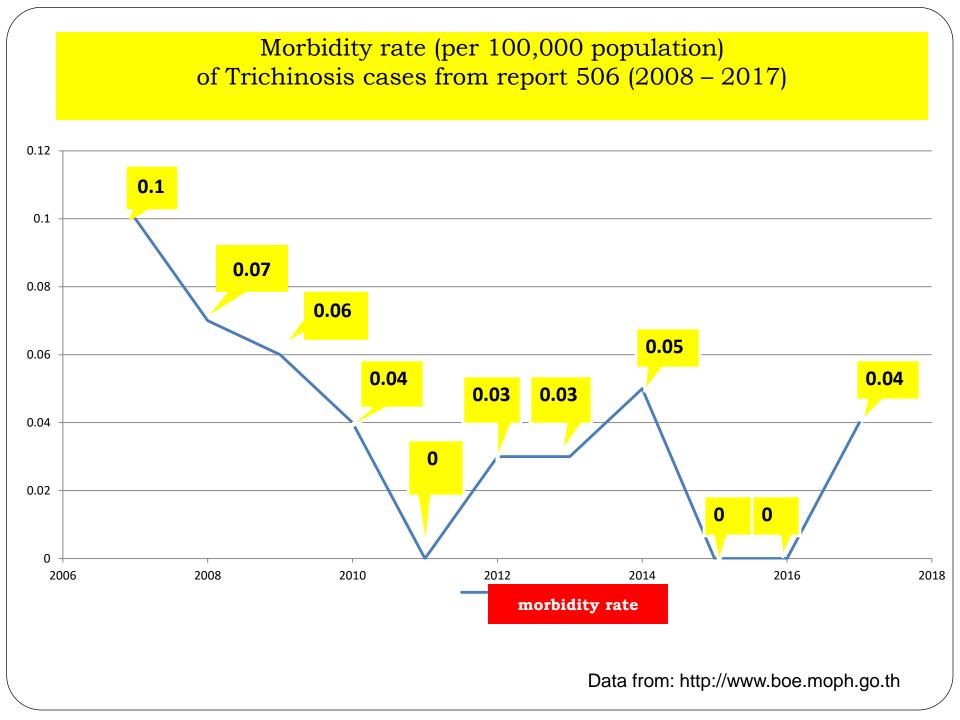




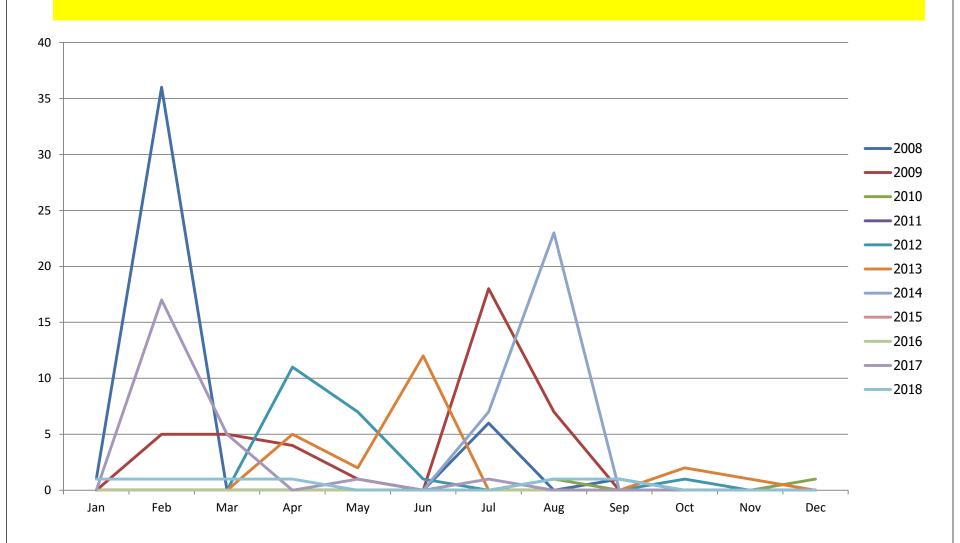
### There has been no report of human Trcihnellosis in Uthaithani Province, since 2008 to date



Data from: http://www.boe.moph.go.th



#### Reported by month of Trichinellosis cases, year 2008-2018



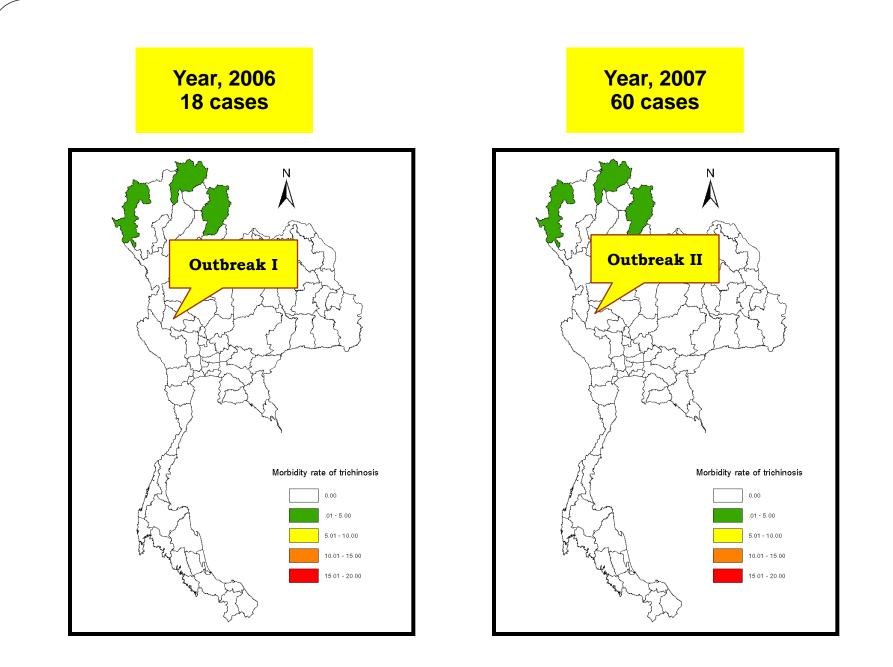
Data from: http://www.boe.moph.go.th

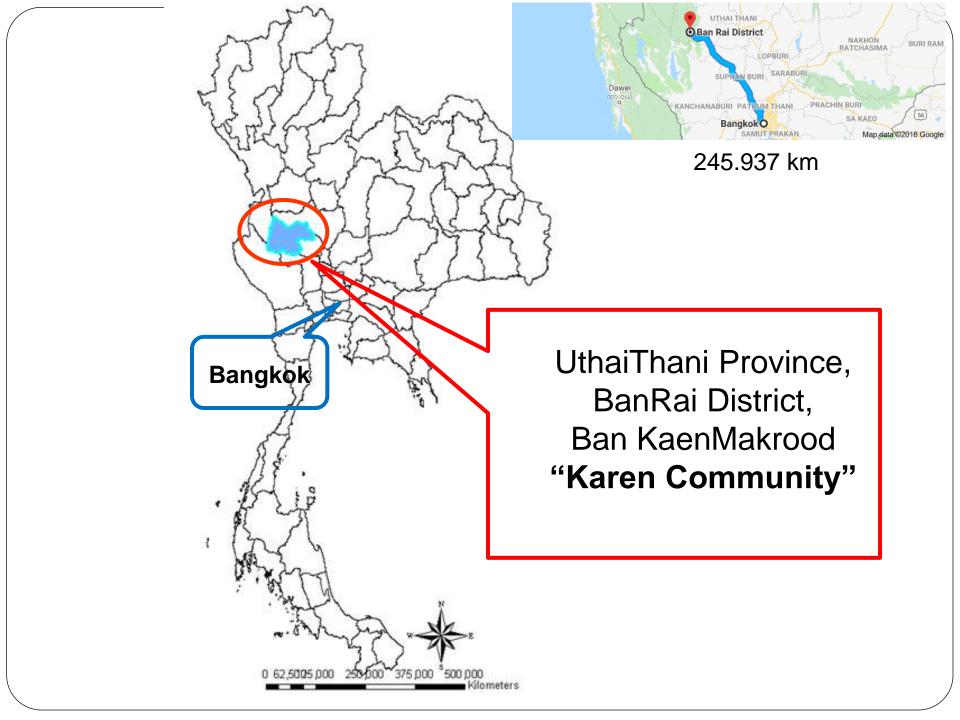
# Outline

- **o** History of Trichinellosis
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Two outbreaks of Trichinellosis papuae in BanRai District, UthaiThani Province, Thailand

> **"BAH CHER CHA YU"** Trichinellosis



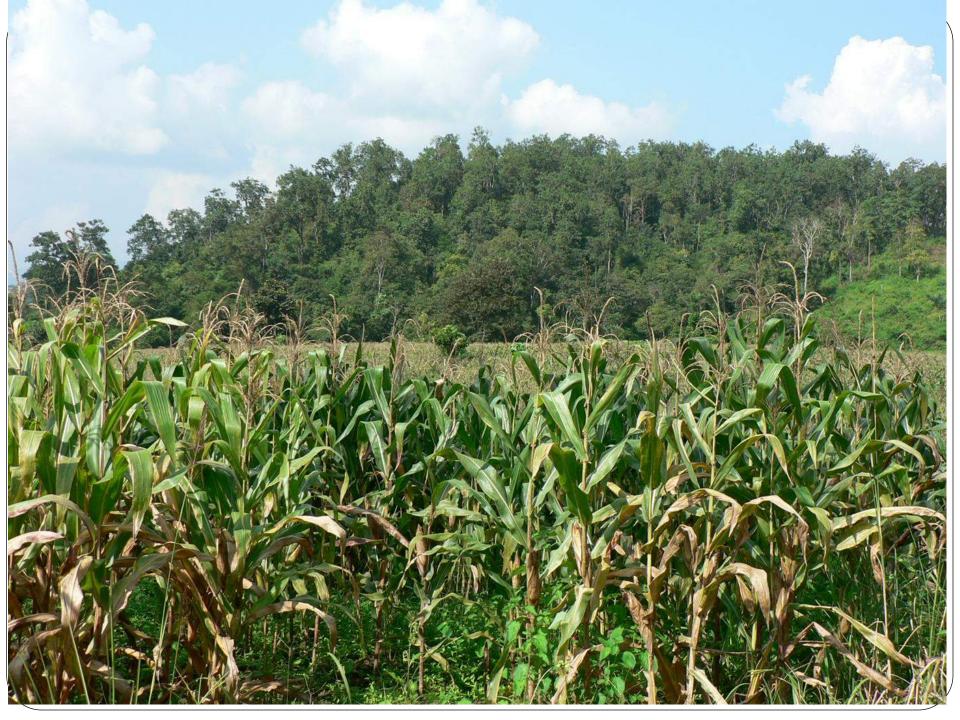


# **Outbreak** I

- Many villagers were suffered of muscle pain and edema, three patients were referred from BanRai hospital and admitted to UthaiThani hospital with clinical signs and symptoms of muscle hypertrophy with severe myalgia and edema on July, 2006.
- Laboratory shown eosinophilia.
- The other 25 patients who presented with the same clinical symptoms but was not severe, they were treated as OPD case at BanRai Hospital.

# **Outbreak I : Case identification**

- All the patients are Karen people and live in KaenMakrood Subdistrict, village no. I.
- The village is located near "Thungyai Naresuan Wildlife Sanctuary or Huai Kha Khaeng Wildlife"
- Some of them were have symptoms of fever, weakness, vomiting or abdominal discomfort starting from 21 May 2006.
- They developed myalgia and edema on trunk, limbs, periorbital or facial edema.

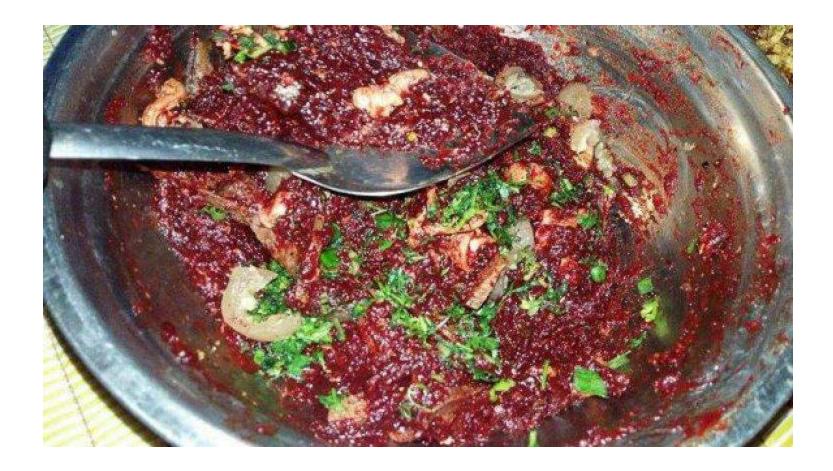








## Local traditional dishes "Lab Moo Dib" (raw pork)



## **Outbreak I : general information**

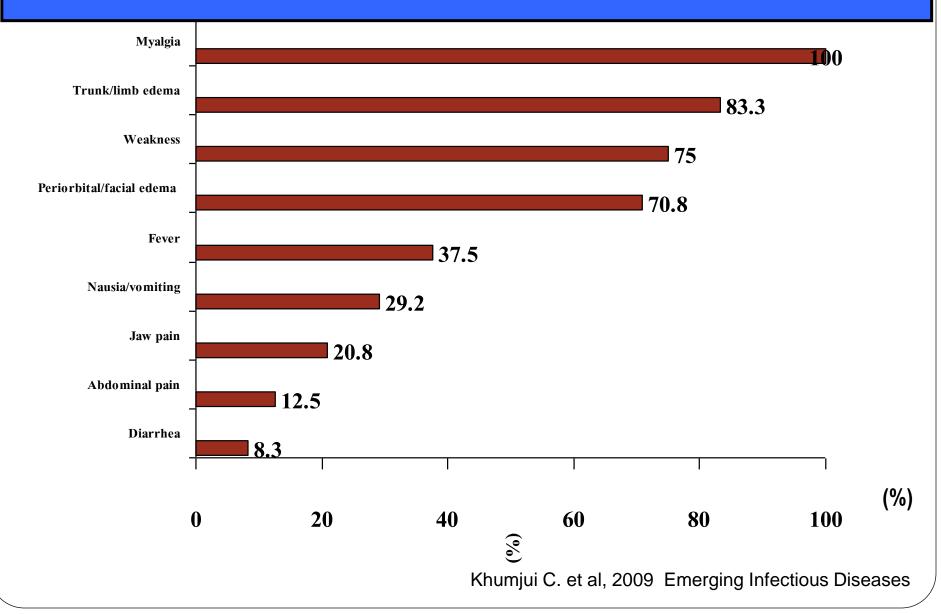
- Among the village no. I, there are 273 population.
- Of 28 suspected patients, 3 cases were clinical severe and admitted to Uthaithani hospital, other 25 were minimal signs and symptoms.
- Among the subjects, median age was 34 years, range 14–55 years and 18 subjects were male.
- The attack rate in the village was 10.3% (28/273); there were no deaths

## **Outbreak I : Clinical manifestation**

- all case-patients (100%) had myalgia.
- trunk/limb edema (83.3%)
- weakness (75.0%)
- periorbital/facial edema (70.8%)
- fever (37.5%),
- nausea/vomiting (29.2%)
- jaw pain (20.8%)
- abdominal pain (12.5%)
- diarrhea (8.3%).

Khumjui C. et al, 2009 Emerging Infectious Diseases

### Clinical manifestation among cases (n=28) at Village 1, Uthaithani province during 24 May 2006-23 June 2006



## **Outbreak I: possible risks associated**

- On May 20, a successful wild boar hunt resulted in distribution of wild boar meat to villagers.
- On May 21, domesticated pig meat was shared among villagers.
- After eating suspected foods on May 20–21, the first case-patient developed symptoms on May 24 (diarrhea and abdominal pain)
- On July, 2006 many villagers developed muscle hypertrophy and painful.

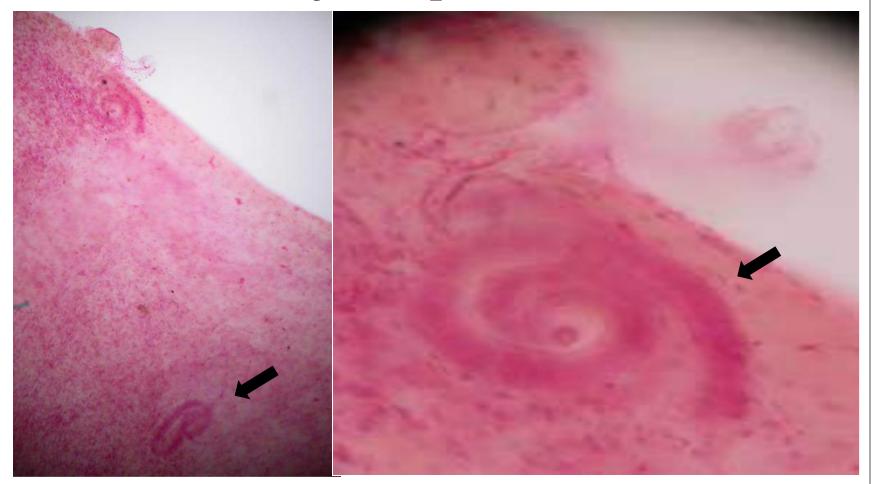
#### **Outbreak I: Clinical briefly**

- Three cases in Uthaithani hospital.
- All case patients had elevated CPK levels (median 830 U/L, range 506–1,208 U/L, reference <50 U/L).
- 19 sera from the patients and 64 sera from the villagers were sent to Department of Helminthology, FTM, MU.
- A piece of human gastrocnemius muscle biopsied specimen from a hospitalized case-patient was conducted and sent to the Department of Helminthology, FTM, MU.

#### **Outbreak I: Laboratory diagnosis**

- Immunoblot tests for human trichinellosis was conducted by using the 109-kDa diagnostic band (sensitivity 100%, specificity 100%)
- The partial cytochrome oxidase subunit I (COI) region was amplified by primers, which were designed from partial COI sequences of mitochondrial DNA from *T. zimbabwensis* and *T. papuae* in GenBank (accession nos. DQ007900 and DQ007899, respectively).

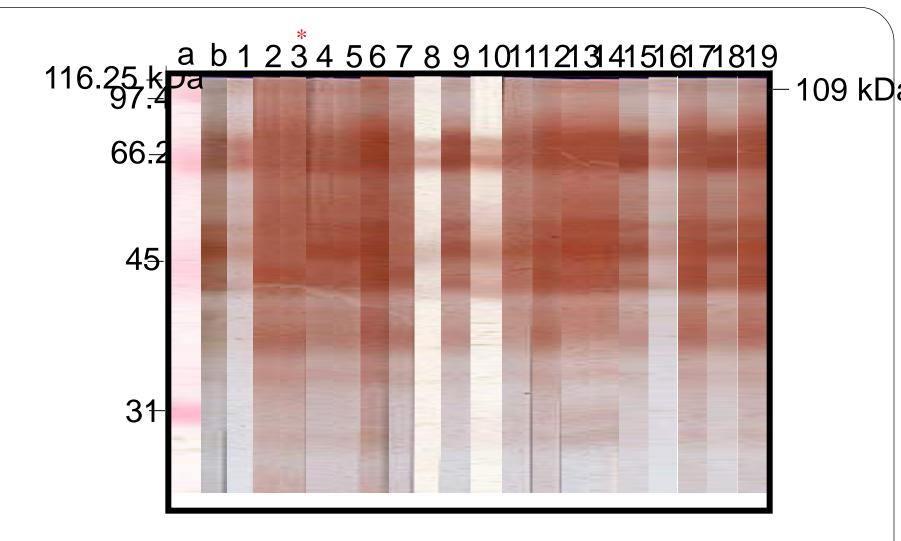
#### Muscle biopsied from patients was examined by compression method



Result shown : Positive for non-encapsulated Trichinella larvae

The detection of Trichinellosis by immunoblot technique;

94.74% (18/19) sera from suspected patients and 21.86% (14/64) sera from villagers were band positive for Trichinellosis at 109 kDa.



- a = broad range molecular weight markers
- b = serum of encapsulated *T. spiralis* larvae-detected case (positive control)
- 1-19 = serum samples of suspected trichinellosis (case # 10 : negative band)

\*case # 3 : found non-encapsulated larvae

#### **PCR Results**

Nucleotide variations of partial CO I sequences of Trichinella spp. (321 base pairs)

**Unknown** CTTTATAGTTTCCCTTTTTGGTCCCTTCCCTGCTCGTTATCTAAAACCC

**T. Zim** .... C .... A ... CT . T ... C . CAA . TTT .... T . C .... T .... G . T . G .... A

T. pseudo TC . CCCGACC . AT . CC . C . AAC . TTCCTTTCAGCTTCC . C . TTAAA

Unknown Sample : Trichinella papuae

## **Outbreak II**

- In September, 2007 at village No.4, same area of KaenMakrood-Subdistrict, BanRai District, about 40 km away from the village No. I, where the first outbreak of *T. papuae* occurred in 2006.
- 34 villagers suffering from one of the symptoms suggestive for trichinellosis were enrolled.
- Twenty-two of them had ingested undercooked pork from a hunted wild-boar.
- Two of them were severe clinical symptoms and admitted to Uthaithani hospital.

## **Patients history**

- In early August 2007, a hunter killed a wild boar and raw pork from this animal was distributed and consumed in the village.
- In the middle of September, a person (the index case) from the village developed clinical symptoms compatible with trichinellosis, and they was hospitalized at the Ban-Rai District Hospital.
- Later, 33 people with similar signs and symptoms visited the same hospital one after another up to September 16, 2007

# Laboratory investigations

- Blood samples were collected from suspected trichinellosis patients for immunodiagnosis testing.
- Two patients who severe clinical symptoms were admitted to Uthaithani hospital.
- One of them was conducted muscle biopsied at his left gastrocnemius muscle.
- 26 serum samples and a piece of muscle biopsied were sent to the Department of Helminthology, FTM, MU.

#### The detection of Trichinellosis by immunoblot technique

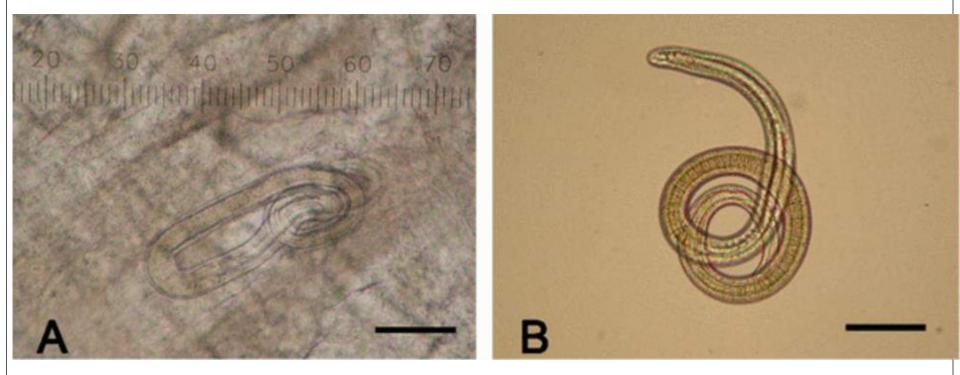
- 26 serum samples were examined for trichinellosis identification
- 20 of 26 (76.9%) were considered to be positive as they recognised the 109 kDa *Trichinella*-specific antigen by Western blot

Patient No.*	Gender	Age	Clinical signs or symptoms	WBC (mm <sup>3</sup> )	Eosinophilia (%)	Eating wild boar meat
1	F	40	GI, My	9,500	51	Y
2	F	11	My	8,600	34	Unknown
3	F	36	Fe, My	8,600	31	Y
4	М	39	Oe, My	6,700	19	Unknown
5	F	39	My	8,300	2	Unknown
6	F	62	Oe, My	8,600	45	Y
7	Μ	14	GI	7,100	8	Y
8	F	34	Oe, My	11,000	21	Y
9	F	45	GI, Oe, My	8,300	25	Y
10	Μ	25	My	15,000	45	Unknown
11	F	44	Fe, GI, Oe, My	11,600	41	Y
12	М	44	Му	6,700	19	Y
13	М	20	My	19,100	64	Y
14	F	44	Oe, My	10,000	11	Y
15**	М	34	GI, Oe, My	20,600	58	Y
16	F	41	Му	10,200	54	Y
17	М	21	My	9,300	62	Y
18	F	32	My	4,100	11	Y
19	F	37	Му	6,000	16	Y
20	М	40	Му	12,400	17	Y

# Muscle biopsied; Compression method results

- A biopsy specimen (approximately 10mm × 10mm × 5mm) from the gastrocnemius muscle was chopped into several pieces and examined by the compression method.
- More than 80 larvae were observed. A few larvae fixed in 10% formalin were examined under a light microscope.
- The average length and width measured on seven larvae was 790µm (range 750–890µm) and 29µm (range 25–30µm), respectively.

# Figure 1 Non-encapsulated muscle larvae in the gastrocnemius muscle biopsy of patient #15:



(A) in situ; and (B) isolated larva. Scale bar: 100  $\mu$ m.

### **PCR Results**

- The partial COI gene sequence of the larvae (GenBank accession no. GQ180180) showed complete identity with the partial COI gene sequences of the larvae of the first **T. papuae outbreak of Thailand** (GenBank accession no. GQ180179) and of the Papua New Guinea T. papuae reference strain (ISS572) (GenBank accession nos. DQ007899 and EF601546)
- The conclusion was that, larvae from a patient was identified as *T. papuae*.

#### Treatment

- Mebendazole (100 mg twice daily for 3 days, followed by 200 mg twice daily for 10 days).
- Prednisolone (15 mg/day for 5 days) was only administered to the individual with severe symptoms (Patient #15).
- The clinical symptoms of all of the patients improved quickly following treatment.

### Outline

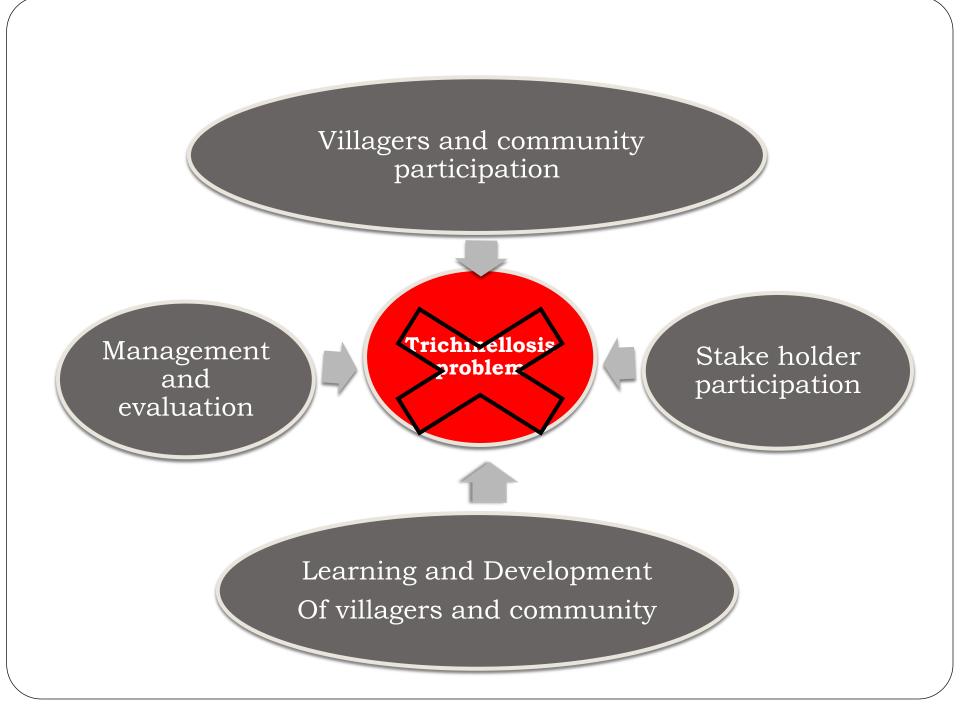
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## **Prevention and control**

- Two outbreaks of human Trichinellosis were occurred the same area in one year.
- How to stop the disease ? Following the Health Education concept (KAP).
  - How to improve their knowledge (K)
  - How to change their attitude to the disease (A)
  - How to change their practice (P)

"Alone we can do so líttle; together we can do so much." <u>Helen Keller.</u>

No one succeeds alone



Villagers or community participation

- Encourage villagers to aware the danger from Trichinellosis disease.
- To make an understanding that, patients with sever Trichinellosis infection may be die
- To make an understanding : eating raw pork or semi-cooked animal meat are risks to infect Trichinellosis



- Joining to stop the disease, prevent reinfection and control the disease.
  - From public health officer staffs
  - From local government staffs
  - From school (teachers and students)
  - From community (Head of the village, villagers)
  - From the temple (monks)
  - From staffs of the university (Mahidol University)

#### Learning and Development

of villagers and community

- Community (villagers) sector
  - Group or mass meeting and brainstorming
  - Sharing ideas to prevent the infection
  - To make the some consensus among villagers to prevent the disease
- School sector
  - Group meeting and brainstorming
  - Teaching by teachers to school children (knowledge gain)
  - Small cartoon book drawing contest by school children about Trichinellosis story
  - Preventing parasites' song implementation

#### Management and evaluation

- To implement programs and activities to villagers and school children as the following
  - Mass meeting and learning to gain their knowledge
  - Starting campaign to prevent re-infection
  - Produce VDO of Trichinellosis, including the ways to prevent of infection
  - Brochure producing in Karen and Thai language
  - Small cartoon book drawing contest
  - Follow up and evaluation of the sustainable
  - Refresh understanding of Trichinellosis among health officers and villagers

## **Activities in community**

- Mass Meeting with Mass Health education implementation.
- Wat BanTai (in KaenMakrood) was the main center for our mass meeting.
- Buddhist Holy Day, Karen people have leave their work, they were free and joining our program preventing Trichinellosis campaign.

# Bra Chur Cha Yu (Trichinellosis)











# Mobile clinic service to villagers in community







#### **Mobile fecal examination service**







#### Drawing small cartoon book



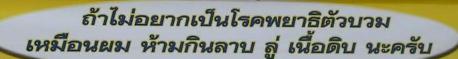






## Brochures were produced in Karen Language





#### ้ำมีอาการตัวบวมปวดกล้ามเนื้อต้องไปพบหมือ**โดยด่วน!**

"อาเมืองเรื่ออยู่ป่า ถ้าโพอิบ ป้าเชอช่าหุ เมืองแหมืองกัญ"



SAUDERS

องค์การบริหารส่วนตำบลบ้านไร่

## Outline

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- The follow up previous Trichinellosis patients and villagers

#### **Follow Up with 19 Patients**

Pt No.	Fever	Nause a	Vomiting	Fatiqu e	Muscle Power	Muscle hypertrop hy	Periorbital swelling
1	Ν	N	N	Y	Normal	Ν	Ν
2	Ν	N	N	N	Normal	Ν	Ν
3	Ν	N	N	N	Normal	Ν	Ν
4	Ν	N	N	Y	Normal	Ν	Ν
5	Ν	N	N	Y	Normal	Ν	Ν
6	Ν	N	N	Y	Normal	Ν	Ν
7	Ν	N	N	Y	Normal	Ν	Ν
8	Ν	N	N	Y	Normal	Ν	Ν
9	Ν	N	N	N	Normal	Ν	Ν

Pt No.	Fever	Nause a	Vomiting	Fatiqu e	Muscle Power	Muscle hypertrop hy	Periorbital swelling
10	N	N	N	Y	Normal	Ν	Ν
11	N	N	N	Y	Normal	Ν	N
12	N	N	N	Y	Normal	Ν	Ν
13	N	N	N	Y	Normal	Ν	N
14	N	N	N	Y	Normal	Ν	N
15	N	N	N	Y	Normal	Ν	Ν
16	N	N	N	Y	Normal	Ν	Ν
17	N	N	N	Y	Normal	Ν	Ν
18	N	N	N	Y	Normal	Ν	Ν
19	N	N	Ν	Y	Normal	Ν	Ν





# 

#### **BEFORE TREATMENT**

#### AFTER TREATMENT





#### **BEFORE TREATMENT**

#### AFTER TREATMENT







# The survey of reservoir hosts around patient's house

• The muscle biopsied from the possible reservoir hosts around the houses were examined by compression method and digestion technique.







#### Artificial digestion of animal tissues

a: twenty grams of meat from each animal for examinationb: separating larvae by Baermann's techinquec: examination under stereomicroscope



#### **Results of animal reservoir hosts tissue examination**

Animal	Number of examined	Organs for examined	Compression		Artificial digestion	
			positive	negative	positive	negative
Wild boar	4	diaphragm, tongue, muscle		/		/
Domestic pig	2	diaphragm, tongue, muscle		/		/
Rat	20	diaphragm, tongue, muscle		/		/

# Evaluation after health education program implementation

Item	During	F/U in 2 years	F/U in 10 years
	outbreaks	after outbreaks	after outbreaks
	(N=229)	(N=196)	(N=40)
<b>Knowledge (K)</b>	47.16%	70.91%	77.5%
(> 50%)	(108/229)	(139/196)	(31/40)
<b>Attitude (A)</b>	37.11%	58.10%	67.5%
(>50%)	(85/229)	(114/196)	(26/40)
<b>Practice (P)</b> (do not eat raw pork or wild animal)	30.13% (69/229)	80.10% (157/196)	87.5% (35/40)

#### The follow up patients and villagers in 2018

Serum	Muscle ex	amination	CBC:	Immunoblot test	
No.	normal	fatique	Eosinophil		
1			4.2	no band detected	
2		$\checkmark$	2.3	no band detected	
3	$\checkmark$		5.0	no band detected	
4	$\checkmark$		4.6	no band detected	
5		$\checkmark$	2.6	no band detected	
6			3.4	no band detected	
7			7.8	no band detected	
8			8.5	no band detected	
9		$\checkmark$	4.7	no band detected	
10		$\checkmark$	1.6	no band detected	
11			0.5	no band detected	
12			3.3	no band detected	
13			2.2	no band detected	

## EMERGING INFECTIOUS DISEASES

## Outbreak of Trichinellosis Caused by Trichinella papuae, Thailand, 2006

Chowalit Khumjui, Pravit Choomkasien, Paron Dekumyoy, Teera Kusolsuk, Wandee Kongkaew, Mutita Chalamaat, and Jeffrey L. Jones

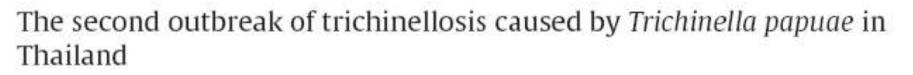
Emerg Infect Dis. 2008 Dec; 14(12): 1913–1915



Contents lists available at ScienceDirect

#### Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh



Teera Kusolsuk<sup>a,\*</sup>, Suthida Kamonrattanakun<sup>b</sup>, Apiluk Wesanonthawech<sup>c</sup>, Paron Dekumyoy<sup>a</sup>, Urusa Thaenkham<sup>a</sup>, Tipayarat Yoonuan<sup>a</sup>, Supaporn Nuamtanong<sup>a</sup>, Surapol Sa-nguankiat<sup>a</sup>, Somchit Pubampen<sup>a</sup>, Wanna Maipanich<sup>a</sup>, Jittima Panitchakit<sup>d</sup>, Gianluca Marucci<sup>e</sup>, Edoardo Pozio<sup>e</sup>, Jitra Waikagul<sup>a</sup>

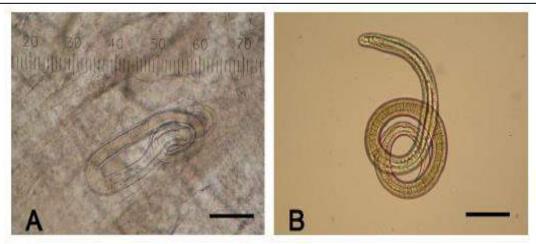


Figure 1. Non-encapsulated muscle larvae in the gastrocnemius muscle biopsy of patient #15: (A) in situ; and (B) isolated larva. Scale bar: 100 µm.

## Conclusions

- Two outbreaks of *T. papuae* have been reported in BanRai District, UthaiThani Province, Thailand in 2006 and 2007
- The sample parasite was confirmed by PCR technique and identified as *T. papuae* and approved by Prof. Edoardo Pozio before published the first reported *T. papuae* in Thailand to the journal
- No One Succeeds Alone to Prevent and Control of Trichinellosis

## Conclusions

- Community participation is an important way to success in control program
- No reported of re-infection of Trichinellosis in BanRai again after health education program implementation within 10 years
- This model have been applied to control the other parasitic infections such as Taeniasis and cysticercosis among the Karen people in Thasong Yang District, Tak province, Thailand



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### Mahidol University Medicine

 All patients and villagers in community, KaenMakrood Subdistrict

# ขาวประชาร่วมใจต่ำนภัยโรคพยาธิตัวบวม (บ้าเชอช่านงุ) 14 กันยายน 2551

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