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**Characterisation of factors influencing trichinellosis in
humans and pigs in Nan Province,
Northern Thailand**

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**Submitted in fulfilment of the requirements of the degree of
Doctor of Philosophy**

**The University of Edinburgh
2011**

Declaration

I declare that the research described within this thesis is my own work and that this thesis is my own composition and certify that it has never been submitted for any other degree or professional qualification.

Wandee Kongkaew

Edinburgh, 2011

To

My parents, sisters and brothers

Abstract

The work presented in this thesis focuses on trichinellosis, a food-borne zoonosis caused by a nematode of the genus *Trichinella*, in Thailand. The main objectives were to characterise human trichinellosis, determine its endemic foci and characterise factors influencing infection in humans and in the pig, which is an important domestic animal reservoir host of *Trichinella*. The investigations comprised (i) a retrospective review to characterise human trichinellosis in Thailand, (ii) a survey to characterise factors influencing trichinellosis in pigs and (iii) quantitative and qualitative studies to characterise the factors influencing trichinellosis in humans.

An analysis of Thai national trichinellosis surveillance data between 1981 and 2008 highlighted the five northernmost provinces in the Northern Region as particularly affected with trichinellosis. The incidence of human trichinellosis in Thailand decreased significantly during this period. Until recently, trichinellosis was found to cluster significantly in these provinces. Domestic pigs and wild boar appeared to be the major sources of infection.

Field studies focused on populations in Nan Province in the Northern Region, where specific foci of human trichinellosis have been observed. A survey of pig production in both lowland and highland areas in five districts of Nan Province found small-scale pig production to be predominant. Production and management practices differed significantly between lowland and highland areas. In the highland areas, pig production was mainly for the owners' own use. The holdings were poorly managed compared with those in lowland areas. A survey of trichinellosis seroprevalence in these five districts showed the disease to be associated with scavenging pigs (OR = 2.96, $p = 0.02$) and older pigs (OR = 1.02, $p = 0.02$). Seroprevalence was estimated with 95% confidence and was in the range 0 - 0.36% in lowland areas and 0.46 - 1.48% in highland areas. A pig acquired from a Thai-Laotian border market was among the sero-positive pigs identified in the survey. A survey for trichinellosis in wild animals ($n = 97$), mostly wild boar ($n = 53$; 55%), using the digestion method did not detect *Trichinella* in any of the animals screened.

A retrospective gender-matched and age-matched case-control study in four trichinellosis-affected communities during 2003 – 2006 showed that consumption of raw wild boar (OR = 2.66, $p = 0.005$) and consumption of raw meat at social gatherings (OR = 3.89, $p = 0.008$) were risk factors, and the belief that alcohol can kill the parasite in raw meat (OR = 0.36, $p = 0.03$) was a protective factor associated with individual trichinellosis cases. Qualitative studies on communities' knowledge, attitudes and practices relating to trichinellosis in 12 villages indicated that the

communities received information about food-borne diseases. However, the practice of raw food consumption continued because of individual taste preferences and the belief that consuming raw food infrequently and in small amounts lessened the risk of eating infected meat. In terms of pig management, although there had been improvements in the practices within Nan Province, with the majority of pigs kept in pens, due to insufficient pig feed and poor survival rates of piglets in inappropriately-designed pens, free-range scavenging still occurred.

For control of trichinellosis to be improved, the factors identified as influencing its maintenance in the study areas must be communicated to the local administrative organizations and veterinary and public health offices. This will enable them to construct and implement guidelines for good management practices in pig farms in the highland areas. Additionally, there is a need to specify a requirement for *Trichinella*-free certification of pigs and meat products sold at the border markets. There is also a requirement for the continued education of the general public regarding the safe consumption of adequately-cooked meat.

Acknowledgements

I would like to thank my supervisors Dr. Mark Eisler, Dr. Michael Thrusfield and Professor Sue Welburn for their invaluable guidance and encouragement throughout my research. Many thanks go to Dr. Kim Picozzi, Dr. Ewan Macleod and Dr. Jenna Fyfe for taking the time to read drafts of the thesis and for always being there to help. I would also like to thank Pauline McManus, the administrative staff, for sharing a fun time with me and librarians at the Royal (Dick) School of Veterinary Studies for help with reference tracking. I thank all of my friends in the postgraduate office who are always supportive and make me smile for the whole period of my study and I will always miss. Special thanks go to Marie Ducrotoy, Mamoonah and Dr. Hamad Chaudhry for helping with English correction, Dr. Niel Anderson and Aman Ullah for helping with installing software programmes and Dr. Kohei Makita for statistical advice in my second year.

I am grateful to the Agricultural Research and Development Agency (public organization), Thailand for funding my study as well as Birrell Gray Trust and Page Bequest Fund for additional funding. The Office of Educational Affairs, Thai Embassy Office in the UK took care of financial issues and supporting documents flawlessly and I am grateful for that with special thanks go to Mr. Natee Bunchit who responded to all enquiries without delay.

The field work carried out in this study was funded by the Department of Livestock Development, Thailand with special arrangement by Dr. Wironrong Hoonsuwan, Dr. Pranee Panichabhongse and Dr. Thammawan Noonthaisong for whose support I am thankful. I am grateful to the Department of Livestock Development, Thailand both for allowing me to pursue a PhD and on providing funds for the field work. Dr. Chaiwat Vitoorakool and staff at the upper Northern Veterinary Research and Development Centre, Thailand must all be thanked for their help performing laboratory testing of animal samples. Special thanks go to my friend, Dr. Karoon Chanachai for his help on obtaining collaboration with the Provincial Livestock Offices and overcoming all obstacles to help smooth my field work as well as always giving me mental support.

This work could never have been flourished without the tremendous and tireless effort of the chief of Nan Provincial Livestock Office, Mr. Tassanai Toh-Anan and Mr. Nopporn Mahakantha and all staff from the Nan Provincial Livestock Office as well as all District Livestock Offices. Their kind-hearted help and support allowed me to fit in and conduct field work with ease in a way that made me regard Nan Province as my second home. I am grateful and thank them all warmly. Special thanks go to Dr. Aomruathai Jaijan and Dr. Penporn Tablerk at Nan Provincial Livestock Office, Mr. Utit Srisitipoj, former chief of Tha Wang Pha District

Livestock Office, Mr. Surachate Pinthip, chief of Thung Chang District Livestock Office, Mr. Vorathep Rachaphan, chief of Chiang Klang District Livestock Office, Mr. Narin Prommuangdee, chief of Muang District Livestock Office, Mr. Dusit Phuthacharoen, former chief of Bo Kluea District Livestock Office and his assistant Mr. Rujanaphob Muangmun. I am grateful to Mr. Jaturapat Kamnama and Mrs. Thanikarn Thaochiang, my assistants, for helping with sample and data collection without ever complaining, Mr. Samart Luninta, Mr. Smarn Chainuan for driving me safely along the mountain range and Bo Kluea Tai Local Administrative Office for providing a safe car and driver to access the remote villages. Trained veterinarians, village heads and farmers in Nan Province are very kind and generous in giving up their time to organise a place for group meetings and for giving information without hesitation, I am very grateful to them all. I am also thankful for Mr. Jaroon Utuporn, chief of the Thoeng District Livestock Office in Chiang Rai Province and Ms. Anongnat Chaiya, member of the Thoeng Local Administrative Organization for helping with data collection in Chiang Rai Province.

I am grateful for the wide range of support with data collection and advice from various offices, all levels of the Ministry of Public Health Offices and officers, Nan Statistical Office, Office of Land Development Region 7, the Department of Social Development and Welfare, Thailand. The Bureau of Epidemiology, Ministry of Public Health, Thailand provided human trichinellosis surveillance data and Nan Public Health Office and Chiang Rai Public Health Office and every District Health Offices provided and gathered data in a case-control study. Nan Statistical Office provided a survey guideline and advices on survey method, Office of Land Development Region 7 provided map of Nan Province and the Department of Social Development and Welfare, Thailand provided data on highland communities in Thailand.

I am grateful to Dr. Chuleeporn Jiraphongsa, the director of the International Field Epidemiology Training Programme, Ministry of Public Health Thailand as well as Dr. Andrew Routh and Dr. Arpassorn Routh for their invaluable advice, comments and correct the draft thesis. I would like to thank colleagues at the Southern Veterinary Research and Development Centre for always supporting my work. Special thanks go to Dr. Anyarat Thiptara my junior colleague for continuing help me in all aspects without reserve and for providing comments and advice on the data analysis. Gratefulness and love goes to my parents, brothers, sisters and my friends who gave me the power to live happily.

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List of abbreviations

AIDS	Acquired Immunodeficiency Syndrome
BoE	Bureau of Epidemiology, Ministry of Public Health, Thailand
B.E.	Buddhist Era
DALYs	Disability-Adjusted Life Years
DLD	Department of Livestock Development, Thailand
DoPA	Department of Provincial Administration, Ministry of Interior, Thailand
EKG	Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GDP	Gross Domestic Product
HIV	Human Immunodeficiency Virus
IAEA	International Atomic Energy Agency
ICT	International Commission on Trichinellosis
ISSR-PCR	Inter Simple Sequence Repeat Polymerase Chain Reaction
MoAC	Ministry of Agriculture and Cooperatives
MoPH	Ministry of Public Health, Thailand
NESDB	Office of the National Economic and Social Development Plan, Thailand
NSO	National Statistical Office, Thailand
OIE	World Organization for Animal Health
OD	Optical Density
PCR	Polymerase Chain Reaction
RCI	Reproductive capacity index
RFLP-PCR	Restriction fragment length polymorphism Polymerase Chain Reaction
TAS	Thai Agricultural Standard
USA	United States of America
WHO	World Health Organization

1. Chapter 1- Introduction

Insufficient resources for the public sector has led to the omission of certain zoonoses from control programmes, in particular where the disease is assessed as having a low economic impact or is a low threat to the health of the general population. Most commonly public education is the only preventive measure in place. However, for various reasons, education may not achieve its desired objective in certain populations who live in areas where the pathogen in question is found in domestic species and who indulge in risk-associated behaviour. In this study one of the food-borne zoonosis, trichinellosis, which has been persistent in northern Thailand since 1962 is explored.

Trichinellosis is a food-borne zoonosis caused by a nematode of the genus *Trichinella*. The parasite has a worldwide distribution and has a wide range of hosts, including mammals, birds and reptiles. The global distribution of *Trichinella* allied with certain cultural eating habits, represents the main factors leading to human infections in both industrialised and non-industrialised countries. In the year 2000, human global trichinellosis cases were estimated at 11 million (Dupouy-Camet, 2000). The economic losses caused by trichinellosis are focused on two main areas. One is the cost of control systems for detecting the infection in potential *Trichinella* carriers. The second is losses due to the occurrence of infections in humans and animals (Pozio, 2000). Global economic losses resulting from this disease, similar to other food-borne diseases, are difficult to assess due to inadequate information (Roberts *et al.*, 1994). Using the information available for the European Union (EU) alone, the cost of detecting *Trichinella* infection in pigs in the EU was about US\$ 570 million per year (Pozio, 1998). Medical costs and productivity losses for each human case occurring in France over a decade ago were about US\$ 3,000 (Ancelle *et al.*, 1990). Estimated

losses caused by trichinellosis in people in the United States of America (USA) were approximately US\$ 1,062 per case (Roberts *et al.*, 1994).

Trichinella spp. is maintained in nature by transmission within wild animal populations, particularly wild carnivores through predation, cannibalism and scavenging behaviours. Transmission within domestic animal populations, particularly domestic pigs, is a result of poor stock (pig) management practices that allow them to consume food contaminated with *Trichinella*-infected meat (Campbell, 1988). The biological characteristics of each *Trichinella* species, human behaviour and culture, the presence of synanthropic animals and the characteristics of the ecosystem are all factors involved in the transmission pattern and cosmopolitan spread of this parasite (Pozio, 2000). The risk of humans acquiring trichinellosis is restricted to areas and cultures where the consumption of raw or undercooked meat is a common practice, especially in areas where meat is not properly examined for *Trichinella* infection. The highest number of human trichinellosis cases worldwide is linked to the consumption of domestic pigs and wild boar infected with *T. spiralis*. To a lesser extent, human trichinellosis cases can be resulted from the consumption of other domestic animals (for example horses, dogs and sheep) and wildlife (for example walrus, bear, deer, etc). Human infections from wild animal reservoirs occur in developed countries where the animals are mainly hunted for leisure, in contrast with developing countries where the animals are hunted for subsistence (Pozio, 2000). Human trichinellosis caused by domestic pigs is currently confined to pigs reared in small farms with poor management or with access to grazing in wild areas (Pozio, 1998).

The relatively simple transmission pathway of *Trichinella*, which can only occur through ingestion of raw infected meat has led to the belief that trichinellosis can be easily controlled and therefore eradicated (Gajadhar & Gamble, 2000; Pozio, 2000). Control measures by meat

inspection and through meat processing have been in place for over a century. Prior to the 1970s, human trichinellosis from infected pork had dramatically declined throughout the world, in particular in Europe and USA (Murrell & Pozio, 2000). There is evidence to support the belief that trichinellosis can be controlled or eradicated from domestic pig populations (Gajadhar & Gamble, 2000; Pozio, 2000). Infection in humans has never been reported to have been caused by domestic pigs from industrialized farms. The intensive farming and its management practices result in the prevention of *Trichinella* transmission to pigs (Pozio *et al.*, 1996; Kapel, 2005). This has enabled at least one country, Denmark¹, to have been granted trichinellosis-negligible risk status for its domestic pig population (Alban *et al.*, 2008) .

However, the current status of human trichinellosis and recent findings on the disease's epidemiology indicate that the control of trichinellosis may be more complex than has been anticipated. Human trichinellosis has not only continued to occur in most parts of the world but has also re-emerged in several countries of Eastern Europe, in some parts of China and in Argentina (Dupouy-Camet, 2000). Additionally, with advanced molecular technology to speciate the species of the genus *Trichinella*, it is now known that it comprises of not one but a number of species. These species have different biological characteristics linked to their survival, maintenance and propagation in diverse ecosystems. Perhaps, surprisingly there have been reports of outbreaks of human trichinellosis caused by infected meat from domestic herbivores such as the horse (in France and Italy) and sheep (in China) (Ancelle *et al.*, 1988; Pozio *et al.*, 1988; Ancelle *et al.*, 1998; Tamburrini *et al.*, 2001; Wang *et al.*, 2007). Infection with *Trichinella* in farmed and wild crocodiles has been reported (Pozio *et al.*, 2005; Pozio *et al.*, 2007). Finally, trichinellosis should no longer be thought of solely as a curable acute infection.

¹The European Commission concerning Denmark's status as a region with negligible risk of *Trichinella*, in July 2007, the European Commission granted status as "negligible risk" to Danish pigs and pork

Follow-up studies have observed a significant proportion of cases with trichinellosis-related complications several years after initial infection (Harms *et al.*, 1993; Kociecka *et al.*, 2001). With regard to all of these aspects, trichinellosis will never be removed without control efforts.

The main purpose of this study was to identify factors influencing *Trichinella* transmission to humans and pigs focusing on endemic foci in Thailand. First, Thai national human trichinellosis surveillance data between 1981 and 2008 were analysed to describe epidemiological characteristic of human trichinellosis in Thailand. Secondly, a survey was carried out in endemic foci to determine the prevalence of trichinellosis in pigs and to identify the production and management factors significantly associated with trichinellosis in pigs. A preliminary survey was performed to examine the prevalence of *Trichinella* infection in wildlife caught for human consumption. This was important to demonstrate the role of wildlife to transmit *Trichinella* to pigs and humans. Thirdly, a study was conducted in trichinellosis endemic foci to examine whether any individual' household characteristics, food consumption habits and knowledge significantly associated with an individual being a trichinellosis case. Finally, a study was conducted to explore knowledge, attitudes and practices relevant to *Trichinella* transmission and control in the community in trichinellosis endemic foci. This work will provide knowledge on factors influencing *Trichinella* transmission specific for trichinellosis endemic foci in Thailand. It will address information required to guide for establishing guidelines and preventive measures for trichinellosis. This hopefully will lead to the meaningful changes taking place in order to prevent *Trichinella* transmission in these areas.

1.1. General introduction to trichinellosis

1.1.1 Early recognition of trichinellosis

Trichinellosis has existed since ancient times, as confirmed by the parasitological analysis of Egyptian mummies (Blancou, 2001; Chastel, 2004). Novel comprehensive analysis of the phylogeny and biogeography of *Trichinella* has concluded that early hominids may have first acquired *Trichinella* on the African savannah several million years ago when meat was introduced into the diet (Zarlenga *et al.*, 2006). Nevertheless, the organism was only discovered in the 19th century after the invention of the microscope. In 1834, larvae of *Trichinella spiralis* were discovered in a middle-aged Italian male cadaver at St Bartholomew's Hospital, London by James Paget, a medical student. The organism was named as *Trichina spiralis* by Richard Owen in 1835 but later changed to *Trichinella spiralis* by Railliet in 1896 because the genus *Trichina* had been already assigned to an insect. In 1846, Joseph Leidy discovered *Trichinella* larvae, which were indistinguishable from those he had seen in human bodies, from the thigh muscle of swine (Gould, 1970).

In 1850, the German scientist Herbst showed that it was possible to transmit *Trichinella* from a dog to a badger by feeding the badger on meat from the dog. He then showed subsequent transmission to a different dog by allowing the dog to feed on meat from the infected badger (Gould, 1970). Ten years later, the German scientists Rudolf Virchow, Rudolf Leuckart, and Friedrich Albert v. Zenker, elucidated the mechanism of infection and the life cycle of *Trichinella*. In addition, Zenker recognized the clinical significance of *Trichinella* infection and concluded that humans became infected by eating raw pork (Gould, 1970). The earliest trichinellosis outbreaks were reported in Germany, and between 1860 and 1880 epidemics of trichinellosis occurred in various parts of the country, producing a large number of cases with case

fatality between 17 and 30% (Gould, 1970). During the same time period, trichinellosis was being reported in humans in several countries, including the United Kingdom, Russia, Canada, the United States, Cuba, and Mexico. From the 1900s it has been gradually recognized in most parts of the world (Gould, 1970; Cook, 2001). The development of trichinoscopic, the method to detect *Trichinella* larvae in meat, and the mandatory test for pigs subject for human consumption has led to the dramatic decrease in the incidence of trichinellosis in humans and pigs (Hinz, 1991).

1.1.2 Trichinella species

Prior to 1972, the genus *Trichinella* was considered to be a single species. There were insufficient distinguishable morphological characters within the group to allow differentiation (Zarlenga & La Rosa, 2000). Nevertheless, explicit differences in the biological profiles among *Trichinella* isolates (for example resistance to heat or freezing, new borne larvae production, infectivity in experimental animals) had been observed previously (La Rosa *et al.*, 1992; Murrell *et al.*, 2000; Zarlenga & La Rosa, 2000). This led to biochemical and genetic techniques being applied to investigate the taxonomy of *Trichinella* (Flockhart *et al.*, 1982; La Rosa *et al.*, 1992; Pozio *et al.*, 1992; Gasser *et al.*, 1998; Rombout *et al.*, 2001; La Rosa *et al.*, 2003; Fonseca-Salamanca *et al.*, 2006).

Blaxter *et al.* (1998) used genetic data to delineate the phylum Nematoda into three classes (Enoplia, Dorylaimia, and Chromadoria), with *Trichinella* spp. being placed in the class Dorylaimia. However a more recent genetic study suggests further DNA sequences are required to resolve placing this parasite in the Dorylaimia (Meldal *et al.*, 2007).

To date, 12 species and genotypes of genus *Trichinella* have been identified) (see Table 1-1) (La Rosa *et al.*, 2003; Pozio *et al.*, 2005; Pozio *et al.*, 2009). They have been segregated into two distinct clades (Mitreva & Jasmer, 2006), Clade I represents species and genotypes where larvae are encapsulated in host muscle tissue. This clade contains five species and four genotypes of uncertain taxonomic status: *T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli* and *T. nelsoni*, genotypes² T6, T8, T9 and T12. Clade II represents three species where larvae are not encapsulated in host muscle tissue: *T. pseudospiralis*, *T. papuae*, and *T. zimbabwensis*.

² T6, T8, T9 and T12 are genotyping variants of *Trichinella* that have yet to be taxonomically defined

Table 1-1 The discovery and speciation methods for species and genotypes of parasites in the genus *Trichinella*

Year	Reference	Species	Speciation methods						
			Morphology	Cross-breeding	Biology	Epidemiology	Zoogeography	Allozymes	Molecular markers
1835	Owen, 1835	<i>T. spiralis</i>	■						
1972	Britov&Boev, 1972	<i>T. native, T. nelsoni</i>		■		■	■		
1972	Garkavi, 1972	<i>T. pseudospiralis</i>	■		■				
1992	Pozio et al., 1992	<i>T. britovi, T6, T8</i>			■	■	■	■	
1999	Pozio et al, 1999	<i>T. papuae</i>			■	■	■	■	■
1999	Nagano et al., 1999	T9							■
2000	Pozio and La Rosa, 2000	<i>T. murrelli</i>		■	■		■		
2002	Pozio et al, 2002	<i>T. zimbabwensis</i>		■	■	■	■	■	■
2008	Krivokapich et al., 2008	T12				■	■		■

Adapted from Pozio *et al* (2009)

1.1.3 Mode of transmission

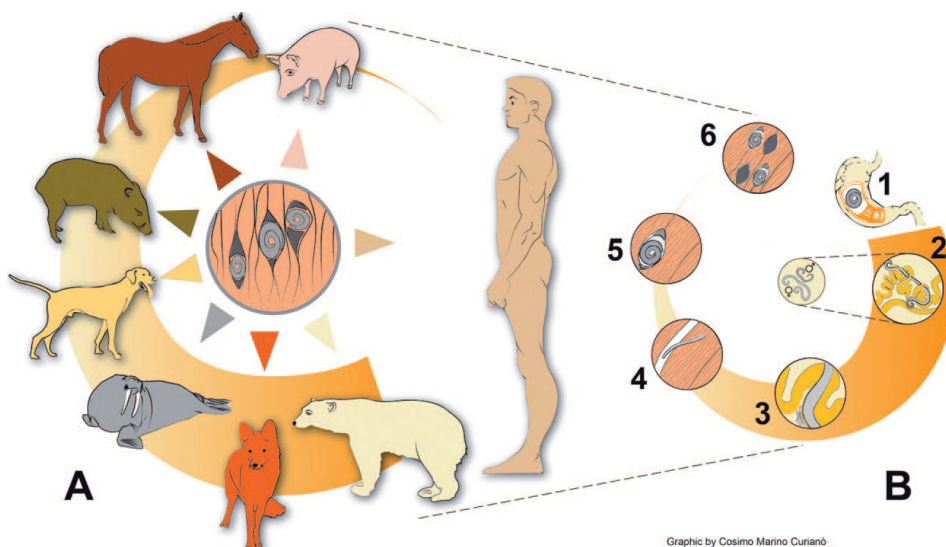
Adult *Trichinella* and their larvae are both capable of infecting a new host. Muscle larvae, as they survive in the hosts and in decaying carcasses, play main role in the transmission cycle. Ingestion of meat contain viable larvae of *Trichinella* is the most important mode of transmission. Faecal transmission may occur in the natural environment because adult *Trichinella* were found in faeces of naturally infected dogs (Rice *et al.*, 1990) and experimental studies showed that both adults and larvae in faeces were able to infect new hosts (Zimmermann *et al.*, 1959). Spindler (1953) (cited in (Zimmermann *et al.*, 1959)) observed *Trichinella* transmission in pigs after they were fed with faeces from dogs, cats, pigs and rats on day 4 – 28 after these animals had consumed trichinae flesh. Vertical transmission through experimental infection has been reported in ferrets, guinea pigs, rats and mice (Cosoroaba & Orjanu, 1998; Webster & Kapel, 2005) . Vertical transmission of *Trichinella* larvae in humans during pregnancy has also been observed (Dubinsky *et al.*, 2001).

1.1.4 Life cycle

According to Gould (1970), *Trichinella* spp. are unique amongst parasitic nematodes with all the stages of the life cycle occurring within a single host (Gould, 1970). The cycle is repeated in another host when it ingests meat containing viable larvae or adult worms contained in, or expelled from, the intestinal tract of infected animals. The life cycle of *Trichinella* (Figure 1-1) begins when a person or an animal ingests meat containing first stage muscle larvae. Larvae are released from cyst in muscle by digestive enzymes in the stomach (pepsin and hydrochloric acid). Freed larvae pass into the small intestine and invade the columnar epithelium (Katz *et al.*, 1989). Within 48 h post infection, they moult four times, mature to adult stage and mate. Female worms can produce 500–1,500

newborn larvae during a life span, before expulsion by the host immune system (Mitreva & Jasmer, 2006). The newborn larvae migrate into tissue; they enter lymphatic, then enter the general circulation at the thoracic duct and are distributed by the circulation into the muscle fibres. In muscle fibers the new born larvae are encysted, undergo development to become infective larvae within 15 days and remain for months to years (Mitreva & Jasmer, 2006).

Figure 1-1 The life cycle of *Trichinella* spp.



Source: Gottstein *et al.* (2009)

A: the life cycle is repeated when humans or animals ingest meat contained viable *Trichinella* larvae or adult worms in intestinal tract of infected animals (rare); B: (1) larvae are freed in the stomach of new host, pass to the small intestine, penetrate mucosal lining, (2) larvae moult four times and reach adult stage within 48 h post infection (p.i.) and mate, female worms release newborn larvae from 5 day p.i. (3) the newborn larvae migrate to tissues and organs through lymphatic and blood vessels throughout the host body, (4) the larvae reach and penetrate striated muscle (5) they grow to the infective stage in muscle cells and (6) calcification process occurs, and have been reported to survive up for up to 30 years (Froscher *et al.*, 1988).

1.1.5 Host range

Trichinella has been described as the least restricted in terms of host range of all parasitic nematodes (see Table 1-2) (Bachman and Rodriguez-Molina (1933) cited on p.61 by Gould (1970)). It can infect

mammals, birds and reptiles. Natural infections with *Trichinella* has been found in 13 different species of birds in seven families (Corvidae, Accipitridae, Falconidae, Stercorariidae, Strigidae, Tytanidae and Cathartidae) and more than 150 mammalian species in the following orders: Marsupialia, Insectivora, Edentata, Chiroptera, Lagomorpha, Rodentia, Cetacea, Carnivora, Perissodactyla, Artiodactyla, Tylopoda and Primates) (Kapel, 2000; Pozio, 2005). Natural infections of *Trichinella* in some reptile species including Nile crocodiles (*Crocodylus niloticus*), monitor lizards (*Varanus niloticus*), soft-shell turtles (*Pelodiscus sinensis*), have also been documented (Foggin *et al.*, 1997; La Grange *et al.*, 2009; Lo *et al.*, 2009).

1.1.6 Geographical distribution

Trichinella spp. have been detected in all continents except Antarctica (Figure 1-2 and Figure 1-3)(Pozio, 2007). *T. spiralis* is the dominant *Trichinella* spp. in domestic habitats. In sylvatic habitats other species have individual ecological niches to which they are adapted, according to their biological characters (Pozio, 2000). The main biological characters that play an important role in the transmission patterns of *Trichinella* species are the reproductive capacity index (RCI)³ of the host, the survival time of muscle larvae in the host and the survival time of muscle larvae in frozen meat or in meat exposed to high temperatures.

The number of newborn larvae produced by females is primarily related to the *Trichinella* genotype and the host species, and subsequently to the level of intestinal immunity, which can increase or reduce the survival time of females in the host gut. The high reproductive capacity index of *T. spiralis* in the domestic pigs and rats is a selective advantage in

³ Reproductive capacity index (RCI) is the number of muscle larvae collected from host muscles divided by the number of infective larvae ingested from a host

domestic habitats. However, the low reproductive capacity index of sylvatic genotypes in the pigs and rats is one of the most important biological characters preventing the passage of these parasites from the sylvatic to the domestic cycle.

The habitat of the host influences the survival time of larvae in muscles, increasing or reducing the role of an infected animal as a reservoir. When a *Trichinella*-infected animal dies, the parasite continues as a free-living stage in the decomposing carcass. During this stage of the natural cycle, the survival time of muscle larvae in frozen muscles or in muscles exposed to high temperatures is related to the parasite species. Thus it represents a key factor for parasite dispersion. Therefore capability to tolerate high or low temperatures and the decomposition of host tissues dictates the survival and transmission of the species in sylvatic habitats (Kapel, 2000; Pozio, 2000).

The relationship between *Trichinella* species and their distribution pattern is supported by field studies. A study conducted in three regions of Finland has shown that trichinellosis prevalence in wildlife varied according to geographical region. In addition, *T. spiralis* was detected more often in domestic and synanthropic animals (rats) than in sylvatic hosts. *T. nativa* was detected only in wildlife. *T. pseudospiralis* was found both in sylvatic and synanthropic hosts. *T. britovi* was detected only in mixed infections with other *T.* species (Oivanen *et al.*, 2002a).

Figure 1-2 The geographical distribution of *T. spiralis* (Tsp), *T. papuae* (Tpa), *T. zimbabwensis* (Tzi) and *T. pseudospiralis* in the United States (TpsN), *T. pseudospiralis* in Europe and Asia (TpsP) and *T. pseudospiralis* in Australia (TpsA)

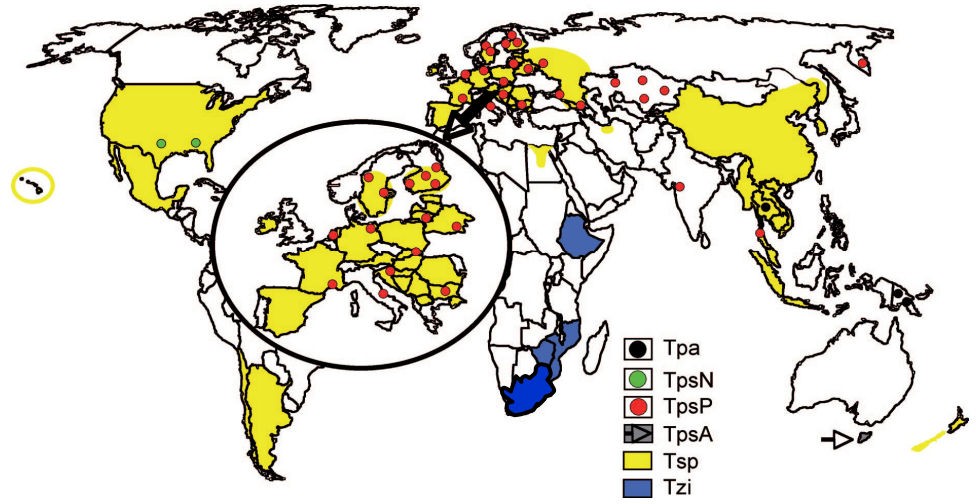
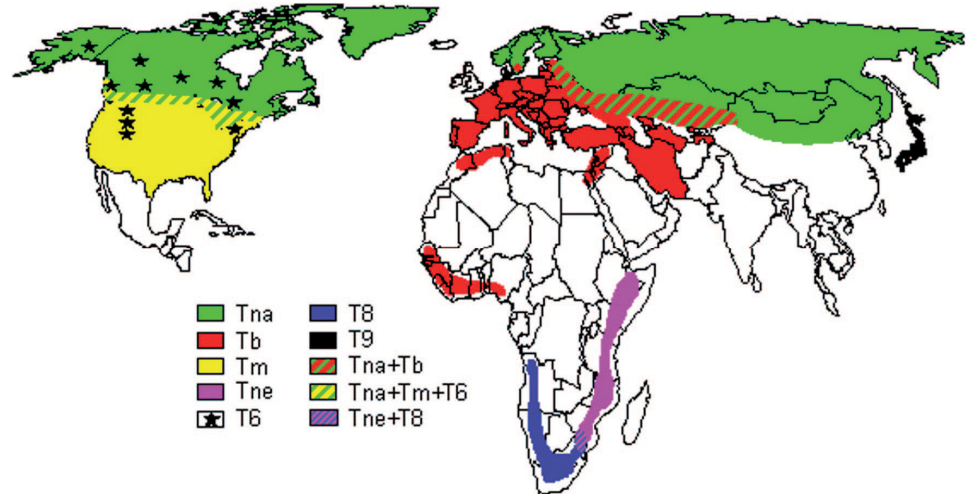


Figure 1-3 A world map shows geographical distribution of *T. nativa* (Tna), *T. britovi* (Tb), *T. murrelli* (Tm) and *T. nelsoni* (Tne), T6, T8 and T9, the recently identified T12 in Argentina is not shown in this map



Source: Gottstein et al. (2009)

Besides the biological of *Trichinella* species and ecosystem, geographical distribution of *T. spiralis* is strongly influenced by human behaviour (Rosenthal *et al.*, 2008). The trade in domestic pigs and migration of brown rats (*Rattus norvegicus*) from Asia to Europe and then the Colonization by Europeans to North, Central and South America and New Zealand was believed to be responsible for the spread of *T. spiralis* to domestic animals followed by transmission to wildlife in all continents. Pozio (2000, 2005) stated that *T. spiralis* did occur in sylvatic habitats of temperate and tropical regions as a result of current or past transmission from the domestic environment. Transmission is rare in cold regions because muscle larvae do not survive for long in frozen carcasses.

Trichinellosis in wildlife occurs virtually throughout the world, mainly in natural ecosystems far from human settlements (Pozio *et al.*, 1996) and involves all species of the parasite. *T. nativa*, the freeze tolerant species (Kapel, 2000), is prevalent in sylvatic carnivores in arctic and subarctic areas of North America, northern Europe and Asia. *T. britovi*, another species tolerant of freezing, is present in temperate regions of mainland Europe and Asia and it has also been recently documented in West Africa (Pozio *et al.*, 2005). *T. murrelli* is widespread amongst wildlife in the United States. The T6 genotype is widespread amongst carnivores in the United States, along the Rocky Mountains, from Alaska to Montana and Idaho. *T. nelsoni* a heat tolerant species, is found in wildlife of Eastern Africa, from Kenya to South Africa. The T8 genotype has only been isolated a few times from carnivores in South Africa and Namibia (Kapel, 2000) while the T9 genotype is found in Japan (Kanai *et al.*, 2006). *T. pseudospiralis* infection in nature has been documented in wild and domestic habitats. It is found in Europe, Asia, the United States, Canada and Australia (La Rosa *et al.*, 2001). *T. papuae* is reported in Papua New Guinea (Owen *et al.*, 2000) and Thailand (Khumjui *et al.*, 2008). *T. zimbabweensis* is present in Zimbabwe, Ethiopia, South Africa

and Mozambique (Pozio, 2000, 2005). T12 has been recently identified and was discovered in a jaguar in Argentina (Krivokapich *et al.*, 2008).

1.1.7 Transmission cycles

Trichinella occur naturally in sylvatic and domestic cycles (Campbell, 1988). Man is an accidental host, acquiring the infection either from domestic or wild animals. The domestic cycle involving human settlements and domestic pig (*Sus scrofa*) is by far the most common and requires no species other than the pig for zoonotic infection. The existence of the domestic cycle is a result of inappropriate human behaviour, mainly related to pig production practices (Pozio, 2000). Domestic pigs may acquire infection from other pigs by ingesting flesh of pigs that die in piggeries, feeding of pig-swill, tail-chewing and eating carcasses of infected rats (Smith, 1975; Hanbury *et al.*, 1986; Schad *et al.*, 1987).

The sylvatic cycle or wildlife cycle operates independently of humans (Campbell, 1988). Transmission occurs amongst wildlife either by ingestion of infected flesh of an animal that has been devoured as prey, through cannibalism or by scavenging of flesh from an infected carcass (Solomon & Warner, 1969; Leiby *et al.*, 1988; Forbes, 2000; Kapel *et al.*, 2003; Pozio *et al.*, 2007).

The domestic and sylvatic cycles of trichinellosis are not exclusive. Flow of transmission between the two cycles does occur. Evidence for natural transmission of *Trichinella* between domestic swine and wildlife has been demonstrated from isolates of *Trichinella* from farm swine and wild mammals trapped on the same premises (Murrell *et al.*, 1987).

Table 1-2 Features of the transmission cycle and natural hosts of *Trichinella* species or genotypes

Species/genotype	Transmission cycle	Natural hosts
<u>Encapsulated</u>		
<i>T. spiralis</i>	Domestic and sylvatic	Swine, rats, seldom carnivores
<i>T. nativa</i>	Sylvatic	Terrestrial and marine carnivores
T6	Sylvatic	Carnivores
<i>T. britovi</i>	Sylvatic, seldom domestic	Carnivores, seldom swine
T8	Sylvatic	Carnivores
<i>T. murrelli</i>	Sylvatic	Carnivores
T9	Sylvatic	Carnivores
<i>T. nelsoni</i>	Sylvatic	Carnivores, seldom swine
T12	Sylvatic	Carnivores
<u>Non-encapsulated</u>		
<i>T. pseudospiralis</i>	Sylvatic, seldom domestic	Mammals and birds
<i>T. papuae</i>	Sylvatic, seldom domestic	Swine, saltwater crocodiles
<i>T. zimbabwensis</i>	Sylvatic and domestic	Nile crocodiles, Nile monitor lizards, lion

Source: Pozio et al. (2009)

1.1.8 Survival of *Trichinella* larvae

Adult *Trichinella* survive in the host small intestine for less than two months post-infection. However, their larvae in host muscle tissues may survive for many years (Froscher *et al.*, 1988). The ability of *Trichinella* muscle larvae to survive in hosts or in decomposing carcasses under different environments is critical in explaining the global dispersion of this parasite. *Trichinella* larvae have been reported to survive in muscles of the human patients for between six to 30 years (Froscher *et al.*, 1988; Dupouy-Camet *et al.*, 2001). *T. spiralis* muscle larvae in pork pieces of 700 g in size and buried at 30 to 100 centimetres depth were able to

preserve their infectivity throughout the whole period of a 90-day assessment (Jovic *et al.*, 2001). *T. spiralis* larvae in rat muscle survived up to 96 hours in an experimental anaerobic sewage digester (Fitzgerald & Prakasam, 1978). *T. spiralis* in rat carcasses experimentally placed to contaminate several types of animal feed (including silage, grained barley, propionic acid-preserved feed and simulated pasture conditions) survived for up to six weeks (Oivanen *et al.*, 2002b). Muscle larvae of *T. papuae* in pig carcasses left under natural environmental conditions in Papua New Guinea survived and remained infective to a new host up to nine days after the pig was slaughtered (Owen & Reid, 2007). *Trichinella* larvae in maggots of *Sarcophaga argyrostoma* kept between 8 – 26°C were able to infect mice. While infective larvae survived in *Sarcophaga argyrostoma* maggots kept at 8°C for up to five days post infection (Maroli & Pozio, 2000). Muscle larvae of *T. nativa*, a freeze-resistant species, retained their infectivity after a seven week freeze and thaw process (Davidson *et al.*, 2008). Larvae of *T. spiralis* and *T. pseudospiralis* both survived freezing at -5 and -18 C for four weeks (Theodoropoulos *et al.*, 2000). Infective *T. spiralis* larvae in the musculature of grizzly bear and wolverine survived at temperatures of -6.5 to -20°C for up to 34 months and four months, respectively (Worley *et al.*, 1986).

1.1.9 Pathogenesis and clinical manifestations

Clinical trichinellosis is dose, host-species and parasite-species dependent. Humans are more susceptible to *Trichinella* than other animals. The minimum amount of *Trichinella* larvae necessary to produce clinical infection in humans has been estimated to range from 70 – 150 larvae (Murrell & Bruschi, 1994). Contrary to that in humans, experimental studies on pigs given 5,000 larvae/kg of body weight, the infected pigs manifested only mild clinical signs of trichinellosis (Kapel

& Gamble, 2000). Experimentally-infected canine puppies, having ingested 5,000 *Trichinella* larvae displayed only minor signs of gastrointestinal disturbance during the first week after infection (Bowman *et al.*, 1991). Cattle infected with 100,000 larvae displayed signs of reluctance to eat and masticate between days 10 – 30 post infection before returning to normal (Smith *et al.*, 1990). Raccoon dogs infected with either *T. spiralis* or *T. nativa* at the dose of 1,000 larvae/kg of body weight had only gastrointestinal signs, loss of appetite and diarrhoea during the early phase of infection (Nareaho *et al.*, 2000).

Clinical presentations of trichinosis in humans range from being asymptomatic infection to death. The severity depends on the number of ingested larvae (Cortes-Blanco *et al.*, 2002; Ozdemir *et al.*, 2005), the *Trichinella* species (MacLean *et al.*, 1989; Pozio *et al.*, 1993) and the age of the infected individuals (Ozdemir *et al.*, 2005). The presence of 1 – 10 larvae per gram of muscle biopsy is usually associated with an asymptomatic infection, while a density of 1,000 or more is critical or fatal (Most, 1978). The clinical presentations of *T. britovi* infection in children were both milder and significantly less common in children aged less than 17 years of age than in adults who had consumed the same amount of infected meat (Ozdemir *et al.*, 2005). The forms of human trichinellosis are summarized in Table 1-3 (Dupouy-Camet & Murrell, 2007).

Table 1-3 Clinical forms of human trichinellosis

Clinical forms	Clinical signs and symptoms
Severe	▪ All relevant signs and symptoms with complications
Moderate severe	▪ All relevant signs and symptoms without complications
Mild/benign	▪ Signs and symptoms are mild; no complications
Abortive	▪ Few mild signs or symptoms lasting for only a few days
Asymptomatic	▪ No signs or symptoms

The incubation period of trichinellosis in humans varies from 1 – 45 days, with an average of 10 – 15 days. In two outbreaks caused by *T.*

britovi in Spain, the time between ingestion of infected meat and the appearance of clinical signs ranged from 15 to 30 days in one outbreak and ranged from 3 to 20 days in another outbreak (Rodriguez-Osorio *et al.*, 1999; Gomez-Garcia *et al.*, 2003). According to Dupouy-Camet *et al.* (2002), the length of the incubation period depends upon the disease severity. The incubation period is shorter in the severe form and lasts about one week, while for the benign and abortive forms the incubation period lasts for 3 – 4 weeks (Dupouy-Camet *et al.*, 2002).

Clinical trichinellosis can be divided into two stages: an intestinal/enteric stage and a muscular invasion/parenteral stage (Capo & Despommier, 1996) (

Table 1-4). Some authors recognise a third, which Gould described as a convalescent stage (Gould, 1970). The intestinal stage customarily sets in from the 2nd to 7th day after ingestion of infected meat. At this stage symptoms may include malaise, anorexia, nausea, vomiting and diarrhoea. Muscular invasion may begin as early as the 7th day after infection and lasts for one to five weeks or longer. Manifestations at this stage may include oedema of the eyelids, pain and swelling of muscles, fatigue, fever, conjunctivitis, haemorrhage and marked eosinophilia. If death occurs then this will normally happen between four to six weeks after infection. Death is associated with myocarditis or other life-threatening complications including respiratory failure, sinus arrest and severe neurological damage (Alvarez-Chacon *et al.*, 1992; Compton *et al.*, 1993). Neurological complications may include peripheral facial diplegia and meningeal disorder. Electrocardiogram (EKG) changes have been reported (Lopez-Lozano *et al.*, 1988). Abortion has been observed in several outbreaks in mothers infected during pregnancy, although the cause of abortion was inconclusive (Cabral-Soto *et al.*, 1990; Ancelle, 1998; Taybouavone *et al.*, 2009). The convalescence stage among the moderately severe cases usually begins during weeks five or six and is usually followed by complete recovery. In some cases, symptoms may

persist for three to four months with an occasional individual sustaining some permanent residual symptoms or disabling complications (Compton *et al.*, 1993).

Long-term complications from trichinellosis or chronic trichinellosis have been studied by several authors. Harms *et al.* (1993) reported, after assessing 128 previously-infected persons and 16 non-infected controls, based on clinical, biochemical, serological, immunologic, neuro-radiological, radiological, and psychological studies that the previously-infected patients had tested parameters that differed significantly from the controls at a point 10 years post-infection (Harms *et al.*, 1993). In a separate study in 25 patients one, two, three and six years after surviving acute trichinellosis a number of conditions were noted. These were recorded in 22 patients (88.0% of the study group) and included muscle complaints (68.2%), cardiovascular complaints (45.4%), generalized weakness (40.9%) and fatigue (31.8%) (Kociecka *et al.*, 1997). Nemet *et al.* (2009) followed the former trichinosis patients from Brasov County in Romania for two years. They suggested that trichinellosis in humans cannot be considered only as an acute disease. Their study demonstrated that 24 months after the onset of illness, about 9% of patients remained ill; some patients were forced to retire from their jobs because of illness. In addition there were a number of deaths from cardiovascular and neurological complications in patients who did not have any pre-existing conditions (Nemet *et al.*, 2009).

Table 1-4 Summary of possible clinical presentations in two phases in patients suffering clinical trichinellosis

Phase	Period post ingestion	Clinical presentations
Enteric	1-2 weeks	Malaise, Anorexia, Nausea, Vomiting, Diarrhoea, Upper abdominal pain, Constipation, Fever
Muscular/Parenteral	2-6 weeks	Diffuse myalgia, Swelling of muscles, Eyelid oedema, Fatigue, Fever, Headache, Conjunctivitis Skin rash, difficulties in swallowing/opening the mouth, Splinter haemorrhage, Paralysis, Insomnia, Weight loss, Hot flashes, Coryza, Hoarseness, Bronchitis, Visual disturbances, Paralysis of the ocular muscle, Endocarditis, Myocarditis, Cardiac failure Marked eosinophilia

Source: Capo & Despommier (1996)

1.1.10 Diagnosis and treatment of human trichinellosis

1.1.10.1. Diagnosis of human trichinellosis

The principles for diagnosis of human trichinellosis include three criteria: clinical signs or symptoms, laboratory testing and epidemiological linkage (Kociecka, 2000). A definitive case of trichinellosis is defined as one that is laboratory confirmed and is clinically compatible with the common signs and symptoms of trichinellosis (fever, myalgia, periorbital oedema and eosinophilia). Laboratory confirmation is made either by identification of *Trichinella* larvae through muscle biopsy or by a positive serological test. In an outbreak, at least one case must be

laboratory confirmed. Any associated patients that have either positive serological tests or clinical compatible illness that have shared an epidemiologically-implicated meal or meat products should also be reported as confirmed cases (CDC, 1996).

There are no pathognomonic symptoms for trichinosis (Anon, 1996; Pozio *et al.*, 2003). The disease is usually detected when a cluster of patients show common signs and symptoms of trichinellosis (Krauss *et al.*, 2003). Delay in final diagnosis and misdiagnosis are common for trichinellosis, with patients having to endure undiagnosed disease for months or years. The algorithms for the probability of human trichinellosis and the criteria for diagnosis have been described in a diagnosis guideline (see Table 1-5, Table 1-6) (Dupouy-Camet & Murrell, 2007).

Table 1-5 Algorithm for diagnosis the probability of human trichinellosis

Group A	Group B	Group C	Group D
i. Fever	i. Diarrhoea	i. Eosinophilia	i. Positive
ii. Eyelid	ii. Neurologic	(>1000	serology
and/or	signs	cells/mm ³)	ii. Seroconversion
facial	iii. Cardiologic	and/ or	iii. Positive
oedema	signs	increased	muscular
iii. Myalgia	iv. Conjunctivitis	total IgE	biopsy
	v. Subungual	levels	
	haemorrhages	ii. Increased	
	vi. Cutaneous	levels of	
	rash	muscular	
		enzymes	

Group A-C refer to clinical signs of trichinellosis or/and laboratory tests

Table 1-6 Diagnosis criteria of human trichinellosis

Diagnosis	Number of clinical signs or laboratory tests ^a			
	Group A	Group B	Group C	Group D
Very unlikely	only one			-
Suspected	1	-	1	-
	or -	2	1	-
Probable	3	-	1	-
Highly probable	3	-	2	-
Confirmed	3	-	2	1
	or ≥ 1		1	1

^a Clinical signs or laboratory tests as were shown in Table 1-5

1.1.10.2. Treatment of human trichinellosis

Treatment regimes for trichinellosis are based on anthelmintics and corticosteroids (Dupouy-Camet & Murrell, 2007). However, there is still disagreement between authorities on most facets of trichinellosis therapy, including when to use corticosteroids and which anthelmintic drugs should be administered, for how long and at what dose (Watt & Silachamroon, 2004). Treatments that are currently recommended include the anthelmintics mebendazole or albendazole in combination with glucocorticosteroids. Mebendazole is administered at a daily dose of 5mg/kg. Up to 20 – 25 mg/kg/day is recommended in some countries. Albendazole can be used at 800 mg/day or 15 mg/kg/day twice a day. These drugs should be taken for 10 – 15 days. The most commonly used steroid is prednisolone. It may alleviate the general symptoms of the disease and is administered at a dose of 30 – 60 mg/day for 10 – 15 days (Dupouy-Camet *et al.*, 2002). A side-effect of albendazole can include a Jarisch-Herxheimer-like reaction⁴ which has been observed in some cases. Failure of mebendazole to alleviate patient symptoms or to kill

⁴ The Jarisch-Herxheimer-like reaction is characterized by severe chills, muscle aches, headache, fever, increased heart rate and increased respiratory rate.

muscle larvae has been reported (Pozio *et al.*, 2001; Jongsuksuntigul & Imsomboon, 2003).

1.2. Detection of trichinellosis in animal reservoirs

Based on the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, there are two approaches to the diagnosis of *Trichinella* infection in animals: (i) direct detection and (ii) indirect detection (OIE, 2008). Direct detection comprises two general methods (the trichinoscopic or compression method and the digestion method). These methods directly identify the first-stage larvae of *Trichinella* in striated muscle tissues of infected animals. Indirect detection is carried out through the presence of antibodies specific for *Trichinella* spp. There are several serological tests available for detection of *Trichinella* spp. infection. The ELISA is the most common test and has been recommended by the OIE as a diagnostic tool for surveillance purposes.

1.2.1 Trichinoscopic or compression method

The trichinoscopic is the simplest method for recovering larvae of *Trichinella* spp. in striated muscle tissues. Pieces of striated muscle from predilection sites are analysed with the aid of a trichinoscope or a dissecting microscope with the compressed translucent tissues being directly visualized. Where the digestion method is used, the test's sensitivity depends on the amount of tissue examined and the site from where the sample is obtained. The compression method is less sensitive when compared with the digestion method (Forbes *et al.*, 2003). Shortfalls in the trichinoscopic method have been reported such as when it failed to detect infection in sample with a larvae density ≥ 6 larvae/g

(Beck *et al.*, 2005b), a level that can cause human trichinellosis. There are also limitations when examining non-encapsulated *Trichinella* larvae (OIE, 2008). Thus the trichinoscopic method is not recommended for routine meat inspection.

1.2.2 Digestion method

The digestion method involves the enzymatic digestion (using a 1% hydrochloric acid/pepsin solution) of individual or pooled muscle tissue samples followed by filtering or sedimentation procedures. The processed samples are finally inspected microscopically for the presence of larvae. Its sensitivity depends on the amount of tissue examined and the site from which the sample was obtained. Predilection sites for *Trichinella* spp. have been recorded as the base of tongue and the crus muscle of the diaphragm, the forearm muscle, the masseter muscle and the caudal muscle of the wild boar, carnivores, herbivores and reptiles respectively (Kapel *et al.*, 1994; Kapel *et al.*, 1995; Reina *et al.*, 1996; Serrano *et al.*, 1999; Oksanen *et al.*, 2000; Kapel, 2001; Pozio *et al.*, 2007). Though, *Trichinella* larvae have been shown to be distributed evenly in muscles of heavy-infected animals, in lighter infections they are more likely to be found in the predilection-site muscles (Kapel *et al.*, 2005). Thus it is recommended to collect samples for *Trichinella* examination with respect to the larvae predilection sites in particular animal species or to take a greater sample weight for analysis in the absence of predilection muscles (Dupouy-Camet & Murrell, 2007). Other factors which may affect the test results include the low intensity of larval burden in wildlife species, the digestibility of the muscle from predilection sites and even the knowledge of non-skilled hunters to recognise and correctly collect the preferential muscles. Therefore, designated preferential muscle sites guidelines have been developed based on all of these factors (Table 1-7).

Table 1-7 Predilection sites for *Trichinella* in muscles of different wild animal species

Animal species	Predilection sites
Wild boar	Forearm, diaphragm
Bear	Diaphragm, masseter, tongue
Walrus	Tongue
Seal	Tongue, diaphragm, flippers, masseter
Fox	Diaphragm, forearm muscles
Raccoon dog	Diaphragm, forearm muscles
Crocodile	Intercostal muscle, tongue, forearm muscle, tail

Source: FAO/WHO/OIE Guidelines for the surveillance, management, prevention and control of trichinellosis; in Chapter III (K. Nöckler and C.M.O. Kapel)

The digestion method can detect as low as three and one larvae/g of muscle tissue when testing 1g and 5g of tissue respectively. It has been accepted that meat containing <3 larvae/g of tissue will not cause clinical infection in humans. Therefore, a sample of pork muscle for testing weighing approximately 1g is recommended. Other animal species may require a greater amount of tissue. Gamble *et al.* (2000) recommended that the quantity of each sample for epidemiological studies in wildlife should be increased to at least 5g. Pozio and Rossi (2008) recommended that the minimum amount of muscle that should be tested from each animal irrespective of the species, is $\geq 10\text{g}$ from the preferential muscles or $\geq 20\text{g}$ from other muscles.

1.2.3 Enzyme-linked immunosorbent assay (ELISA)

The ELISA is the only recommended serological test for the ante-mortem detection of *Trichinella* infection. It is also recommended for

epidemiological studies (OIE, 2008). Sera and muscle juice⁵ have been reported to give comparable results (Kitamura & Ishii, 1993; Kapel *et al.*, 1998; Beck *et al.*, 2005a; Nockler *et al.*, 2005). The test can detect an infection with a larvae density of <1 larvae/g of muscle tissue (van der Leek *et al.*, 1992). It is not recommended for individual carcass inspection in food animals because there is the potential of false-negative ELISA results that may be obtained in recently-infected animals with low-grade infection (Gamble *et al.*, 1983). False negative results prior to sero-conversion occur up to the 4th and 5th week post infection in experimentally infected pigs, with correlation to the infection dose (Nockler *et al.*, 1995). A low rate of false positives results are reported but these can be confirmed through Western blots (Frey *et al.*, 2009a). ELISA is not a recommended test for trichinellosis in equines (Pozio *et al.*, 2002) because it can only detect antibodies for a short period of time (18 – 20 weeks) (Sofronic-Milosavljevic *et al.*, 2005).

1.2.4 Polymerase chain reaction (PCR)

A few studies have shown that PCR can be used to detect *Trichinella* larvae in muscle tissue, however, this method lacks sensitivity and is not practical for routine testing of food animals (OIE, 2008). Viveros *et al.* (2001) used PCR to examine muscle samples obtained from 170 slaughtered horses from the rural and the federal abattoirs and compared the results with ELISA test from serum from the same horse that muscle same sample was obtained. The ELISA test identified specific antibodies against *Trichinella* in 17% of serum samples obtained from rural abattoir and 7% of the serum samples obtained from federal abattoirs. Meanwhile PCR studies in the same animals identified *Trichinella*-positive in 15% of muscle samples obtained from rural abattoirs and 2% of muscle samples obtained from federal abattoirs (Viveros *et al.*, 2001).

⁵ Muscle juice is a liquid from muscle tissues

Currently PCR analysis can be used to identify the genus of *Trichinella* based on a single larva (Pozio & La Rosa, 2003). Several PCR derived analysis methods have been developed *e.g.* inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) (Fonseca-Salamanca *et al.*, 2006), multiplex-PCR (Zarlenga *et al.*, 1999) and restriction fragment length polymorphism-PCR (RFLP-PCR)(Wu *et al.*, 1999).Two PCR methods are routinely used for *Trichinella* species identification at the International *Trichinella* Reference Centre in Italy, the multiple-PCR and the RFLP-PCR (Pozio & La Rosa, 2003). PCR is useful for species identification and to trace the source of infection, determine the course of infection, estimate the potential risk for pigs and understand the epidemiology of the infection (Pozio & La Rosa, 2003).

1.2.5 Western Blot

As with PCR, Western Blots are not used in a routine testing of food animals. Although international regulations and guidelines have approved serological surveillance using ELISA to demonstrate the absence of *Trichinella* spp. in a domestic pig population the ELISA test does not yield 100% specificity, and therefore, a complementary test is needed to confirm the diagnosis of any initial ELISA sero-positivity. Recently the Western Blot assay method has been validated to confirm samples that reacted positively in an initial ELISA test (Frey *et al.*, 2009b). Frey *et al.* (2009b) reported that the diagnostic sensitivity and specificity of the Western Blot ranged from 95.8% to 96.0% and from 99.5% to 99.6%, respectively. Nockler *et al.* (2009) examined 144 field-infected pig sera and 159 experimentally-infected pig sera and revealed a 96.8% sensitivity and 97.9% specificity for the ELISA test and 98.1% sensitivity and 100% for the Western Blot method .

1.3. Trichinellosis control measures

van Knapen (2000) reported that there were reasons why trichinellosis is still a world-wide problem. Proper control requires an expensive, large scale inspection system. Although this is quite effective in stopping *Trichinella* infected meat entering the food system, in poorer countries there is often not enough money to develop such systems. In addition, private home slaughtering is still widely practised in some countries. As home slaughtered meat is not subjected to veterinary inspection, this represents another avenue where infected meat can enter the food chain. Various preventive measures are available or being developed in places where meat inspection systems do not exist, the most common one is processing methods and consumer education (Table 1-8).

Table 1-8 Interventions for prevention and control of trichinellosis at various stages from farms to tables

Type of interventions	Target places for intervention			
	Farm/Origin	Slaughterhouse	Food factory	Home
Education on good management practise in the farm				
Certified <i>Trichinella</i> -free farm				
Education on good practise in hunting				
Tested at slaughterhouse				
Certified <i>Trichinella</i> -free meat/processed-food				
Education on safe cooking procedures				
Education on safe eating behaviour				

1.3.1 Processing methods and consumer education

In all area where methods of *Trichinella* control measures have not been fully implemented, consumers should be adequately informed of the risks

and educated in proper meat preparation (Gamble *et al.*, 2000; van Knapen, 2000). Acceptable methods for consumer preparation of meats which may pose a public health risk include: cooking meat to an internal temperature of 71°C (160°F) or freezing the meat at -15°C or below for three weeks (for cuts up to 15 cm in thickness) or for four weeks (for cuts up to 69 cm thickness). The freezing method is not recommended in areas where freeze-resistant *Trichinella* spp. is present.

The International Commission on Trichinellosis (ICT) is a scientific organization established in 1958. Its principle purpose is to research and control *Trichinella* and trichinellosis in humans and animals (<http://www.med.unipi.it/ict/>). It has recommended three methods that are considered reliable to render meat, particularly pork, safe from *Trichinella*. The methods include cooking, freezing and irradiation (Gamble *et al.*, 2000).

Each species of *Trichinella* differs in its ability to survive in the muscle tissue of various hosts (Worley *et al.*, 1986; Hill *et al.*, 2007). As pork is the major food source, as well as being the major source of human trichinellosis, the standard guidelines to inactivate the parasite's muscle larvae is based on the inactivation of larvae in pork. In Europe, where trichinellosis is caused by the consumption of raw horse meat became a public health problem (Ancelle *et al.*, 1998; Maillot, 1998), the guidelines for control of *Trichinella* in pigs are applied to the control of *Trichinella* in horse meat (Gamble *et al.*, 2000; Kapel, 2005).

The standard guidelines for the cooking and freezing temperatures and times to inactivate *T. spiralis* larvae in pork (Table 1-9) were set by the United States Department of Agriculture (Anon, 1990). Hill *et al.* (2009) reported recently that the freezing time-temperature combinations proven to inactivate *T. spiralis* in pork were also sufficient to inactivate the

larvae of the North American genotypes *T. nativa*, T6, *T. murrelli* and *T. pseudospiralis* similarly in pork.

Table 1-9 Methods of inactivating *T. spiralis* larvae in pork by heating and freezing

Minimum internal temperature for cooking		Minimum time for cooking	Minimum internal temperature for freezing		Minimum time for freezing
°F	°C		°F	°C	
120	49.0	21 hr	0	-17.8	106 hr
122	50.0	9.5 hr	-5	-20.6	82 hr
124	51.1	4.5 hr	-10	-23.3	63 hr
126	52.2	2 hr	-15	-26.1	48 hr
128	53.4	1 hr	-20	-28.9	35 hr
130	54.5	30 min	-25	-31.7	22 hr
132	55.6	15 min	-30	-34.5	8 hr
134	56.7	6 min	-35	-37.2	30 min
136	57.8	3 min			
138	58.9	2 min			
140	60.0	1 min			
142	61.1	1 min			
144	62.2	Instant			

For irradiation, the ICT currently recommends irradiation at a level of 0.3 kGy⁶ to render meat safe from *Trichinella*, but only for meat products in sealed packages (Gamble *et al.*, 2000). Gamma irradiation with a dose of 15 – 30 krad⁷ (0.15 – 0.3 kGy) can block the maturation of *T. spiralis* muscle larvae in pork, while a dose of 30 krad can be delivered to split market-weight hog carcasses with acceptable uniformity (Brake *et al.*, 1985). The wholesomeness of irradiated food has been carefully evaluated for over 50 years and the FAO/IAEA/WHO Expert Committee on Food Irradiation concluded in 1981 that irradiation of food

⁶ Gray (Gy) is the SI unit used to measure the dose of radiation. One Gy dose of radiation is equal to 1 joule of energy absorbed per kg of food material; 1 kGy = 1,000 Gy

⁷ Rad is the non SI unit used to measure the dose of radiation; 1 krad rad = 0.01 kGy

commodities up to an overall average dose of 10 kGy presents no toxicological hazard and introduces no special nutritional or microbiological problems (WHO, 1981; Farkas, 2006). The guidelines set the irradiation dose requirement for prevention of reproduction of food-borne parasites at 0.1 – 0.3 kGy (Farkas, 2006).

Curing and smoking according to commercial standards are able to inactivate *Trichinella* larvae (Worley et al., 1986). However, the effectiveness of the methods of destroying *Trichinella* larvae are all time, temperature and salt-concentration dependent (Zimmerman, 1971; Smith et al., 1989; Lin et al., 1990; Medina-Lerena et al., 2009). In the view of the ICT these processing methods cannot be controlled reliably (Gamble et al., 2000). Trichinellosis outbreaks involving consumption of processed meat products do occur occasionally (Landry et al., 1992; Tiberio et al., 1995; Milne et al., 2001; Gomez-Garcia et al., 2003; da Cunha-Bang & Lunding, 2009). As a result, the ICT recommended that only meat free from *Trichinella* should be used to prepare cured, dried and smoked products (Gamble et al., 2000).

Similar to processed-product preparation methods, microwave cooking is not recommended to render meat safe from *Trichinella* as the microwave oven does not evenly distribute heat throughout the meat. Zimmermann (1983) reporting that infective *Trichinella* larvae were recovered from pork that was cooked in a microwave oven (Zimmermann, 1983).

1.3.2 Certification by freezing (pork)

Freezing pork to inactivate *Trichinella* larvae has been used as an alternative to individual carcass testing for the purpose of international trade (van Knapen, 2000). Meat from wild boars, game or horses can harbour cold tolerant species of *Trichinella*, therefore, such meat can not be certified *Trichinella*-free through freezing (Kapel, 2005).

1.3.3 Individual carcass inspection

The prevention of human trichinellosis by appropriate meat inspection is a classic example of a successful veterinary public health measures (van Knapen, 2000). All countries in the EU perform mandatory official inspection of slaughtered animals intended for export to prevent distribution of infected meat to consumers (Webster *et al.*, 2006). Two methods are used in meat inspection: the trichoscopic and the digestion method. However, the only approved method for meat inspection in the EU is the digestion method (Kapel, 2005). The amount of meat that must be tested per animal is 1g for pigs, 5g for horses and wild boar and 10g for all other game (Kapel, 2005).

1.3.4 *Trichinella*-free farming

It is believed that the modern pork production systems in developed countries have eliminated trichinellosis as a food safety risk. This led to the initiative of *Trichinella*-free farming as an alternative to individual carcass inspection in the USA and the European countries (Kapel, 2005; Pyburn *et al.*, 2005). The knowledge of the risk factors that lead to the exposure of pigs to *T. spiralis* was used to develop an objective audit of on-farm production practices that could be applied to pork production sites. The on-farm audit includes aspects of good management practices involving farm management, bio-security, feeding and feed storage, a rodent control programme and general hygiene. The programme will document the safety of pork produced under scientifically proven production methods by ensuring the risk factors exposing swine to *T. spiralis* have been eliminated (Pyburn *et al.*, 2005). A pioneer on-farm audit in the USA pig farms observed <4% of farms met all of the criteria. The most common deficiency was inadequate rodent control (Gamble *et al.*, 2001).

1.3.5 Anthelmintic prophylaxis in pigs

The use of anthelmintics in feed as a control measure against trichinellosis in pigs has been reported in China. The prevalence of trichinellosis in pigs was substantially lowered after the use of anthelmintic prophylaxis. However, drug residues and public health safety were issues discouraging these practices (Wang & Cui, 2001b). An experimental study to assess the efficacy of flubendazole (mixed with food) against experimentally induced trichinellosis in pigs showed that the efficacy of the regime against adults and larvae of *Trichinella* depended on the duration of administration and dose rate of the drug (Marinculic *et al.*, 2001). For example, a dose of 31 mg/kg flubendazole administered for 14 days was 72.35% effective, while the higher dose of 62 mg/kg increased efficacy to 87.77% (Marinculic *et al.*, 2001).

1.3.6 Vaccination in pigs

Experimental vaccine trials to prevent *T. spiralis* infection in pigs and rats have demonstrated successful outcomes (Smith, 1987; Marinculic *et al.*, 1991; Wang *et al.*, 2006a). However, the low prevalence of infection in any pig herd means the use of vaccination as a method of controlling trichinellosis in pigs is unlikely to be practical in the commercial sector. Smith (1987) showed that pigs vaccinated with *T. nativa* were protected from *T. spiralis* infection. In the experiment, pigs were orally vaccinated against *T. spiralis* infection with 23,000 larvae of *T. nativa* and challenged on day 28 with 5,500 larvae of *T. spiralis*. The number of larvae established in the muscle of vaccinated non-challenged and vaccinated challenged pigs was negligible and comparable. Highly significant infections were established in the non-vaccinated challenged pigs. Marinculic *et al.* (1991) reported that pigs vaccinated with *T. nativa*

had fewer numbers of muscle larvae, compared with pigs vaccinated with *T. spiralis*. They also established high levels of immunity against challenge infection with *T. spiralis*. This immunity in pigs was expressed in accelerated expulsion from the intestine of the adult parasites used in the challenge infection and reduced numbers of muscle larvae (Marinculic *et al.*, 2001). A DNA vaccine has been developed and showed a significant reduction of larval burden in muscle of experimental mice (Wang *et al.*, 2006a).

1.3.7 Community trichinellosis control programme

Nunavik comprises the Inuit communities in northern Quebec, Canada. Between 1982 and 1999 nine out of 11 outbreaks of trichinellosis were caused by consumption of walrus meat. According to Proulx *et al* (2002), a trichinellosis prevention programme for residents of Nunavik, was initiated in 1992. The programme offered all municipalities the opportunity to test selected samples of meat from newly harvested walrus for the presence of *Trichinella* larvae. At the time of a hunt, all butchered pieces of meat are tagged. A fist-sized piece of meat should be obtained from the tongue, digastricus, intercostals and pectoral muscle from each walrus to use for detection of *Trichinella* larvae. Once the hunters return to the community the samples are flown to the laboratory centre. Hunters are advised not to distribute or consume any walrus meat before the results of the analyses are made available. The communities involved have complied with the programme.

The effectiveness of the programme depended on various factors. These included the validity of the test used, the proportion of harvested walrus from which the samples are tested, compliance with the distribution and restrictions on consumption of temporarily stored walrus meat, the delay

between the arrival of the walrus harvest in the community and the availability of test results, the efficacy of the local communication system, and compliance with any subsequent health advisory notices in the event that positive test results are obtained (Proulx *et al.*, 2002).

1.3.8 Surveillance of trichinellosis in animals

Surveillance of trichinellosis in animals, particularly pigs, has been conducted through routine mandatory meat inspection in some countries (Webster *et al.*, 2006). All the countries in the EU perform mandatory official inspection of slaughtered animals intended for export to prevent distribution of infected meat to the consumer (Webster *et al.*, 2006). In addition, some countries in the EU *e.g.* Germany requires all animals destined for human consumption to be examined for *Trichinella* (Webster *et al.*, 2006). In Canada trichinellosis surveillance has been conducted through national surveillance programme and through prevalence study of *Trichinella* in wild boar and domestic pigs (Gajadhar *et al.*, 1997). Trichinellosis surveillance in pigs in China has been conducted through routine inspection and an epidemiological surveys (Cui *et al.*, 2006a).

1.3.9 Risk-based surveillance in wildlife

Wide-ranging surveillance of trichinellosis in wild animals is limited because many wildlife species are protected. In addition to routine inspection of game animals, there are alternative opportunities for surveillance of trichinellosis in wild animals in several world regions as follows: inspection from wild game meat during the hunting season, special study projects in wildlife populations, comprehensive studies of trichinellosis prevalence in wildlife in the natural environment or in captivity, wild animals killed or found dead following accidents and wild

animals killed as part of a control and eradication programmes (Yepez-Mulia *et al.*, 1996; Perez-Martin *et al.*, 2000; Kobayashi *et al.*, 2007).

It is only in the EU that surveillance of trichinellosis in wildlife is compulsory. The regulation (Commission Regulation (EC) No 2075/2005 on *Trichinella* in meat intended for human consumption) recommended a risk-based wildlife monitoring programme in the areas where wildlife and holdings applying for *Trichinella*-free status coexist, or in a region where the risk of *Trichinella* in domestic swine is officially recognised as negligible (Pozio & Rossi, 2008). The aims of the wildlife monitoring programme are to determine the presence/absence of *Trichinella* spp. in the susceptible animals in a certain area and to establish the prevalence of the infection if infections are detected (Pozio & Rossi, 2008).

1.4. Epizootiology of trichinellosis

1.4.1 Trichinellosis in pigs

The prevalence of trichinellosis in domestic pigs varies between different pig productions systems. Pigs in outdoor farms or backyard pigs have a higher tendency of trichinellosis than commercial pigs. In the Netherlands, the prevalence of *Trichinella* infection in pigs from different housing systems (40 organic, 9 free-range and 24 intensive farms) was examined. A total of 845 serum samples were tested for antibodies specific for *Trichinella* using ELISA assays. The seropositivity of *Trichinella spiralis* antibodies was 0.12 – 0.35% (depending on whether the cut-off value was put at the 99.5% or 97.5% level). The tendency for *Trichinella* sero-positivity was greatest in organic pig farming (van der Giessen *et al.*, 2007).

The epidemiology of swine trichinellosis in China during 1999–2004 was reported based on a sero-prevalence surveys and slaughter house inspection (Cui *et al.*, 2006a). The sero-prevalence of swine trichinellosis in four provinces varied from 1.63 – 15.21%. The prevalence of *Trichinella* infection in swine slaughtered at abattoirs in seven provinces varied from 0.0001 – 23%. In Nepal a 1% infection rate of *Trichinella* through sero-prevalence, was observed from samples obtained from 400 slaughtered pigs in Kathmandu Valley. The pigs had been reared indoors (Sapkotal *et al.*, 2006).

In the USA a prevalence survey of trichinellosis in pigs was conducted in New England and New Jersey where 4,078 pigs from 156 farms were examined. A total of 15 sero-positive pigs on 10 farms were identified, representing a prevalence rate of 0.37% and a herd prevalence of 6.4% . In Canada, sera from 14,408 market sows from the Canadian domestic swine herd were tested for trichinellosis. Three sera (0.02%) gave low-level reactions on the competitive ELISA (Appleyard *et al.*, 2002). A study in 12 trichinellosis-endemic provinces in Argentina found the infection in pigs (59%; n = 164) and pork products (68%; n = 56) (Krivokapich *et al.*, 2006).

1.4.2 Trichinellosis in wild animals

The prevalence of trichinellosis in wildlife varies between different animal species and between different habitats (van der Giessen, 2001; Oivanen *et al.*, 2002a; Pozio *et al.*, 2004; Hurnikova *et al.*, 2006). Several trichinellosis prevalence surveys in wild boars in European countries have taken place between 1981 – 2006. Using the digestion method and with sample sizes greater than 1,000 (regardless of survey period) they showed that prevalence in wild boar varied from 0 – 8.6%: Switzerland 0%, n = 1,458; Italy 0%, n = 1,508; Sweden 0.05%, n = 8,000; Slovakia 0.06%, n = 70,568; Spain 0.3%, n = 29,333; Spain 0.8%, n = 2,216; and Romania 8.6%, n = 29,825 (Rossi & Dini, 1990; Perez-Martin *et al.*,

2000; Pozio *et al.*, 2004; Frey *et al.*, 2009a; Garcia-Sanchez *et al.*, 2009; Hurnikova & Dubinsky, 2009). At the same time and using similar diagnostic tests, the prevalence of trichinellosis in red foxes, the principle host of sylvatic *Trichinella* in the EU, in each country in EU was found to be greater than that in the wild boar. The prevalence of trichinellosis in the red foxes ranged between 0.05 – 4.8%: Norway 4.8%, n = 393; Spain 2.6%, n = 227; Netherlands 3.9%, n = 276; Sweden 4.5%, n = 1,800; Denmark 0.05%, n = 6,146; and Ireland 0.9%, n = 454 (van der Giessen *et al.*, 1998; Enemark *et al.*, 2000; Perez-Martin *et al.*, 2000; Pozio *et al.*, 2004; Rafter *et al.*, 2005; Davidson *et al.*, 2006).

In countries outside the EU, surveillance of trichinellosis has been conducted through periodical surveys. In Canada during 1972 – 1975, prevalence surveys observed *Trichinella* infection in various wild animals including grizzly bear (35%), marten (33.1%), wolverine (27.3%), bobcat (16.0%), black bear (12.0%), coyote (10.1%), lynx (9.8%), cougar (9.8%), weasel (9.1%), skunk (3.8%), mink (2.4%), shrew (0.3%), white-footed mouse (0.6%), ground squirrel (0.4%) and red squirrel (0.4%) (Schmitt *et al.*, 1976). In Japan, samples of wildlife from annual control and elimination programmes were examined for *Trichinella* infection (Kobayashi *et al.*, 2007). Of the 1,080 raccoons, 113 raccoon dogs, 41 wild boars, 14 martens, five Siberian weasel, seven minks and one red fox, *Trichinella* larvae were identified only from six raccoons and one raccoon dog.

1.4.3 Trichinellosis in rats

Prevalence of trichinellosis in rats has been reported from several countries. A study in Finland found an infection rate of 13% among 767 rats from 12 out of 13 rubbish dumps (Mikkonen *et al.*, 2005). In six provinces or autonomous regions of China the prevalence rates of *T. spiralis* infection in rats ranged from 1.98% to 15.06% (Wang & Cui, 2001b). Additionally, Wang *et al.* (2007) also reported that *Trichinella*

larvae were detected in 1.5% (9/587) of house rats (*Rattus norvegicus*) and in 0.8% (3/369) of wild rats (*Apodemus chevrieri*). One study in endemic areas of Argentina found three *Trichinella*-infected rats (33%; n = 9) (Krivokapich *et al.*, 2006). Another study found four of 26 rats (15.4%) infected with *Trichinella* in the area of Rio Negro Province where two human trichinellosis cases were detected (Larrieu *et al.*, 2004).

Whether rats are a reservoir or accidental host/vector of *Trichinella* infection continues to be debated. It was proposed that at least two criteria must be accomplished to define a host species as a reservoir of *Trichinella* in a given area: firstly the area where the host species is detected should be similar in size or larger than the area where *Trichinella* is present; and secondly the host species should maintain the infection for years, independently of the presence of *Trichinella* in other animals living in the same area. In all other cases, the host species should be considered as a vector of the parasite to the other host or as a victim (Pozio, 2005). Previous studies have showed that rat could be a reservoir, accidental host and vector of *Trichinella*. The role of the rat as a vector of *T. spiralis* was shown in some swine herds of the Atlantic provinces of Canada, where a pest control program forced rats to migrate from *Trichinella* positive pig herds to *Trichinella* negative pig herds. The negative pig herds subsequently become positive (Smith *et al.*, 1976). On the other hand, several studies have also demonstrated that infected rats were only detected on farms with *T. spiralis* positive pigs and low sanitation or formerly with high prevalence and low sanitation (Hanbury *et al.*, 1986; Schad *et al.*, 1987; Stojcevic *et al.*, 2004). A longitudinal investigation, using tracer pigs, was performed to determine whether rodents and/or other wild animals were involved in transmission of *T. spiralis* on the pig farm. Tracer pigs exposed to rodents and wild animals did not become infected with *T. spiralis*. Meanwhile, none of the rodents trapped and examined were found to be infected (Hanbury *et al.*, 1986).

Another study explored the prevalence of *Trichinella* infection in rats in two endemic villages. Rats were collected on 60 farms with different levels of sanitation and with, or without, *T. spiralis*-infected pigs. Infected rats were detected only on farms with *T. spiralis*-positive pigs and low sanitation or formerly with low sanitation but not on farms with *T. spiralis*-negative pigs (Stojcevic *et al.*, 2004).

1.4.4 Trichinellosis in dogs and cats

In some areas of the world meat from dogs and cats is consumed and there are also several reports of human trichinellosis caused by ingestion of dog meat (Ozeretskoykaya *et al.*, 2005; Wang *et al.*, 2007). In particular areas, dog carcasses left on the ground are the source of *Trichinella* transmission to wildlife (Kapel, 1997). Oivanen *et al.* (2005) examined the prevalence of *Trichinella* infection in domestic dogs in Finland. Altogether 727 dog serum samples were tested serologically by ELISA with ES-antigen, 7.3% of dogs were classified positive for trichinellosis. A study in Greenland examined the prevalence of *Trichinella* infection in sled dogs by the digestion method. About 11.3% of dogs were infected with *Trichinella* (Moller, 2007). In China the prevalence of trichinellosis in dogs slaughtered in abattoirs was 16.2% (5654/34,983) (Wang *et al.*, 2007). The infection rates ranged from 1.2% to 44.8%, with the highest prevalence located in northeast China. The prevalence in dog meat sold at markets in five provinces or autonomous regions was 3.5% (988/27,898). *Trichinella* infections in cats were examined using the digestion method. The infection were found in 10 provinces/autonomous regions/municipals with infection rates ranging from 1.6% to 87.5% (Wang *et al.*, 2007). In Lithuania *Trichinella* larvae were identified from muscle samples of four out the 86 (4.6%) stray cats (Senutaite & Grikieniene, 2001).

1.5. Epidemiology of trichinellosis

Human trichinellosis cases have been documented in 55 (27.8%) countries worldwide (Pozio, 2007b). The presence of *Trichinella* in wild and/or domestic animals is not always linked to human infection; with raw meat eating habits playing an important role in the transmission.

The distribution of current human trichinellosis in different regions of the world has been reviewed (Murrell & Pozio, 2000). Since the 1980s, Eastern Europe has experienced a marked increase of trichinellosis compared with other regions. For example, in Romania the incidence of human trichinellosis has increased 17-fold and the prevalence of trichinellosis in pigs has increased by 50%. The high prevalence of trichinellosis in pigs and wild animals in Eastern Europe has resulted in important human trichinellosis outbreaks in other part of Europe (Murrell & Pozio, 2000).

The USA has experienced both a rising importance of wild animals as sources of human trichinellosis and a dramatic decrease of trichinellosis prevalence in domestic pigs. In Canada the highest prevalence of human trichinellosis occurs in the eastern arctic, Northern Quebec and the Rocky Mountain regions of British Columbia and Alberta, some 72% of the reported human cases originating in the Northwest Territories and Quebec where the infection rate was 200 times the national average. Trichinellosis is endemic in Mexico and Argentina where the prevalence of trichinellosis was high in backyard pigs and pigs from small farms (Murrell & Pozio, 2000).

Increase in cases of trichinellosis has been reported from eastern and western Asia. Lebanon reported two large outbreaks of human trichinellosis. China has shown an increasing trend of trichinellosis due to socio-cultural changes from rising affluence. In Southeast Asia outbreaks of human trichinellosis have been recorded in Thailand.

Trichinellosis probably occurs in other countries but there was no official reports (Murrell & Pozio, 2000).

In several countries where human trichinellosis has been reported, infections occur only among ethnic minorities and tourists. The native inhabitants do not consume uncooked meat or meat of some animal species (Pozio, 2007). For example, data from the National System of Epidemiological Surveillance indicated that most of the reported cases (91%) in Argentina were registered in only three provinces (Buenos Aires, Cordoba and Santa Fe), suggesting human trichinellosis was focussed in the central area of the country (Bolpe & Boffi, 2001). In China foci of trichinellosis are located in the South-eastern, the Central and Northeastern Regions (Wang & Cui, 2001a). A survey in rural communities in Papua New Guinea found that the sero-prevalence of human trichinellosis was substantially higher in the villages nearest to the hunting areas where the wild pigs are infected with *T. papuae* (Owen *et al.*, 2005).

1.6. Human behaviour influencing the occurrence of trichinellosis

Murrell and Pozio (2000) reviewed the epidemiology of trichinosis worldwide and mentioned several factors, derived from human behaviour and activities. According to Murrell and Pozio (2000), humans are involved trichinellosis in several ways. There are the misperceptions that the pig is the main source of human trichinellosis or that trichinellosis is not a problem in today's pig production. This has led to the role of the other animals as source of human infections being over-looked and to a relaxation in meat inspection for *Trichinella*. The improvement in immunodiagnostic tools has resulted in better recognition of the infection in humans and animals. The global increase in animals and meat has transferred *Trichinella* from endemic to non-endemic regions. Changing livestock production practices, the increasing number of outdoor pig

farms and the increased use of animal protein in horse feed have increased the risk of *Trichinella* transmission to livestock. Migrating humans brought their high-risk food habits and animal rearing practices to other countries. Lastly, the practices of home slaughtering pigs, increased consumption of game meat, increased numbers of farms breeding wild animals for recreational hunting and increased number of hunters and their tendency to leave game carcasses in the field have resulted in the transmission of *Trichinella* to humans, domestic animals and wildlife.

1.7. Research methodologies

Baum (1995) mentioned that epidemiological methods have been seen as the gold standard for investigating public health problem. This is also true for research about trichinellosis in which the majority of previous research has been based on quantitative approaches using epidemiological study. Thrusfield (2007) has described four main types of epidemiological study: experimental studies; cross-sectional studies; case-control studies; and cohort studies. In experimental studies the subjects are randomly allocated to various groups, according to factors assign by the investigator. An example is a study to compare the efficacy and tolerability of two anthelmintic therapies in trichinellosis patients in France. The 46 patients were allocated into two groups, the first 26 patients received thiabendazole therapy and the next 18 received albendazole therapy (Cabie *et al.*, 1996). Cross-sectional studies are the studies that investigate relationships between disease and risk factors in a specific population. Many investigations used cross-sectional studies to determine prevalence and identify risk factors of trichinellosis both in humans and animals. Moller (2007) studied the status of *Trichinella* infections in humans, sled dogs and seals in Greenland. De-la-Rosa *et al.* (1998) investigated the presence of antibodies against *Trichinella spiralis* in humans in a semi-rural county of Mexico and the association of

antibodies to several social, hygienic and dietary factors. A study in the adult population of Nunavik aimed to detect antibodies against *Trichinella* sp., *Toxoplasma gondii*, *Toxocara canis*, *Echinococcus granulosus*, *Leptospira* sp., *Coxiella burnetii*, *Brucella* sp. and *Francisella tularensis*. The study also investigated the association between socio-demographic characteristics, domestic environment, and nutrition and the above mentioned diseases or infection (Lévesque *et al.*, 2007). Gamble *et al.* (1999) studied to determine *Trichinella* infection in a selected group of farm raised pigs in New England and New Jersey, USA, and to determine risk factors for the presence of *Trichinella*-seropositive pigs. In Germany Wacker *et al.* (1999) investigated seroprevalence and prevalence of *Trichinella* infection in foxes. Case-control studies compare a group of diseased subjects with a group of non-diseased subjects with respect to exposure to risk factors. A study in Laos investigated the possible occurrence of vertical transmission of *Trichinella* in humans using a case-control study. Cases were eight pregnant women who have been confirmed positive for trichinellosis and their children and controls are trichinellosis-unexposed pregnant women and their children. Prevalence of *Trichinella*-specific IgG antibodies in children from both group of women was compared (Taybouavone *et al.*, 2009). Cohort studies compared a group exposed to factors with a group not exposed to the factors with respect to the development of a disease. In Croatia, Venus *et al.* (2008) investigated the role of pest control as a preventive measure in the control of trichinosis using a cohort study. In this study the incidence of trichinosis was compared between domestic pigs from the County areas exposed to regular pest control measures with domestic pigs from County areas where pest control measures were not or were occasionally performed.

Qualitative research has gained recognition in the investigation for public health problems in recent decades (Baum, 1995). This is because it is

becoming obvious that health of people is not only related to an absence of disease but also to complex issues such as social, economic, political and environmental factors. Qualitative research offers several tools for investigating these issues which are beyond the scope of quantitative research (Baum, 1995). Several methods are named in qualitative research, to name a few, ground theory, phenomenology, ethnography and case study. Patton (1990) has grouped these methods, according to the way they utilized qualitative data, into three types: in-depth, open-ended interviews with individual or groups; direct observation on people's activities, and behaviour; and written data such as clinical or programme records, personal dairies, official reports or publication, and open-ended written responses to questionnaire survey. In terms of data collection in a qualitative research, three main methods have been addressed (Patton, 1990): observation, in-depth interview and focus group interview. Observation method refers to a method of directly observing operations and activities. This method provides opportunity to collect a wide range of data of individual's or individuals' behaviour. It also allows the researcher to learn about the actual behaviour that participants may be unaware of or that they are unwilling or unable to discuss in an interview or focus group. In-depth interview is a conversation between a skilled interviewer and an interviewee that aims to elicit rich, detailed material that can be used in analysis. Focus groups combine elements of interview and observation. The group interactions in a focus group generate data and insights that would be unlikely to emerge without the interaction found in a group. The technique allows observation of group dynamics, discussion, as well as the behaviour and attitudes of participants.

The methods mentioned above have been widely used to investigate public health problems. However, none of which have been documented to have been used with an emphasis on issues specific to trichinellosis. For example, experts were consulted by means of in-depth interview

coupled with self-administered questionnaire to investigate the question ‘what are the risk of infectious diseases of animals (wildlife, livestock, and domestic animals) for human public health in Europe’ (van der Giessen *et al.*, 2004). Ellis and Butow (1998) examined knowledge of and attitudes towards randomized clinical trials by focus group discussion. In this study 21 mothers or grandmothers of children attending a local primary school and 20 breast cancer patients identified from the medical records were participated. One of an examples use of the observation method in qualitative research was conducted by a research team from the North Carolina BEAUTY and Health Project in USA (Solomon *et al.*, 2004). The team conducted an observational study in 10 North Carolina beauty salons to gain insight into naturally occurring conversations between cosmetologists and customers, and to assess features of the salon environment that might be used to inform the development of salon-based health promotion interventions.

The research designs and strategies of inquiries for this current study were decided based on the consideration that both the risks for disease maintenance and transmission and the people’s perception to the risk of diseases as well as to prevent the disease are to be identified and understood for the promotion of appropriate sustainable preventive measures. Thus, the research employed both quantitative and qualitative approaches. Quantitative research to investigate disease prevalence and risk factors of trichinellosis used epidemiological study: cross-sectional study (1.7.1) and case-control study (1.7.2). Qualitative research to investigate people’s perception to the risk of diseases as well as to prevent the disease used a qualitative study method, a focus groups interview (1.7.3).

1.7.1 Cross-sectional survey

A cross-sectional survey is a survey of a population at a single point in time (Jekel *et al.*, 2007). It involves the selection of a sample of individuals from a larger population. Each individual is determined simultaneously for the presence or absence of disease and the hypothesized risk factor (Thrusfield, 2007). It is considered the best way to determine prevalence and it is useful at identifying associations (Mann, 2003). This study method has the advantages of being quick, cheap and easy to perform (Mann, 2003; Jekel *et al.*, 2007). Only one group of subjects is used, data are collected only once and multiple outcomes can be studied (Mann, 2003). It is also useful for measuring health status and planning for health services (Majeed *et al.*, 2000). A cross-sectional survey can be taken to determine the knowledge, attitudes and health practices of population regarding particular diseases e.g. rabies (Matibag *et al.*, 2007), HIV/AIDS (Bui *et al.*, 2001) or other health related problems (Chang, 2006).

In a cross-sectional survey data about the exposure to risk factors and the presence or absence of disease are collected simultaneously, thus it does not offer evidence of a temporal relationship between risk factors and disease. This is a major disadvantage of this study method. Another disadvantage is that chronic diseases are more likely to be found by a survey than severe diseases. This is because people live longer with chronic diseases while people with severe diseases may die before they can be surveyed (Jekel *et al.*, 2007).

1.7.2 Case-control study

A case-control study is designed to help determine if an exposure is associated with an outcome e.g. disease or condition of interest (Lewallen & Courtright, 1998). In contrast with cross-sectional study, case-control study is usually retrospective (Mann, 2003). In a case-control study, the cases are a group known to have the outcome and the controls are a group known to be free of the outcome. The study determines the relative importance of any exposures in relation to the presence or absence of the disease (Mann, 2003). Case-control studies look back in time to learn the exposures history in the cases and the controls and compares the frequency of the exposure in the case group to the control group (Lewallen & Courtright, 1998).

The advantages of case-control studies are that they can obtain findings quickly and in an inexpensive way, as only a few subjects are required, it is an efficient study for rare diseases because the study require smaller sample size compare with other types of study and it can study multiple exposures (Lewallen & Courtright, 1998). There are, however, some problems associated with case-control studies, for example it is difficult if record keeping is either inadequate or unreliable and selection of control groups can also be difficult (Lewallen & Courtright, 1998; Thrusfield, 2007). Case-control studies rely on recall or records for information on past exposure (Thrusfield, 2007) and this can lead to bias. Two major forms commonly encountered of bias in case-control studies are sampling bias and observation and recall bias (Mann, 2003). Mann (2003) suggests overcoming the sampling bias by randomly selecting cases from all of the patients with disease. To select controls to be representative of the same population as cases, four techniques are suggested:

1. Sample controls the same way as the cases, for example, from the same hospital.

2. Select match or un-matched controls from the unaffected population. Over-matching may occur if the controls are closely matched to the cases. They may not represent the general population and this problem causes the true differences to be underestimated.
3. Use more than one control group.
4. Use a population-based sample for both cases and controls.

Recall bias can be overcome by using recorded data. The success of this strategy is limited by the availability and reliability of the data collected. The use of the blinding technique is suggested to overcome the problem of observer bias. This means participants and observer are not aware of their status and the study hypothesis. This technique is impractical when cases present with physical signs (Mann, 2003).

1.7.3 Focus groups interview

Focus groups are one of the main methods used in a qualitative research (McKinlay, 1993; Pope & Mays, 1995). It is defined as a method of group interview which explicitly includes and uses the group interaction to generate data (Kitzinger, 1995; Pope & Mays, 1995). People are encouraged to respond to the researchers question by talking to one another instead of responding individually. The idea behind this method is that group processes can help people to explore and clarify their views in ways that would be less easily accessible than in a one-to-one interview (Kitzinger, 1995).

Focus groups have advantages for researchers because they do not discriminate against people who cannot read or write. They can encourage participation from people reluctant to be interviewed on their own or who feel they have nothing to say (Kitzinger, 1995). People's knowledge and attitudes are not entirely enclosed in reasoned responses

to direct questions, in this sense focus groups can reveal the issues of what people know or experience that could not be obtained through other conventional data collection methods. Morgan (1993) says

“Focus groups are useful when it comes to investigating what participants think, but they excel at uncovering why participants think as they do”(Morgan, 1993).

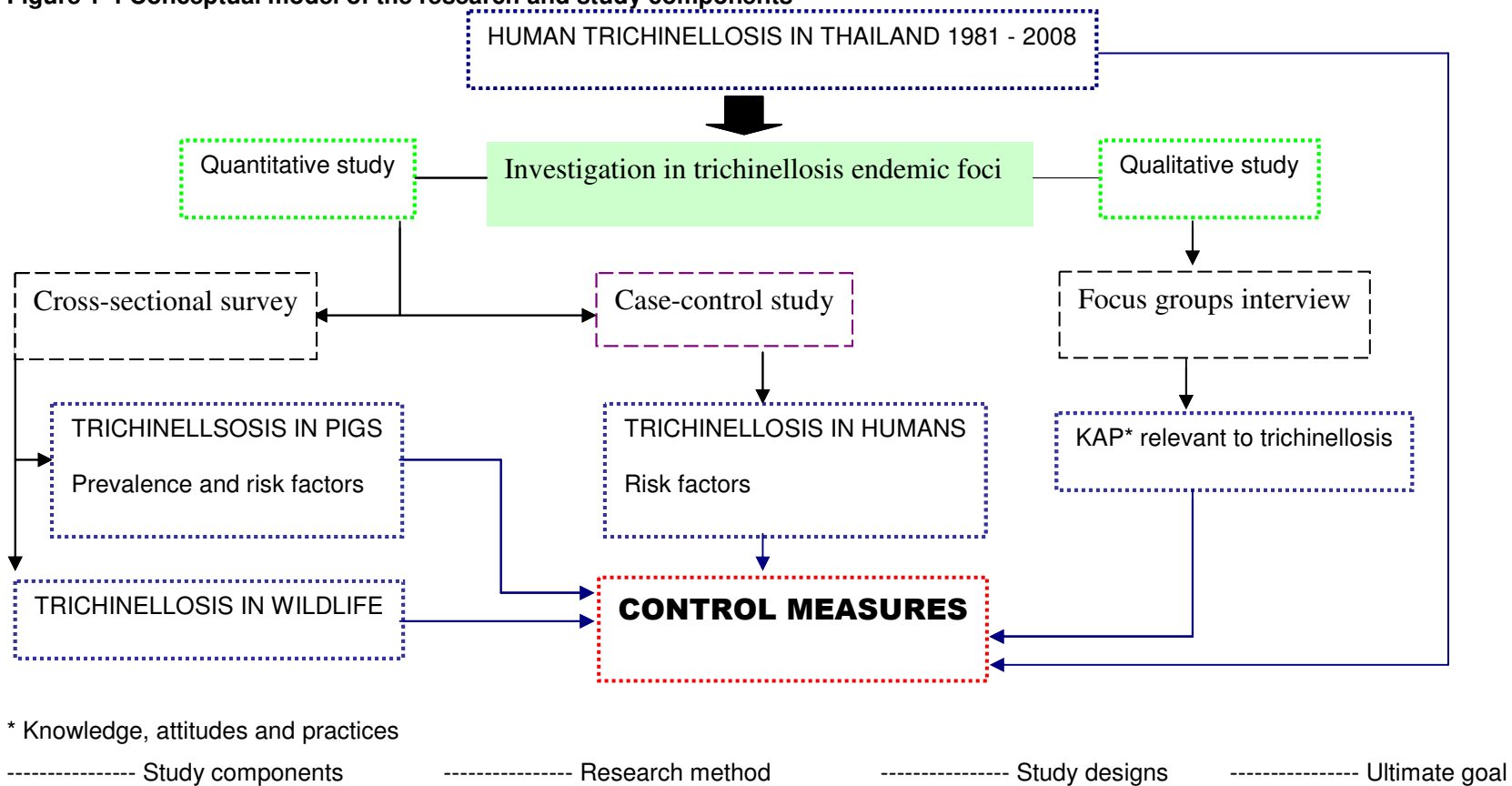
Another advantage for focus groups is that through interpersonal communication some cultural values or group norms could be identified and this makes focus groups useful in cross cultural research and work with ethnic minorities (Kitzinger, 1995).

Although the focus group method has many advantages, as with all research methods there are limitations. The pitfall of focus group method is that it is not useful for hypothesis testing in the traditional experimental design nor does it is appropriate for drawing inferences about large populations (Basch, 1987). The expression of group norms may silence individuals with a different voice (Kitzinger, 1995). Morgan (1997) points out that it is not appropriate for the use of focus groups with a small and unrepresentative samples as the only evidence to support a decision. In focus group the researcher has less control over the data produced than in one-to-one interview or quantitative studies (Gibbs, 1997). Gibbs (1997) also points out that it should not be assumed that the individuals in a focus group are expressing their own definitive individual view. They are speaking in a specific context, within a specific culture, and so sometimes it may be difficult for the researcher to identify an individual message. Additionally focus group can be difficult to assemble. It may not be easy to get a representative sample and focus group may discourage certain group from participation for example those who are not confident or those who have communication problems (Gibbs, 1997).

1.8. Thesis structure

In this thesis, background information on the study areas and the study methods used to collect the data are incorporated in the following four data chapters. The first data chapter (Chapter 2) gives a profile of Thailand, its characteristics that are relevant to *Trichinella* transmission and the infrastructure that is in place for the control of trichinellosis. Analysis is then carried out on human trichinellosis surveillance data collected between 1981 and 2008 in Thailand in order to characterise the epidemiological characteristics of human trichinellosis over this time period. Chapter 3 aims to characterise factors influencing trichinellosis with emphasis on domestic pigs in trichinellosis endemic foci. This chapter includes characteristics of Nan Province, the study area under investigation in northern Thailand, followed by an investigation to characterise the nature of pig holdings, estimate the sero-prevalence of trichinellosis in pigs, characterise sero-positive pigs, identify the risk factors that lead to trichinellosis in pigs, and estimate the prevalence of trichinellosis in wild animals caught for human consumption. Chapter 4 incorporates a case-control study to explore the risk factors for human trichinellosis and the changes in the trichinellosis risk-associated behaviour of individuals after experiencing the infection. In Chapter 5, information on the knowledge, attitude and practices relevant to trichinellosis are explored in various ethnic communities in Nan Province to expand on the information about the factors influencing trichinellosis. Finally, all data chapters are drawn together in Chapter 6 to make recommendations for future preventive measures in trichinellosis endemic areas in Thailand.

Figure 1-4 Conceptual model of the research and study components



2. Chapter 2- Human trichinellosis in Thailand

2.1. Study Aims

This study analyses the human trichinellosis surveillance data collected between 1981 and 2008 in Thailand in order to characterise the epidemiological characteristics of human trichinellosis over this period.

2.2. Introduction

Trichinellosis was first recognised in Thailand in 1962 when the outbreak occurred in a village in Mae Hong Son Province, Northern Region. Fifty six patients were observed, of which 11 proved fatal (Menakanit *et al.*, 1962; Boonthanom & Nawarat, 1963). Following that first reported outbreak, trichinellosis has continued to occur (Dissamarn & Indrakamhang, 1985). It was addressed as public health problem and preventive measures have been implemented. Trichinellosis has also been included in the national disease surveillance system.

Public health surveillance is normally undertaken for three primary purposes (Anon, 1992). Firstly, it is used to explore the ongoing pattern of disease occurrence and the potential for disease occurrence within a population. Secondly, surveillance provides knowledge about the natural history, clinical spectrum, and epidemiology of a disease, which may lead to the development of prevention and control measures. Lastly, it provides baseline data that can be used to assess prevention and control measures. Surveillance data can be used to monitor health events and provide linkage to prevention and control programmes. Surveillance information is analyzed by time, place, and person. The analysis of surveillance data begins by characterizing the pattern of disease reports by person, place, and time. Surveillance data can then be analysed to detect unusual variations or test a hypothesis.

Routine national surveillance for human trichinellosis surveillance has been undertaken in many countries worldwide, including in USA, Canada, Germany, France, Romania, Russia and Thailand. Information from surveillance studies has shown that epidemiological characteristics of trichinellosis are diverse both within and between countries (Hinz, 1991; Anon, 1999; Appleyard & Gajadhar, 2000; Roy *et al.*, 2003; Ozeretskovskaya *et al.*, 2005; Blaga *et al.*, 2007). Surveillance may detect a decreasing or increasing incidence caused by factors such as changes in practices in pig husbandry (Roy *et al.*, 2003), political climate (Blaga *et al.*, 2007), socio-economic changes (Ozeretskovskaya *et al.*, 2005) and changes from control measure implementation (Hinz, 1991). Surveillance may also play a key role in the design of control measures (Proulx *et al.*, 2002).

Surveillance data related to human trichinellosis cases in Thailand have been analysed periodically (Khamboonruang, 1991; Kaewpitoon *et al.*, 2006) and there have also been numerous outbreak investigations reported in the literature (Doege *et al.*, 1969; Suriyanon & Klunklin, 1972; Limsuwan & Siriprasert, 1994; Nontasut *et al.*, 1999). These studies have suggested non-random spatial patterns of trichinellosis and the association of trichinellosis with social gathering events. For example, Takahashi *et al.* (2000), in a review of trichinellosis status in Asia and the Pacific Rim, mentioned that trichinellosis in Thailand occurred most frequently in the provinces next to Laos, Myanmar and Cambodia. These are remote and highland areas where free-ranging pigs are raised and where local trade between countries occurs in both domestic and wild animals (Takahashi *et al.*, 2000). Khamboonruang (1991) reported that in Thailand most trichinellosis occurred in rural areas and were linked to food served during festivities such as Thai New Year, wedding ceremonies and other occasions.

Surveillance data up to the year 2008 showed that trichinellosis has continued to occur in Thailand. For this reason, the study in this chapter retrospectively investigated trichinellosis in humans in Thailand from surveillance data between 1981 to 2008. Herein, the ecosystem and human behaviour related to trichinellosis are introduced. Following this, public health and veterinary services and other factors involved in the control of trichinellosis, such as the food-safety policy and the production systems for the domestic pigs are described.

2.2.1 Profile of Thailand

Thailand is a member of the Southeast Asian Nations, which includes 11 countries: Brunei, Cambodia, East Timor, Indonesia, Laos, Malaysia, Myanmar (Burma), The Philippines, Singapore, Thailand and Vietnam (Figure 2-1).

2.2.1.1. Location

Thailand is located in the middle of the Indochinese Peninsular lying between latitudes 5°37' and 20° 27' N, longitudes 97°22' and 105°37' E, covering an area of approximately 513,115 km². About 84% of the area falls within the mainland section and the rest (16%) falls within the Peninsular South. Twenty three of its 76 provinces have access to the coastline (Arbhabhirama *et al.*, 1988). Land boundaries are shared with Myanmar to the west and northwest, Laos to the north and northeast, Cambodia to the east, and Malaysia to the south (Figure 2-1). Twenty-nine Thai provinces have an international boundary.

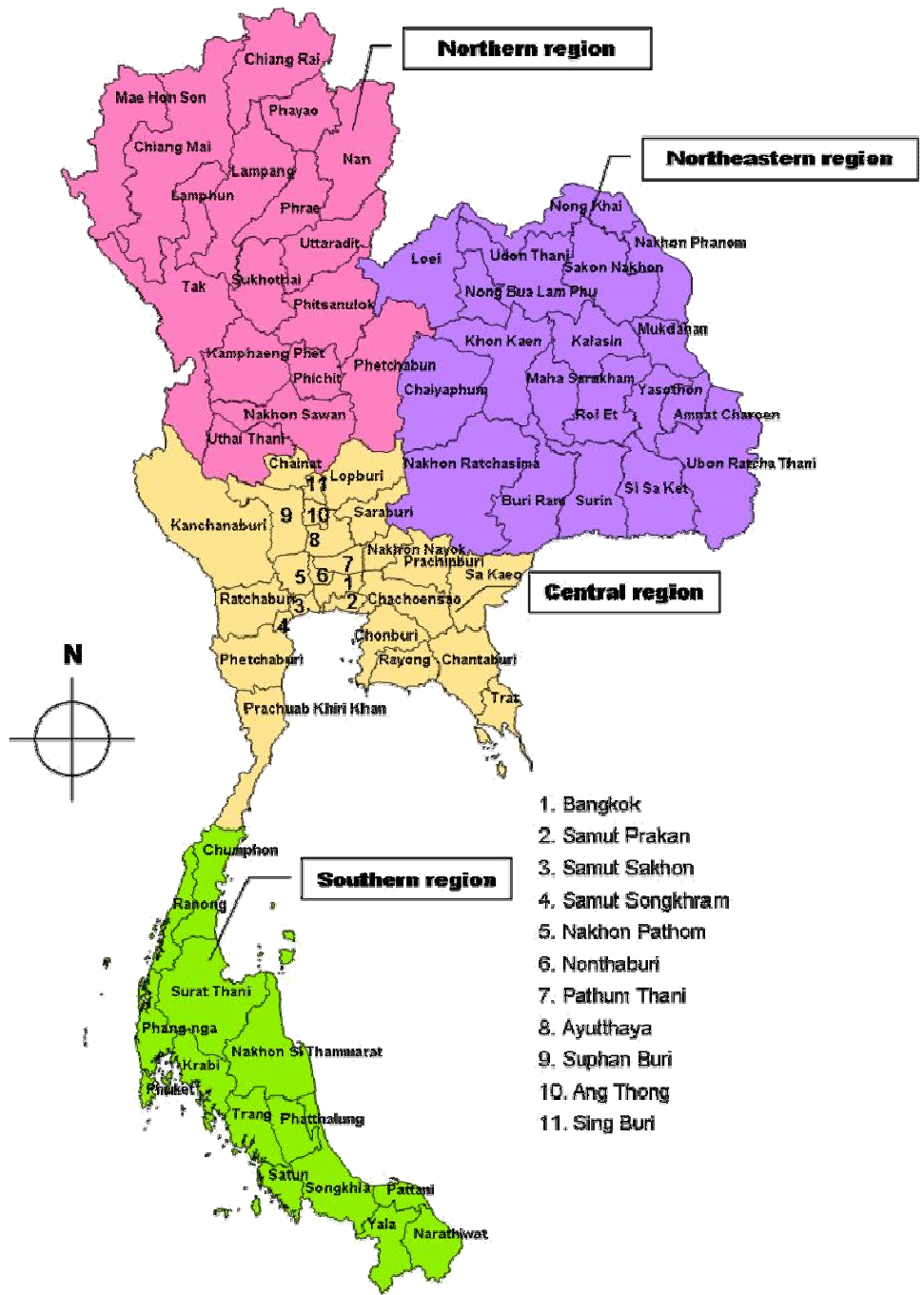
Figure 2-1 Map of Thailand and adjacent Southeast Asian Countries



Source: <http://www.nationsonline.org>

Thailand can be roughly divided into four geographical regions: the northern mountain ranges and valleys, the northeast plateau, the central plain, and the peninsular south (Blanchard, 1958). Two additional regions may, with more refined differentiation be added. These are the western mountains and the east coast (Arbhabhrama *et al.*, 1988). Using the climatic patterns and meteorological conditions as the criteria Thailand can be divided into five parts: northern, north-eastern, central, eastern and southern parts (Thai Meteorological Department, 2005). The National Statistical Office of Thailand has divided Thailand into four regions: north, northeast, central and south, as shown in the map (Figure 2-2), and these are the regions referred to in this study, unless otherwise stated.

Figure 2-2 Map of Thailand with four regional divisions and provinces



2.2.1.2. Administrative system

The structure of the national administrative system of Thailand consists of the central administration, the provincial administrations and the local administrations.

The central administrative system, according to the Reorganization of Ministries and Departments Act of B.E. 2545 (2002), consisting of 20 ministries. The provincial governmental functions as various ministries and departments as delegated to the provincial level, under the supervision of the provincial governor with assigned officials from various central administrative agencies. The provincial administration consists of 75 provinces (Changwat), 795 districts (Amphoe) and 81 sub-districts (King-Amphoe). The districts and sub-districts both comprise of Tambons. Each Tambon contains an average of 10 villages (Moo-Ban). The village is the smallest administrative area in Thailand. For the local administrative system, the local administration involves the autonomous administrative authority of the people in each administrative locality, under the law. Four types of local administrative bodies in Thailand exist, Provincial Administration Organizations (75), Municipalities (1,129), and other types of local authorities as designated by law, for example Bangkok Metropolitan Administration (n = 1), Pattaya City (n = 1), and Tambon Administration Organizations (n = 6,742) (National Statistical Office, 2000).

Although the public administration system of the country is highly centralized, the development policy and planning in Thailand is a combination of top-down and bottom-up approaches. The capacity of the local government has gradually strengthened since the 5th National Economic and Social Development Plan (NESDB, 2006).

Separation between urban and rural areas in Thailand is based on its administrative area character. Urban includes entire area of Bangkok and

municipalities, the rest are regarded as rural areas (National Statistical Office, 2000).

2.2.2 Characteristics of Thai ecosystem and people relevant to *Trichinella* transmission

2.2.2.1. Climatic, flora and fauna

The tropical climate of Thailand provides mild temperature across the year with, high humidity and rainfall, that support a biologically diverse flora and fauna. The climate of Thailand is divided into three seasons in the upper mainland section, and two seasons, the summer and the rainy season, in the lower mainland section and the Peninsular South (Arbhabhira *et al.*, 1988). The rainy or southwest monsoon season from mid-May to mid-October is the wet period with an abundance of rain occurring over the country. The winter or northeast monsoon season is the period from mid-October to mid-February with a mild temperature particularly between December and January in upper Thailand, and with a great amount of rainfall in southern Thailand on the east coast. The summer or pre-monsoon season from mid-February to mid-May is the transitional period from the northeast to southwest monsoons. The mean temperature all year round is about 23.1 – 29.6°C. Most areas of the country receive 1,200 – 1,600 mm. amount of annual rainfall (Thai Meteorological Department, 2005).

Forest covers about 158,652 km², or 31% of the country's area. The greatest proportion of forest is in the Northern Region, accounting for 52% of regional area and over a half of Thailand forest area (National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Thailand, 2006). Thailand has 15,000 species of plants that live in more than 10 different types of forest

habitats, ranging from tropical evergreen forest to mangrove, making up about 8% of the flora species found on Earth. In terms of natural fauna, Thai mammals number about 265 species (Lekagul & McNeely, 1977), of which there are about 70 species of rodents, 36 species of carnivores, 18 species of ungulates and 13 species of primates. Approximately 900 species of birds occur in Thailand, of which 578 are resident forest species (Round, 1985). There are also at least 300 species of reptiles from three orders and 23 families and 100 species of amphibians (Thirakhupt, 2000). Although, the overall wildlife population in Thailand has not been well estimated, their abundance can be assumed especially in the period prior to the 1960s, before a rapidly expanding human population and rapid deforestation. Pendleton (1962) described the abundance of carnivore predators in the forest of the northern and north-eastern Thailand in the past with the following phrases “*in Northern Thailand and Khorat (Northeastern), the farmer, where possible, does not go into the forest without his dog, so that if he meets a tiger, the dog will be attacked first.....the southern edge of Khorat there are settlements in which there is not a single dog: all have been killed by wild animals*”. Between 1987 and 1989, at least 26 carnivore species were found in the forests of Thailand, consisting of those in five families: Canidae, Felidae, Viverridae, Mustalidae and Ursidae (Rabinowitz & Walker, 1991). Srikosamatara (1993) has studied the density and biomass of large herbivores and mammals in a dry tropical forest in western Thailand and estimated the population of sambar deer (1.9 – 4.2 per km²), barking deer (3.1 per km²) and wild boar (<0.5 per km²) (Srikosamatara, 1993).

Sylvatic cycle of trichinellosis in Thailand can be maintained separately from domestic cycle in the Thai natural system. The degree of the interaction of wild and domestic animals between the forest and human habitation interface is theoretically limited by law enacted to protect Thai flora and fauna starting in the 1960s. In 1960, the government enacted

the Wild Animals Reservation and Protection Act, establishing hunting limitations, capture and trade restrictions and making provision for the wildlife areas. In 1962, the National Park Act was passed. The national parks and wildlife sanctuaries encompass about 4.3 million hectares or 8.5 % of Thailand. However, there are still problems of encroachment, illegal sport hunting and poaching, illegal logging and the harvesting of forest products, encroachment for agriculture and for the development of resorts and estates. Forest clearances for farmland have occurred. These human activities inevitably break down the natural barrier of the wild and domestic habitats and enable the inter-transmission of *Trichinella* between wildlife and domestic animals.

2.2.2.2. People, socio-economic and culture

Until the 1960s, Thailand's economy depended heavily on agriculture and most of the workforce was agricultural. Since the 1960s, Thailand has promoted industry and by the end of the 1980s, Thailand was transformed from an agricultural to an industrial and service-based economy. The Thai economic system began to shift to the economic development era with National Economic and Social Development Plans, from the 1st plan (1961 – 1966) through the current 10th plan (2007 – 2011) (NESDB, 2006). The output of non-agricultural sectors has exceeded that of the agriculture sector since 1985 (Phananirami, 1995). Although agriculture continued to grow, it could not compete with non-agricultural sectors, which grew even faster. Agriculture's share of GDP fell from 37 % in 1961 to 13 % in 1991 (Yamada, 1998). Nonetheless, agriculture still employs the majority of the labour force. For instance, the share of employment in agriculture, manufacturing and service sectors in 1992 were 61.04, 15.15 and 23.77 %, respectively (Phananirami, 1995).

In the agricultural census year 2003, there were about 5.8 million agricultural holdings in Thailand, accounting for 114.5 million rai (1 rai = 0.16 ha or 1,600 m²), proportionate to 35.7% of country areas. Seventy-four percent of agriculture holdings own tenure. The land use in agriculture activities comprising; rice (52.9%), para-rubber (8.9%), permanent crops (10.5%), field crop (18.5%), vegetable crops, herb, flowers and ornamental plant (1.4%), planted-forest (0.6%), pasture (1.0%), pen (0.9%), freshwater culture (1.1%) and other (4.2%). About 20.7% of holdings obtained household income from agriculture only, 79.3% receive income from agriculture and other sources(National Statistical Office, 2003) .

The overall Thai population in 2000 was 60,606,947, about 7.4 and 2.3 times higher than that in 1909 and 1960, respectively (National Statistical Office, 2000). The average sex ratio of the population overall was 97 male to 100 female. About two thirds of the population (69%) lived in rural areas while the rest were in urban areas. Of the overall 2000 Thai population, 18.8% were in the Northern Region, 34.2% in the Northeastern Region, 33.7% in the Central Region (10.4% in Bangkok Metropolis) and 13.3 % in the Southern Region (National Statistical Office, 2000). Extensive family planning programmes during the past two decades have reduced the population annual growth rate from 2.7 % in 1970 to 1.05 % in 2000. Life expectancy increased from 58.0 to 63.8 years for males and from 69.9 to 74.9 years for females during the period 1974 – 1996 (National Statistical Office, 1996). In the year 2000, 90.8 percent of Thais over six years old are literate. The literacy rate was 96.2 % in men and 89.2 % in women, 82.1 % among people 60 – 64 years old and 39.4 % among people over 85 years old. The overall literacy rate was slightly higher in the urban areas (93.9%) than in the rural areas (89.4%) (National Statistical Office, 2000).

In 1994, about 12.4 million Thais lived under the poverty line, by 1996 this number had decreased to 9.8 million. The Asian Economic Crisis in 1997 resulted in a rising number of the poor to 11.0 million in 1998 and 12.8 million in 2002. The economic recovery in later years reduced the number of the poor to 9.5 million, 7.1 million and 6.1 million in 2002, 2004 and 2006, respectively (National Statistical Office, 2008).

An average monthly income and expenditure per capita was substantially different between people living in Bangkok and its vicinity compared to people living in the regions. The lowest average monthly income and expenditure per capita was observed in the Northeastern Region (National Statistical Office, 2008). Problems and constraints at the village-level that required government interventions are being collected annually. The most common problem across the nation indicated largely dependent of the Thais in agriculture, for example, in 2001 the most important problem was the price of agriculture products (54.4%). Other problems included insufficient funds, price of fertiliser and pesticide, unemployment, drugs, transportation, flooding, no agriculture land tenure, drought and plant diseases.

Thai population made up of people from various ethnic backgrounds who lived in the region particularly in the adjacent countries (Blanchard, 1958; Pendleton, 1962). The major religion in Thailand is Buddhism. Among the three religions most practised in Thailand, Buddhism accounted for 93.7%, Islam 5.4% and Christian 0.9% Muslims are concentrated in the five southernmost provinces closest to Malaysia. Significant numbers of previously animist groups, hill tribes, live in the highland areas⁸ (Department of Social Development and Welfare, 2002).

⁸ Highland area as is defined by Office of Registry, Department of Provincial Administration, Ministry of Interior, Thailand, means the area occupied by hill tribes or other ethnic minorities or the areas of habitation composing of over 35% of slope or locating above 500 metres over sea level. These areas are in 20 provinces as follows: Kanchanaburi, Kamphaeng Phet, Chiang Rai, Chiang Mai, Tak, Nan, Prachuab Khiri Khan, Phayao, Phitsanulok, Phetchaburi, Phetchabun, Phrae, Mae Hon Son, Ratchaburi, Loei, Lampang, Lamphun, Sukhothai, Suphan Buri and Uthai Thani.

The Thai hill tribes are at the centre of attention in relation to trichinellosis because their pigs are believed to be the major source of domestic trichinellosis in Thailand. Young (1962) has classified the Thai hill tribes into three groups based primarily on their linguistic roots: Tibetan-Burma (Akha, Lesu, Lahu, Karen), Yao-Meo-Pateng (Hmong, Yao), and Wa (Lua, Khmu). They can be categorized into two geographical groups: low hill (Karen, Lua, Khmu) and high hill (Hmong, Yao, Lahu, Lesu and Akha) (Saihoo, 1970). The high hill group engaging in high-altitude agriculture, migrated to Thailand from Myanmar, Laos and southern China within the last century. The low-hill group are considered to be indigenous to Thailand and the adjoining areas of Myanmar and Laos. There are nine hill tribe groups in Thailand, comprising Hmong, Yao, Lua, Khmu, Karen, Lahu, Lesu, Akha and Mlabri (Department of Social Development and Welfare, 2002). Thai hill tribes live in 3,429 villages of all 3,881 villages in highland areas in 20 provinces of Thailand, 14 provinces are in the Northern Region. The overall population of Thai hill tribes in 2002 was 923,257. The largest group is Karen (47.4%), followed by Hmong (16.7%) and Lahu (11.1%) (Department of Social Development and Welfare, 2002).

A traditional Thai meal consists of rice and small dishes of meat, fish and vegetables (Kosalwat, 2002). Between 1960 and 1995 the average daily intake of meat or poultry by Thai people has increased some four-fold from 17.5g to 71.4g, while, the average daily intake of fish and shellfish has not changed (remaining around 46g) (Anon, 1986, 1997). Thai people in different regions have speciality dishes particular to their own localities and environments. A common feature among them is that they are spicy/hot (Chu, 1968). The Office of the National Culture Commission described food on a regional basis as follows (Office of the National Culture Commission, 1996).

1. Northern region: A northern meal consists mainly of glutinous rice and various kinds of dips, curries, spicy pork sausage,

- fermented pickled ground pork (*nhaem*), dried meat, fried pork and vegetable. The favourite meats are pork and beef and insects.
2. Northeastern region: The north-eastern people prefer steamed glutinous rice to boiled rice. Spiced raw ground meat (*larb, luh*), papaya salad, roasted fish or chicken and fermented fish dip are the most common dishes taken with steamed glutinous rice. Along with the preferred domestic meats, beef, chicken and pork less conventional meats, including frog, snake and insects are eaten.
 3. Central region: Dishes favoured by the Thai people in the central plain are a mixture of those from all regions but eaten with boiled rice. An average evening meal may consist of three to five dishes of curries, soup and fried food.
 4. Southern region: Dishes from the south are spicier than those from other regions. Boiled rice is generally eaten with several curries and fresh vegetables. Seafood is often the main component of southern dishes.

Three of the local recipes of the North and Northeast Regions that contribute towards trichinellosis in Thailand are named *larb, luh*, and *nhaem*. *Larb* is made of ground raw meat mixed with spices, lemon juice and fish sauce. *Luh* is *larb* mixed with fresh blood. *Trichinella* larvae in both dishes are still active and can infect pigs (Dissamarn *et al.*, 1966). *Nhaem* is made of ground raw meat mixed with glutinous rice, salt, nitrate and spices. It is wrapped in banana leaves (or a plastic bag) and kept at room temperature for three to five days, allowing fermentation to take place. *Trichinella* larvae may live for up to 12 days in *nhaem* (Keittivuti, 1983). Any type of meat can be used to prepare these three dishes. They can be eaten cooked or completely uncooked, depending on the taste of the consumer. These recipes are common not only to Thais but also people in neighbouring countries as they have been associated with trichinellosis outbreaks among Southeast Asian immigrants (Thai,

Laotian, Cambodian and Vietnamese) to the United States and Canada (McAuley *et al.*, 1992).

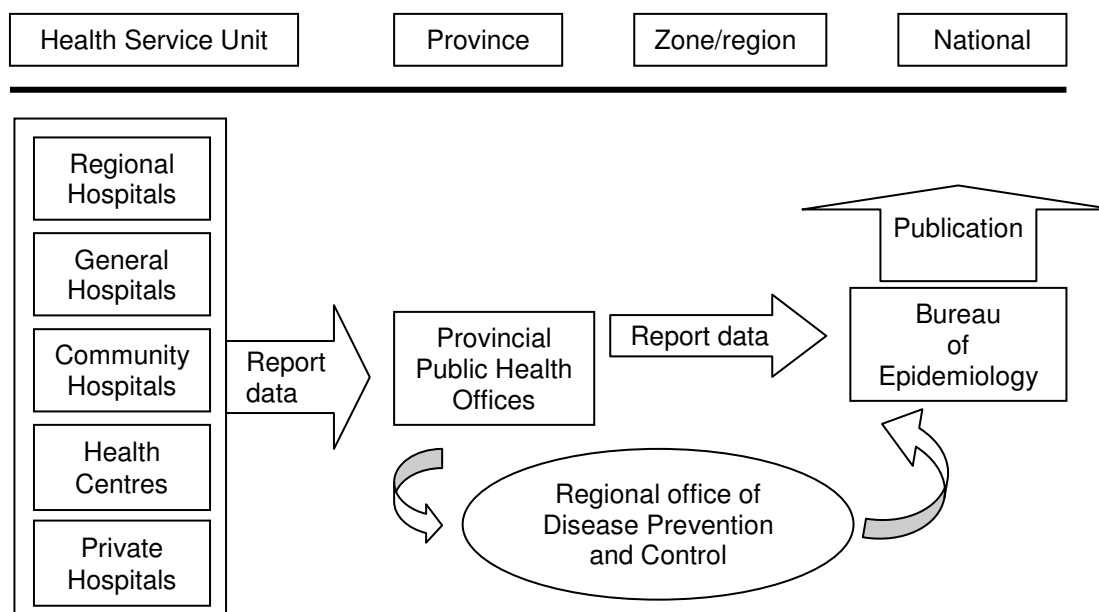
2.2.3 Public health

As a result of the National Economic and Social Development Plan, which was established in 1961, Thailand has a developed public health system and other public services. Currently, the country is in its tenth National Economic and Social Development Plan (2007 – 2011) (NESDB, 2006). The public health system has been improved significantly, (as have) the veterinary services. Every district has its own community hospital (Bureau of Policy and Strategy, 2006). Public health programmes and activities in Thailand are traditionally implemented by the public sector. The Ministry of Public Health (MoPH) serves as the prime agency responsible for the health of all Thais. This provides a base-line of care, but for more complex socially-related public health problems, the MoPH needs to draw on the services of collaborators. These are numerous and include relevant governmental agencies, private agencies and non-governmental organizations. For public health care, a universal coverage health care scheme was implemented in 2002. By 2003 it covered the majority of the whole nation, leaving only 5% of the total population without any health insurance coverage (Bureau of Policy and Strategy, 2006).

2.2.3.1. National Diseases Surveillance System

A National Disease Surveillance System was established in 1969 and currently covers 84 Reportable Diseases, including trichinellosis (Bureau of Epidemiology, 1981-2008). The epidemiological surveillance networking in Thailand comprises of all the related healthcare services in the communities and the Provincial Public Health Offices in the provinces. It utilises the Bureau of Epidemiology as the national reporting centre (Figure 2-3).

Figure 2-3 Schemology of the public health epidemiological surveillance network in Thailand



Source: Second FAO/WHO global forum of food safety regulator, Bangkok, Thailand, 12-14 October 2004

2.2.3.2. Public health status

In 1999, the major and increasing causes of death among Thai citizens were non-communicable diseases, accidents, and HIV/AIDS (Bureau of Policy and Strategy, 2006). The prevalence of communicable diseases, which used to be significant health problems, with the exception of the re-emerging diseases associated with HIV/AIDS such as tuberculosis were declining. The disease burdens to society in terms of disability-adjusted life years (DALYs) from non-communicable diseases were twice those from communicable diseases. In 1999 the breakdown DALYs on a percentages basis were 58.3% for non-communicable diseases, 27.7% for communicable diseases and 14.0% from accidents (Bureau of Policy and Strategy, 2006). Diarrhoea, the leading cause of mortality in Thai people up to the 1960s, decreased from 28.1 per 100,000 of the population in 1967 to less than 0.33 per 100,000 in 2002 (Bureau of Policy and Strategy, 2006).

2.2.3.3. Food-borne diseases

Thailand has encountered a wide range of food-borne diseases from the past until the present day. In the 1960s, diarrhoea was the fourth and enteritis/dysentery was the sixth principle causes of death in Thailand. Between 50 – 90 % of the population was infected with intestinal parasites in the middle of the 20th century (Blanchard, 1958), however, incidence of intestinal parasitic infections have declined (Figure 2-4). For diarrhoea, although the mortality rate has decreased significantly, the incidence rate is still higher than the target of not exceeding 1 episode/ person/year (Bureau of Policy and Strategy, 2006). Bad diet among the Thais is less a matter of poverty than of clinging to traditional habits (Chu, 1968). Thais in the Northeast and the Northern Regions, for example, eat raw fish. This is a source of the liver fluke (*Opisthorchis viverrini*) and a cause of opisthorchiasis, both being proven risk factors for cholangiocarcinoma among Thais (Sripa *et al.*, 2007). In 2006, the opisthorchiasis rates per 100,000 of the population were 0.90 in the North and 1.12 in the Northeast, over 15 times greater than that in the South and Central Regions (Bureau of Policy and Strategy, 2006) . Taeniasis was also common among the natives of the North and Northeastern Regions where the infection rate was even greater among those minority ethnic groups residing near international borders (Waikagul *et al.*, 2006).

Figure 2-4 Prevalence of specific helminthiasis in Thailand during 1981 – 2001

Helminthiasis	Prevalence (%)		
	1981	1991	2001
Hookworm disease	40.56	27.96	11.4
Ascariasis (round worm)	4.04	1.46	1.2
Trichuriasis (whipworm)	4.46	4.34	1.5
Liver fluke – whole country	14.7	15.2	9.6
- Northeast	34.6	24.01	15.7
- North	5.6	22.9	19.3

Source: Department of Disease Control, MoPH

2.2.3.4. Food safety policy

Food-borne diseases impose a substantial burden on the health care systems and markedly reduce the economic productivity of Thailand. Thus, food-borne diseases need to be addressed at the national level. Laws and regulations have given responsible organizations the authority to protect consumer health and to monitor and control all communicable diseases in Thailand. These include e.g. the Epizootic Diseases and Zoonoses Control Act B.E. 2499 (1956), the Food Act B.E. 2522 (1979) and the Communicable Diseases Act B.E. 2523 (1980). Through provisions under these laws there are two major responsible organizations for food safety and public health: the MoPH and the Ministry of Agriculture and Cooperatives (MoAC).

In 1999, the Department of Livestock Development (DLD), MoAC enacted a regulation establishing standards for swine, poultry, and cattle farms. The Farm Standard, which is based on good agricultural practice, is intended to improve the quality and safety of livestock products produced in Thailand. The products from Standard Farms are certified by the DLD ensuring that they are clean and safe when they reach consumers (FAO, 2003). Later, the standard criteria of good management practices for pig abattoir⁹ following the Thai Agricultural Standard (TAS) were established (National Bureau of Agricultural Commodity and Food Standards, 2006). The MoPH has implemented a food safety programme since 2003. This programme has through combined efforts of all the sectors concerned, placed emphasis on all three categories of food, namely fresh food, processed food, and cooked-for-sale food. The government declared the year 2004 “Food Safety Year”. This was aimed at making Thailand a country producing food with both quality and

⁹ Thai Agricultural Standard (TAS) on Good Manufacturing Practices for Pig Abattoir is the criteria for certifying and practical guiding of pig abattoir. The guidelines cover all steps starting from picking up live pig at farm and transferring to abattoir and thereafter distributing of pig carcass, pork and their products to the market in order to provide safe and quality products that fit for consumption and are acceptable domestically and internationally

safety levels conforming to international standards, with the intention of promoting food exports and tourism.

2.2.4 Veterinary service and pig production systems

2.2.4.1. Veterinary services in Thailand

The Department of Livestock Development, Ministry of Agriculture and Cooperatives are directly responsible for the animal health services and the promotion of animal products in Thailand. Within the DLD, there are nine regional livestock offices, 76 provincial livestock offices, district livestock offices, eight regional veterinary research and diagnostic centres that provide veterinary investigations, and a commercial-scale vaccine production unit. The DLD staff included veterinarians and animal husbandry scientists. Apart from permanent staff, the DLD also trains village key persons to carry out routine vaccination and to detect and notify any outbreaks of contagious diseases (FAO, 2003).

2.2.4.2. Pig production systems

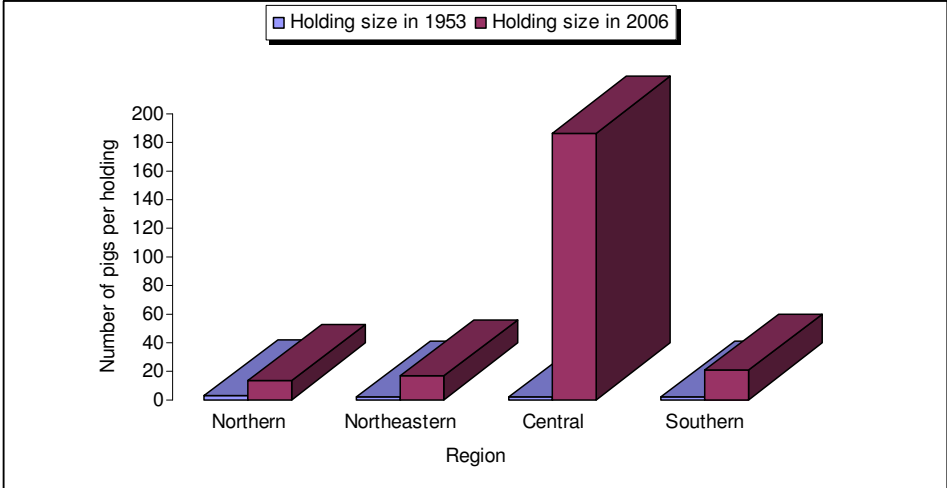
In the 1950s Blanchard (1958) described the livestock of Thailand as consisting of mainly of cattle and pigs. A Thai farm survey conducted in 1953 showed that there were 3,589,036 cattle, 4,343,693 buffalo and 3,232,507 pigs (Pendleton, 1962). Pigs were being raised in all parts of Thailand, with about one third of the total pig production being in the Central Region. Pig production systems were dominated by backyard farming, where farmers kept a few native pigs¹⁰. Although about 50% of all Thai farmers reported owning pigs, only 2.3% reported eating meat from home-raised animals, pigs were sold to markets (Pendleton, 1962). In contrast to pig production in other areas of the country, pigs raised by hill tribe groups were mostly for the farmers' own use, either as food or

¹⁰ Thai-native pigs are black in colour, with an arched back and attain market size (70 kg) in approximately 12 months.

for ritual sacrifice. They were generally slaughtered by the farmers with only a few being produced for sale or barter (Young, 1962; Kunstadter, 1967).

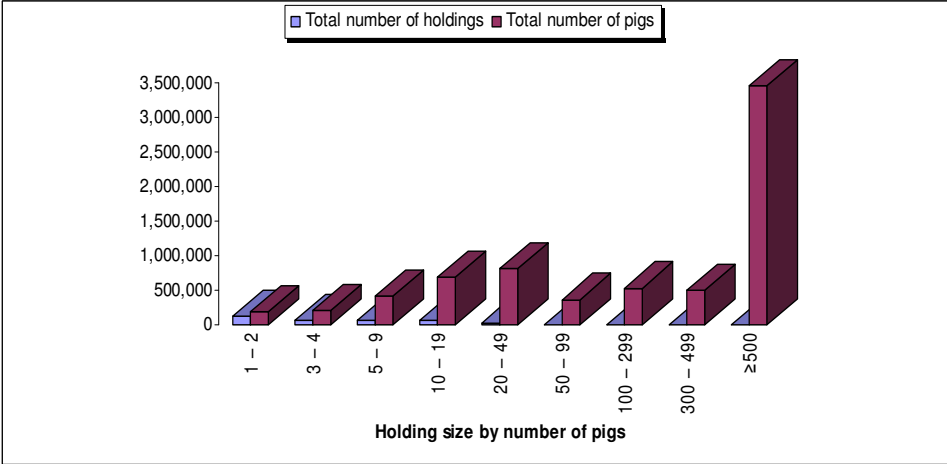
Modern intensive pig production schemes in Thailand began in 1973 (Tisdell *et al.*, 1997). However, large-scale or industrialised pig farming was slow to develop up until the 1980s, after which it rapidly increased. It was driven by changing consumer preferences, the degree of competition within the industry and public concern for environmental pollution by the pig farms. Changes included the breeds of animals used for production, their nutrition, housing and farm management systems and initiation of vaccination programmes, with waste treatment being addressed through changes in disposal systems (Cameron, 2000). The average holding size has significantly increased when compared with that in 1953 (see Figure 2-5). In recent times, about 80% of pigs produced in the country are from intensive farming systems and 56% of these are from farms with over 1,000 pigs. The remainder are from small farms with 50 – 200 pigs and medium sized farms with 201 – 1,000 pigs (Cameron, 2000). Over time, backyard pig production has become less significant in terms of production, as has the role of native-pigs. The National Agricultural Census of Thailand showed that holdings with less than 50 pigs accounted for about 32% of pig production in 2003 (Figure 2-6) (National Statistical Office, 2003). Pig production in Thailand is sufficient for local consumption. About 1% of pork is being exported to countries in the South East Asian Region. Endemic foot- and-mouth disease across the region is one of the main obstacles preventing Thailand competing in the broader international trade of pig and pig products.

Figure 2-5 Comparison of pig holding sizes in the different regions of Thailand between 1953¹ and 2006²



Sources: ¹Division of Agricultural Economics, Ministry of Agriculture, Thailand, Thailand Economic Farm Survey 1953, ²Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand, Livestock Statistics 2006

Figure 2-6 Pig population in Thailand, total number of holdings and total number of pigs stratified by size of holdings



Source: 2003 Agricultural census whole kingdom, National Statistical Office, Ministry of Information and Communication Technology, Thailand

2.2.5 National surveillance for human trichinellosis in Thailand

Thailand implemented a national trichinellosis surveillance system in 1969, when human trichinellosis became a Notifiable Disease. The national trichinellosis surveillance system is a passive system that relies on existing resources at the provincial level. The Provincial Public Health Offices report trichinellosis cases weekly to the Bureau of Epidemiology (see Figure 2-3 Schemology of the public health epidemiological surveillance network in Thailand).

A trichinellosis case is defined as one that is clinically compatible with the common signs and symptoms of trichinellosis among symptomatic persons and which has been confirmed in the laboratory. Laboratory confirmation is made by identification of *Trichinella* larvae from a muscle biopsy or by a positive serological test. In an outbreak setting, at least one case must be laboratory confirmed and any patients that share an epidemiological implicated meal or meat products with a confirmed case, can be reported as a case if they have either a positive serological tests or show clinical symptoms. Once a case of trichinellosis is confirmed, it is reported to the Provincial Public Health Office in a communicable disease report form (Report 506). The Provincial Public Health Office and the Regional Office of Disease Prevention and Control will investigate, to identify new cases, confirm the outbreak and to guide prevention efforts. Cases are reported weekly from Provincial Public Health Office to Bureau of Epidemiology (BoE) by electronic mail or fax and the report is then verified by BoE staff.

Surveillance data are published weekly, monthly and annually. Details include number of cases and deaths by province, month and age group.

The Annual Epidemiological Surveillance Report includes an annual trichinellosis surveillance data and additional report narrative. The report narrative contains the total number of cases from reported cases and non-reported cases from active case finding, the number of outbreaks, and number of cases by gender and food sources.

2.3. Materials and Methods

2.3.1 Study method

The study method was a retrospective review of human trichinellosis cases that have been reported to Bureau of Epidemiology, Ministry of Public Health, Thailand between 1981 and 2008.

2.3.2 Data collection

Data for the study comprises of trichinellosis cases, human population data and geographic location data. These data were obtained from Thai government publication via the online database and paper-based systems.

2.3.2.1. Surveillance data

Surveillance data were obtained from the Bureau of Epidemiology, Ministry of Public Health (<http://www.moph.go.th>). The data describes trichinellosis cases and the potential sources of *Trichinella* in these outbreaks. Cases of trichinellosis by province and month are tabulated in the annual surveillance reports. These were used to characterise trichinellosis epidemiology by time, place and age of cases. Of the 5,455 reported trichinellosis cases, ages were available for 5,445 (99.8%) cases. The ages were categorised into seven groups: (i) 0 – 14, (ii) 15 – 24, (iii) 25 – 34, (iv) 35 – 44, (v) 45 – 54, (vi) 55 – 64 and (vii) ≥ 65 years old. Most of the outbreak investigation reports were summarized in narrative reports (P. Chumkasian, personal communication). Data in the narrative

reports were used to provide information on the potential sources of the outbreaks along with the gender and ethnicities of cases. Additional data on individual cases during the period January 2003 to August 2008 were obtained. These were used to characterise the occupation and the location (rural, urban) of each case.

2.3.2.2. Demographic data

Annual human population data were obtained from the Department of Provincial Administration, Ministry of Interior (<http://www.dopa.go.th>). Annual age-stratified population data were obtained from the Bureau of Epidemiology, Ministry of Public Health (<http://www.moph.go.th>).

2.3.2.3. Geographical coordinates

The geographical coordinates for each province in Thailand were used for cluster analysis. The location of provincial centroids, a polygon's mean centre which is based on the weighted average of its x and y coordinates, was computed from the polygon of each province using ArcGIS software (ArcGIS 9.3). A digital map of the different Thai provinces (scale 1: 50,000) was obtained from the Land development Department, Ministry of Agriculture and Cooperatives, Thailand.

2.3.3 Statistical analysis

Surveillance data was analysed using various statistical methods to describe the following epidemiological items.

- i. Frequency of trichinellosis
- ii. Trend in the incidence over time
- iii. Seasonal variations
- iv. Spatial patterns
- v. Space – time clustering
- vi. Characteristics of cases

2.3.3.1. Frequency of trichinellosis

Trichinellosis cases occurred at national, regional and provincial levels and were described using the incidence rate (*I*). Incidence rate measures the rapidity with which new cases of disease develop over time (Thrusfield, 2007). It is summarized by the formula:

$$I = \frac{\text{number of new cases of disease that occur in a population during a particular period of time}}{\text{the sum over all individual of the length of time at risk of developing disease}}$$

In this study, the numerator is the number of cases, the denominator is approximated from the average number at risk; an average of the number at risk at the start of the time period and the number at risk at the end of the time period (Thrusfield, 2007). For example, the incidence rate of human trichinellosis in Thailand between 1981 – 2008 is calculated from the overall number of cases divided by the average of population in 1991 and 2008. The incidence rate is calculated per 1,000,000 person-year. In addition, fatal cases are measured by the case fatality (*CF*). Case fatality as defined by Thrusfield (2007) is the proportion of diseased animals (individuals) that die:

$$CF = \frac{\text{number of deaths during a particular period}}{\text{number of cases during that particular period}}$$

Case fatality, measures the probability of death in diseased animals (individuals), can take values between 0 and 1 or 0-100% (Thrusfield, 2007). In this study case fatality is calculated based on (i) number of annual death and cases and (ii) overall deaths and cases between 1981 – 2008. The incidence rates and case fatality are computed in Microsoft Excel (Microsoft Office Excel 2003, Ink).

2.3.3.2. Trend in the incidence of trichinellosis

To investigate for annual trends of trichinellosis incidence, a relationship between the annual cumulative incidences (*CI*) and time in a yearly

interval was assessed for linear association using regression analysis. Cumulative incidence is the proportion of non-diseased individuals at the beginning of a period of study that becomes diseased during the period (Thrusfield, 2007):

$$CI = \frac{\text{number of individual that become diseased during a particular period}}{\text{number of healthy individual in the population at the beginning of that period}}$$

It is a proportion that can take values between 0 and 1 (0-100%). In this study, the annual cumulative incidence of trichinellosis was estimated from number of cases that occurred in a year divided by number of population at the beginning of that year. For example, cumulative incidence of trichinellosis in 1981 is calculated by dividing overall trichinellosis cases in 1981 by population in 1981. It is multiplied by 1,000,000 because the incidence is small.

Regression analysis is a statistical technique for investigating the relationship between two or more variables. When observations (the dependent variable), y , are made at defined intervals (the independent variable), x , the regression analysis is applied to detect an association between these two variables (Thrusfield, 2007). The equation for regression line of linear regression of y on x is:

$$y = \alpha + \beta x$$

Where β is the regression coefficient and α is the intercept defining the point of interception on the y axis by the regression line (Thrusfield, 2007). If there is a significant linear relationship between observations and times, β will not be equal to zero. To determine whether there is a significant linear relationship between annual cumulative incidence of human trichinellosis in Thailand from 1981 – 2008 and time the null hypothesis (H_0) is that the regression coefficient of the regression line is equal to zero and the alternative hypothesis (H_1) is that the regression

coefficient of the regression line is not equal to zero. The null hypothesis is accepted or rejected based on P value of the F -test or t -test (Kirkwood & Sterner, 2003; Thrusfield, 2007) greater than 0.05 or smaller than 0.05, respectively (Statacorp LP, College Station, TX, USA).

Regression analysis based on the assumption of normality. The linear normality test was conducted using a Shapiro-Wilk test in Stata (Statacorp LP, College Station, TX, USA). If the P value from a Shapiro-Wilk hypothesis test for the normality of the annual cumulative incidence data was greater than the critical value, 0.05, the null hypothesis (H_0) that the annual cumulative incidence data are normally distributed is accepted.

2.3.3.3. Seasonal variation of trichinellosis cases

The seasonal variations of monthly trichinellosis cases were assessed by (i) the simple average method in which monthly average cases and standard deviations were computed and (ii) the ratio-to-moving average method in which monthly cases were expressed as an index. Seasonal index is an average that can be used to compare an actual observation relative to what it would be if there was no seasonal variation. In this study, the monthly indices showed how each monthly value relates to the annual average median number of cases equal to 100. The calculation based on the ratio-to-moving average method is done in three steps (Lapin, 1978; Ernst & Fabrega, 1989) using a 12-month seasonal cycle. Firstly an average of monthly cases over a 12-month period, centred on the 12th month (the centred 12-month moving average) is obtained. Then, the original monthly cases data (the time series data) are divided by the respective centred 12-month moving average, this yields the ratio-to-moving average (Frechtling, 1996). An average of the ratio-to-moving average for each month is a seasonal index for the month. These values were adjusted so that the total value of the seasonal indices over the year (12 months) equals 1200 and the average value of each monthly seasonal

index is 100 (see Appendix 1& Appendix 2). The seasonal indices, the monthly average cases and the standard deviation were computed using Microsoft Excel (Microsoft Office Excel 2003, Ink).

2.3.3.4. Spatial patterns of trichinellosis cases

Spatial patterns of trichinellosis cases and risk of trichinellosis in each province were explored and assessed. Incidence rates at province-level were calculated in four equal time periods in order to visualize the spatial pattern in the small periods, 1981 – 1987, 1988 – 1994, 1995 – 2001 and 2002 – 2008 (Microsoft Office Excel 2003, Ink). Based on annualized average incidence, all provinces were grouped into four categories: non-endemic area, low endemic area with annualized average incidence between 0 and 5 per 1,000,000, medium endemic area with the incidence between 5 and 10 per 1,000,000, and high endemic area with the incidence over 10 per 1,000,000. The four types of provinces were colour-coded on the maps (Anselin, 2005; Fang *et al.*, 2006). To assess the risk of trichinellosis in each province, excess hazard maps were produced. The excess hazard represents the ratio of the observed incidence at each province over the average incidence of all ‘endemic areas’ (Fang *et al.*, 2006). An excess hazard equal to one means the province has trichinellosis incidence as expected, the excess hazard more than one and less than one refer to the province with higher incidence than expected and lower incidence than expected, respectively (Anselin, 2005; Fang *et al.*, 2006).

2.3.3.5. Cluster detection

To detect clusters of trichinellosis cases in a space-time setting, SaTScan, scan statistic was used (Kulldoff M. and Information Management Services, Inc. SaTscanTM v.8.0). Kulldoff (2001) developed a space-time scan statistic to test for geographical cluster/s, to identify their approximate location and identify cluster/s in time in data sets. It performs better than a purely spatial analysis in data set of an extensive

time period, in which the latter has a low power to detect recent emerging clusters (Kulldoff, 2001). The space-time scan statistic is defined by a cylindrical window with a circular geographic base and with height corresponding to time. The base is centred on one of several possible centroids located throughout the study region, with the radius varying continuously in size. The height reflects any possible time interval of less than or equal to half the total study period. The window is then moved in space and time so that for each possible geographic location and size, it also visits each possible time interval (Kulldoff, 1997, 2001). The space-time scan statistic compares observed and expected number of events (*i.e.* cases) inside and outside the scan window to detect cluster that are least likely to have occurred by chance. For each location and size of the scanning window, the null hypothesis is that the risk within and outside the window is not different and the alternative hypothesis is that there is an elevated risk within the window as compared to outside. The statistical significance for each cluster is obtained through Monte Carlo hypothesis testing where results of the likelihood function are compared for a large number of random replications of the dataset generated under the null hypothesis. The *P* value is obtained by comparing the rank (*R*) of the maximum likelihood from the real data set with the maximum likelihoods from the random data sets. The *P* value represent: $R / (1 + \text{number of simulation})$. In SaTScan, the number of replications must be at least 999 to ensure excellent power for all types of data sets (Kulldoff & Information Management Service, 2009).

Trichinellosis data of yearly count cases in Thailand from 1981 – 2008 were geographically aggregated to province centroid. The data were divided into four equal time-periods, so that the location of clusters can be compared with the excess risk map. The data were assessed separately for potential clustering in space and time using SaTScan 8.0. Cases are assumed to be Poisson distributed with constant risk over space and time under the null hypothesis, and with different risk inside and outside at

least one of the cylinders under the alternative hypothesis. The maximum spatial cluster size was chosen to correspond to 10% of the Thai population (to maximize the number of spatial cluster detect), the maximum temporal cluster size was set to 50% of the study period. By performing the space-time analysis in SaTScan, a space-time scanning window with prior defined maximum size was initiated at the centroids of the 76 Thai provinces. For each cylindrical window the numbers of cases inside and outside the cylinder are noted, together with the expected number of cases reflecting the population at risk. On the basis of these numbers, the likelihood is calculated for each cylinder. The cylinder with the maximum likelihood, and with more than its expected number of cases, is denoted the most likely cluster. This study is set to generate 999 simulations, the smallest recommended number of simulation (Kulldoff, 2009), and the cut-off values whether to reject or not reject the null hypothesis is 0.05.

2.3.3.6. Characteristic of trichinellosis cases

Characteristic of trichinellosis cases namely: sex, age, occupation and residential area, and potential sources of *Trichinella* infections were characterised. The occurrence of trichinellosis in male and female cases each year was described in ratio (a value obtained by dividing one quantity by another (Thrusfield, 2007)). Data were assessed whether trichinellosis cases occurred equally between male and female individuals by applying a test for goodness of fit. The test measures how closely the observed results (number of male cases and number of female case) agree with the expected results (number of male cases and female cases are equal, therefore sex ratio = 1:1). A conventional measure of goodness of fit, the χ^2 value, is calculated from the number of observed cases in each class (sex) compared with the number expected in each of the class on the basis of the hypothesis that cases occurred equally between the classes. In symbol, the calculation of χ^2 can be represented by the expression (Hartl, 2010):

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

Where Σ means the summation over all the classes of gender, χ^2 is calculated using the observed and expected number. The closer the observed numbers are to the expected numbers, the smaller the value of χ^2 . A χ^2 value equal to zero means that the observed and expected number fit perfectly (Hartl, 2010). In this study, the probability of observing a $\chi^2_{df=1}$ greater than 5% ($P < 0.05$) is a critical value to determine whether or not trichinellosis cases occur equally in men and women.

The frequency of trichinellosis occurring in different age groups is described by age-specific incidence rate between 1981 – 2008. Age-specific incidence rate is calculated in a similar manner to the crude incidence rate, except the numerator and denominator apply to one or more categories of a population with specific host attributes (Thrusfield, 2007). For this study, age-specific incidence rate was calculated for seven age groups, following the manner that surveillance data were recorded (but age groups under 15 years old were collapsed into one group), as follows: 0 – 14, 15 – 24, 25 – 34, 35 – 44, 45 – 54, 55 – 64 and over 64. In addition, rate ratio (relative risk) was calculated and graphically showed in time trend. The incidence rate of age group 0 – 14 was use as a base-line. The rate ratio is defined as (Kirkwood & Sterne, 2003):

$$\text{rate ratio} = \frac{\text{rate in exposed group}}{\text{rate in unexposed group}}$$

Time trend of trichinellosis occurrence in each age group was assessed in four equal time periods between 1981 – 2008: 1981 – 1987, 1988 – 1994, 1995 – 2001, and 2002 – 2008. ANOVA tests were conducted to

determine for (i) the differences in the average incidence rate from one age group to another and (ii) the differences in the average age-specific incidence rate from one time period to another. The *F* ratio, the between group variance divided by within group variance, determines the probability that one would obtain a high ratio by chance. The higher the *F* ratio value the smaller the probability that the different would occur by chance. In this study, the null hypothesis was stated no different in the average age-specific incidence rate from one age group to another and there is not different in the average age-specific incidence rate from one time period to another. The null hypothesis is accepted if the probability that the difference between variance by chance is greater than 5%. The test was performed using statistical software package, Stata (Statacorp LP, College Station, TX, USA).

Characteristics of trichinellosis by occupations and residential area and the potential sources of human trichinellosis were described in frequency or percentages. Potential sources of the outbreaks including exposure to domestic and wild animal meats were examined from the narrative reports of the outbreak investigation. Potential sources of outbreaks were described by the number of outbreaks related to each particular potential source.

2.4. Results

There were 5,455 reported cases of human trichinellosis as published in the Annual Epidemiological Surveillance Report from 1981 to 2008. A further 433 cases were identified through active case finding during outbreak investigations. These were mentioned in the report narrative but were not included in the report total. Data from the 5,455 cases that were included in the report total were analyzed here.

2.4.1 Annual human trichinellosis cases and deaths

Between, 1981 and 2008, trichinellosis was reported every year except in 2001 when there were no reported cases of trichinellosis in Thailand (see Figure 2-7). The mean annual number of trichinellosis cases during 1981 – 2008 was 194.8 (SD = 137.8); the highest annual cases were 557 in 1983. There were a total of 25 fatalities, all of which occurred prior to 1998; 23 fatal cases occurred between 1981 – 1990, the two remaining fatal cases occurred in 1992 and 1997 (Figure 2-8). Case fatality by year varied from 0 - 4.3%. Overall case fatality rate was 0.5% (25/5,455).

Figure 2-7 Annual number of human trichinellosis cases in Thailand during the period 1981-2008

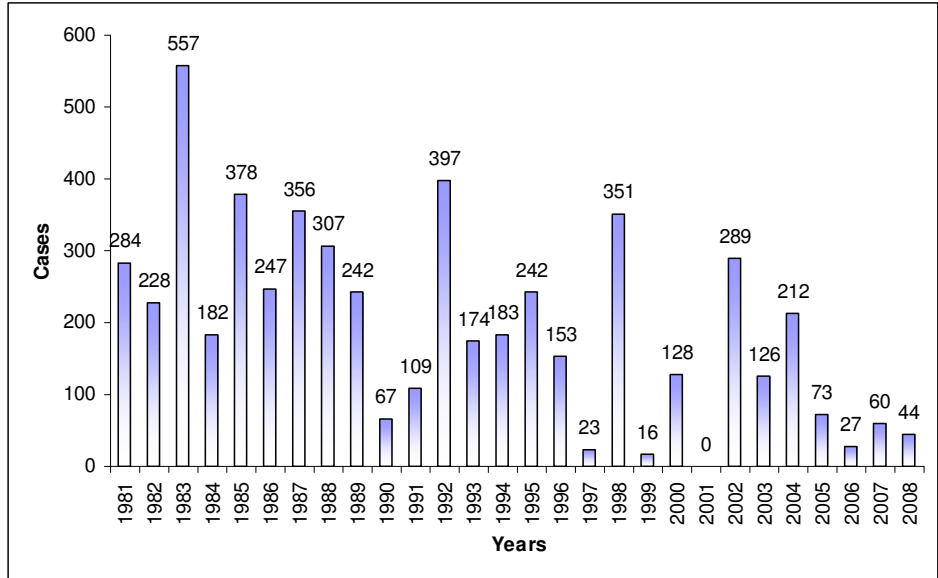
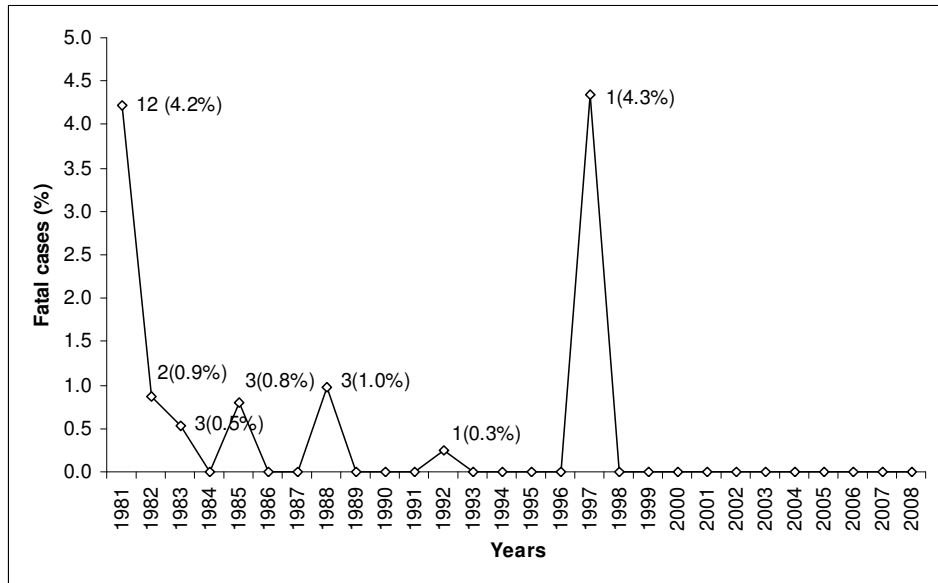


Figure 2-8 fatal cases and case fatality of human trichinellosis in Thailand between the period 1981 and 2008



2.4.2 Distribution of human trichinellosis

The regional distribution of trichinellosis cases over study period are shown in Table 2-1. Most cases occurred in the Northern Region (n = 5,108, 93.63%). The smallest number of reported cases was observed in the Southern Region (n = 97). The incidence rate of trichinellosis in Thailand between 1981 – 2008 was 98.27 per 1,000,000 person-year. The highest incidence was in the Northern Region, with an incidence rate of 471.82 per 1,000,000 person-year or 16.85 per 1,000,000 person-year annually. This rate was about five times higher than the whole country incidence rate (471.82/98.27). The lowest human trichinellosis incidence rate was observed in the Central Region (1.89 per 1,000,000 person-year). Northern Region was the only region where the annual incidence rate of trichinellosis was higher than one per 1,000,000 person-year.

Table 2-1 Rates of human trichinellosis (per 1,000,000 person-year) in Thailand between 1981 and 2008

Region	Person-year	Cases	Rates (1981 – 2008)	Annual rates
North	10,826,087	5,108	471.82	16.85
Northeast	18,800,813	215	11.44	0.41
Central	18,544,768	184	1.89	0.07
South	7,338,862	97	13.22	0.47
Total	55,510,530	5,455	98.27	3.37

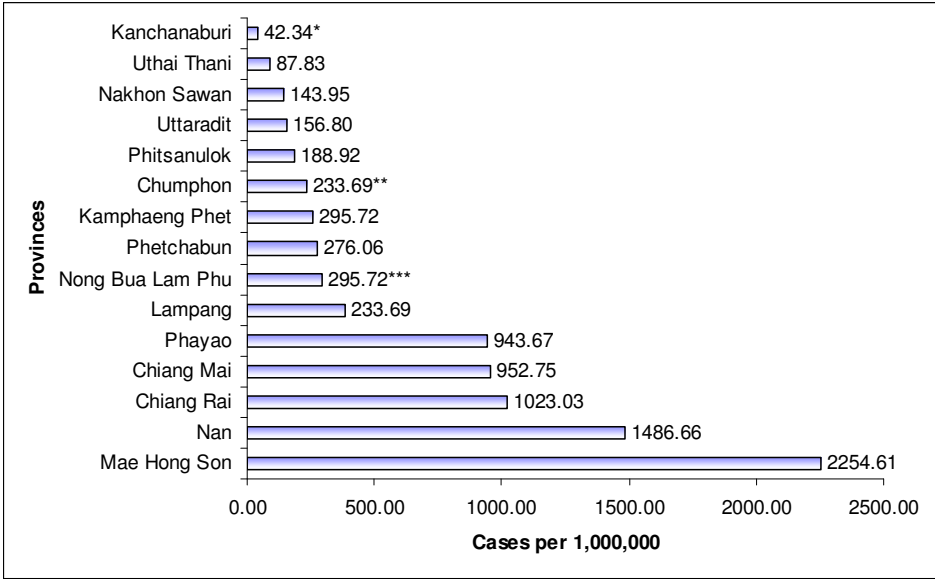
2.4.3 Provincial distribution of human trichinellosis

Cases of trichinellosis were reported by the provinces within the Northern Region every year, with the exception of 2001. Provinces in the Northeast, Central and Southern Region only reported cases of trichinellosis in eight of the 28 years under study; none of these

individual regions reported cases in more than four years (1985, 1989, 1991, 1992, 1994, 2004, 2005 and 2006). Trichinellosis occurred at least one year in 28 years in 31 out of 76 provinces in Thailand (see Appendix 3).

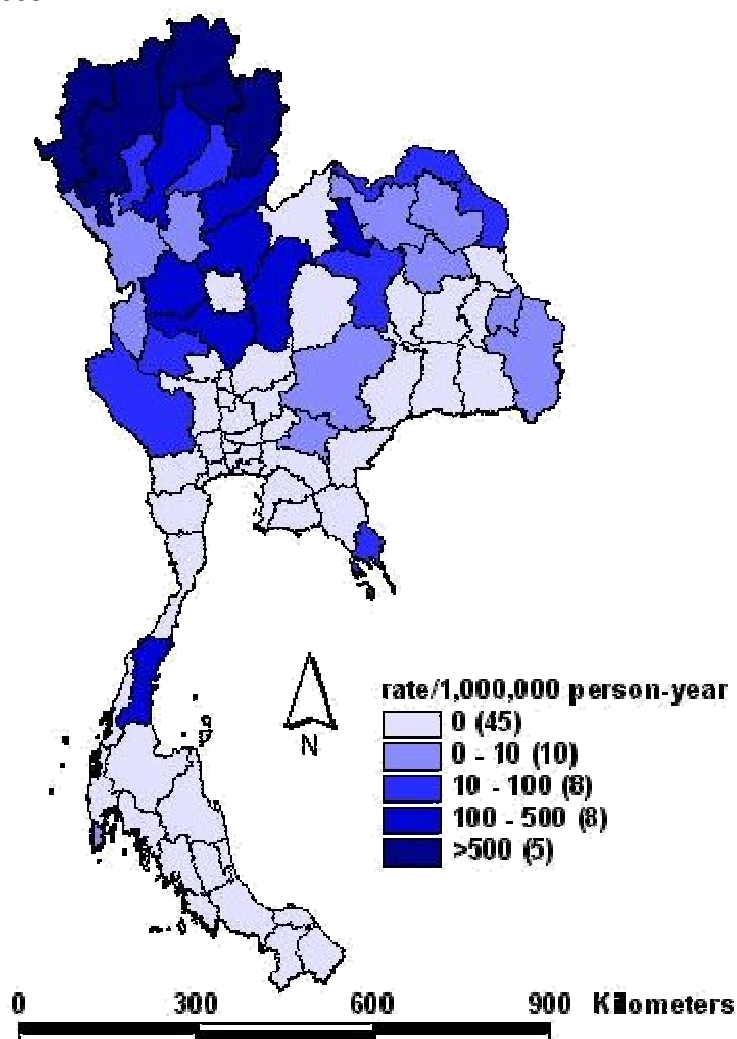
Trichinellosis occurred in 31 of the provinces between 1981 and 2008: 16 provinces in the Northern Region, 10 provinces in the Northeastern Region, three provinces in the Central region and two provinces in the southern Region (Figure 2-10). The incidence of trichinellosis at the province-level ranged from zero to 2254.6 per 1,000,000 person-year, annualized incidence ranged from zero to 75.9 per 1,000,000 person-year. The highest incidence rates (per 1,000,000) were observed in the five northernmost provinces: Mae Hon Son (2254.61), Nan (1486.66), Chiang Rai (952.75), Chiang Mai (943.67) and Phayao Provinces (943.67). The incidence in other provinces was at least four times less than the incidence in the five northernmost provinces ($943.67/233.69$). Twelve of 15 provinces with highest incidence of trichinellosis in Thailand between 1981 – 2008 were in Northern Region (Figure 2-9).

Figure 2-9 The Fifteen provinces in Thailand with the highest incidence rates of human trichinellosis, 1981 – 2008



* a province in Central Region
 ** a province in Southern Region
 *** a province in Northeastern Region
 All other provinces are found within the Northern Region

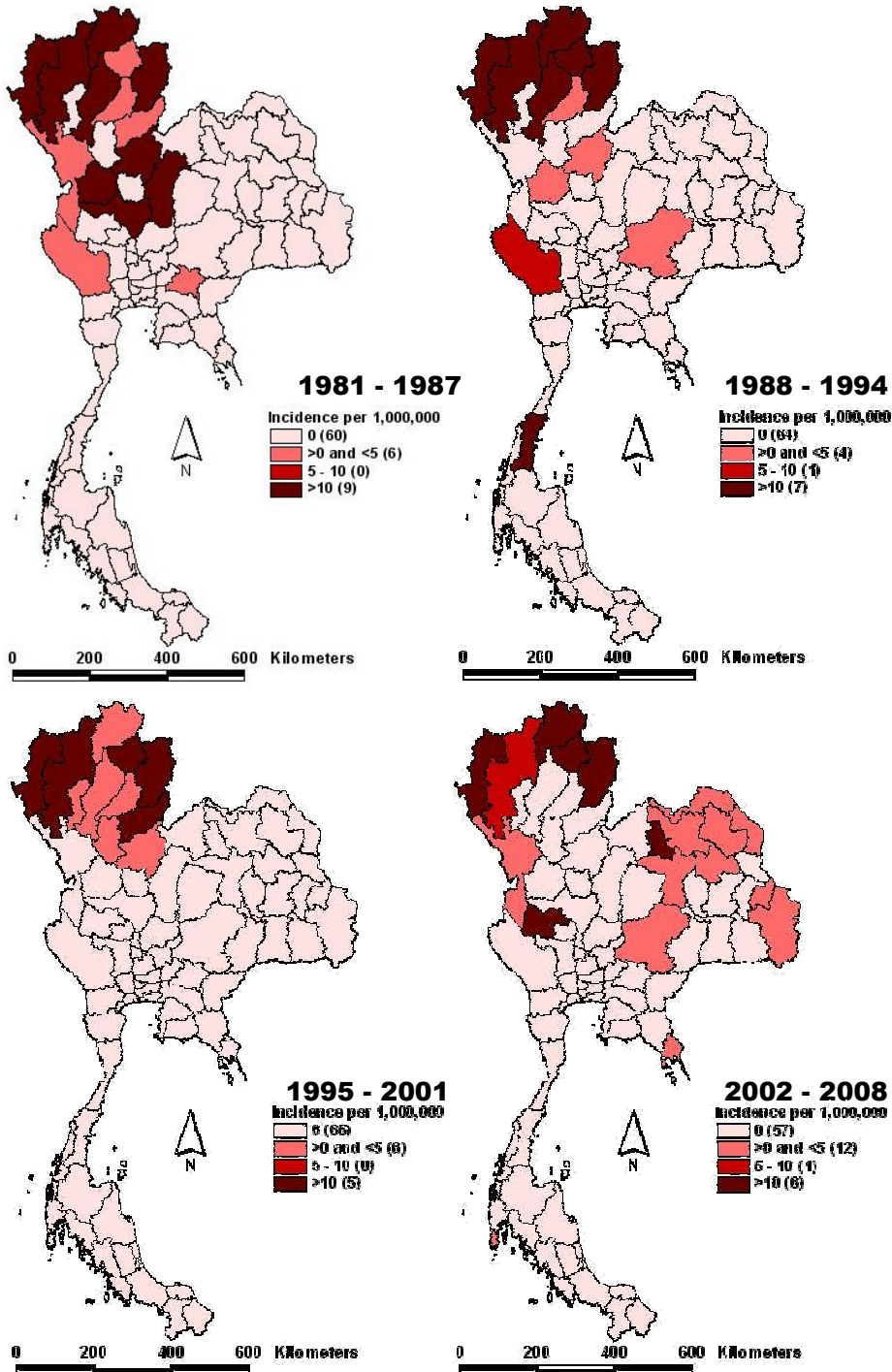
Figure 2-10 Incidence rates of human trichinellosis in Thailand between 1981 – 2008



Annualized incidence rate by province in the four time-periods is shown in Figure 2-11. Trichinellosis affected 15, 12, 11 and 19 of the provinces in the periods 1981 – 1987, 1988 – 1994, 1995 – 2001 and 2001 – 2008, respectively. Based on the assumption in this study, six, four, six and twelve provinces were classified as low endemic areas (incidence rate >0 and <5 per 1,000,000 person-year), while nine, seven, five and six provinces were classified as the endemic areas (incidence rate >10 per 1,000,000 person-year) in the four respective time-periods. Only one province in the period 1988 – 1994 and one province in 2002 – 2008

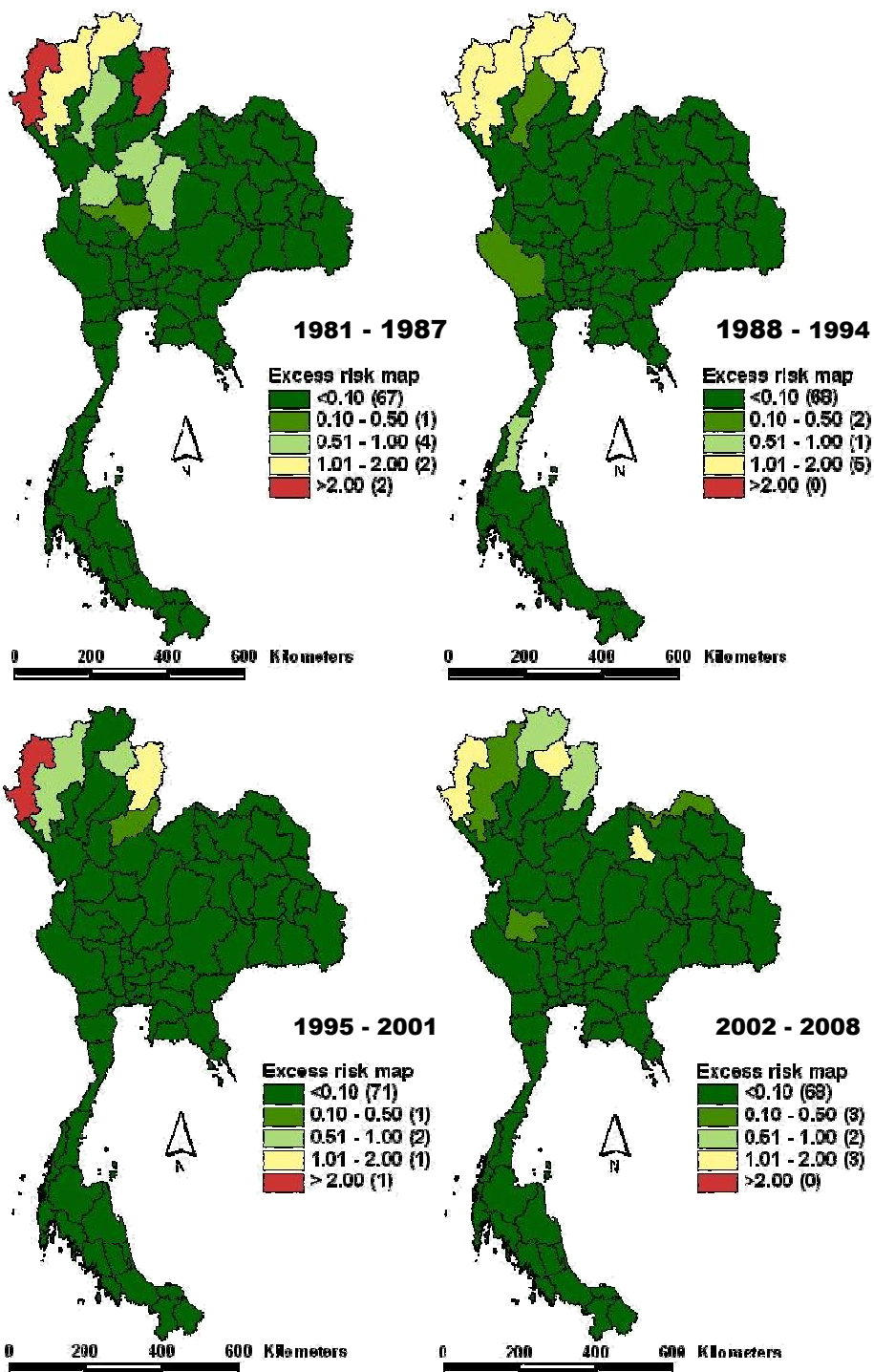
were classified as the medium endemic area (incidence rate 5 – 10 per 1,000,000 person-year).

Figure 2-11 Annualized incidence rates of human trichinellosis at province-level from 1981 – 1987, 1988 – 1994, 1995 – 2001 and 2002 – 2008



The excess hazard map, based on the ratio of the incidence rate in the province to the incidence rate in the 'endemic areas' (the provinces with incidence rate exceed 10.0 per 1,000,000 person-year) shows the distribution of provinces with excess risk of trichinellosis, excess risk value more than 1, in yellow and red (Figure 2-12). Overall there were 14 provinces which had higher incidence than expected (excess risk value more than one) in 1981 – 1987, 1988 – 1994, 1995 – 2001 and 2002 – 2008. They were four of the northernmost provinces, five of the northernmost provinces, two of the northernmost provinces and two of the northernmost provinces and one province in the Northeastern Region, respectively.

Figure 2-12 Excess hazard map of trichinellosis in Thailand from 1981 – 1987, 1988- 1994, 1995 – 2001 and 2002 - 2008



2.4.4 Cluster analysis

In 1981 – 1987, 1988 – 1994, 1995 – 2001 and 2002 – 2008, there were 2,782, 1,479, 913 and 831 trichinellosis reported cases in Thailand, respectively. In these periods the incidence rates (per 1,000,000 person-year) of trichinellosis in Thailand were estimated as 6.0, 4.0, 2.0 and 2.0.

The spatio-temporal scan statistics based on a 1-year temporal with a circular spatial maximum radius corresponding to 10% of population at risk, and a maximum temporal window corresponding to 50% of the study period detected a most likely cluster and identified secondary clusters in the four time-periods. Only the clusters with significant excess risk ($P < 0.05$) were reported. Overall, there were four most likely clusters and 11 secondary clusters (Table 2-2). Only five clusters were detected outside the Northern Region. Geographical distributions of the clusters are showed in the choropleth maps (Figure 2-13).

In the four time-periods, the most likely clusters were detected in the Northern Region and included any of the five northernmost provinces. These results correlated with the excess hazard areas in Figure 2-12. The most likely clusters extended for over a year. In 1981 – 1987, 1988 – 1994, 1995 – 2001 and 2002 – 2008, the most likely cluster were observed between 1985 – 1987, 1992 – 1994, 1996 – 1998 and 2002 – 2003, the clusters encompassed five provinces for all of the periods except in 1996 – 1998 in which the cluster encompassed two provinces. All of these clusters included all or at least three of the northernmost provinces.

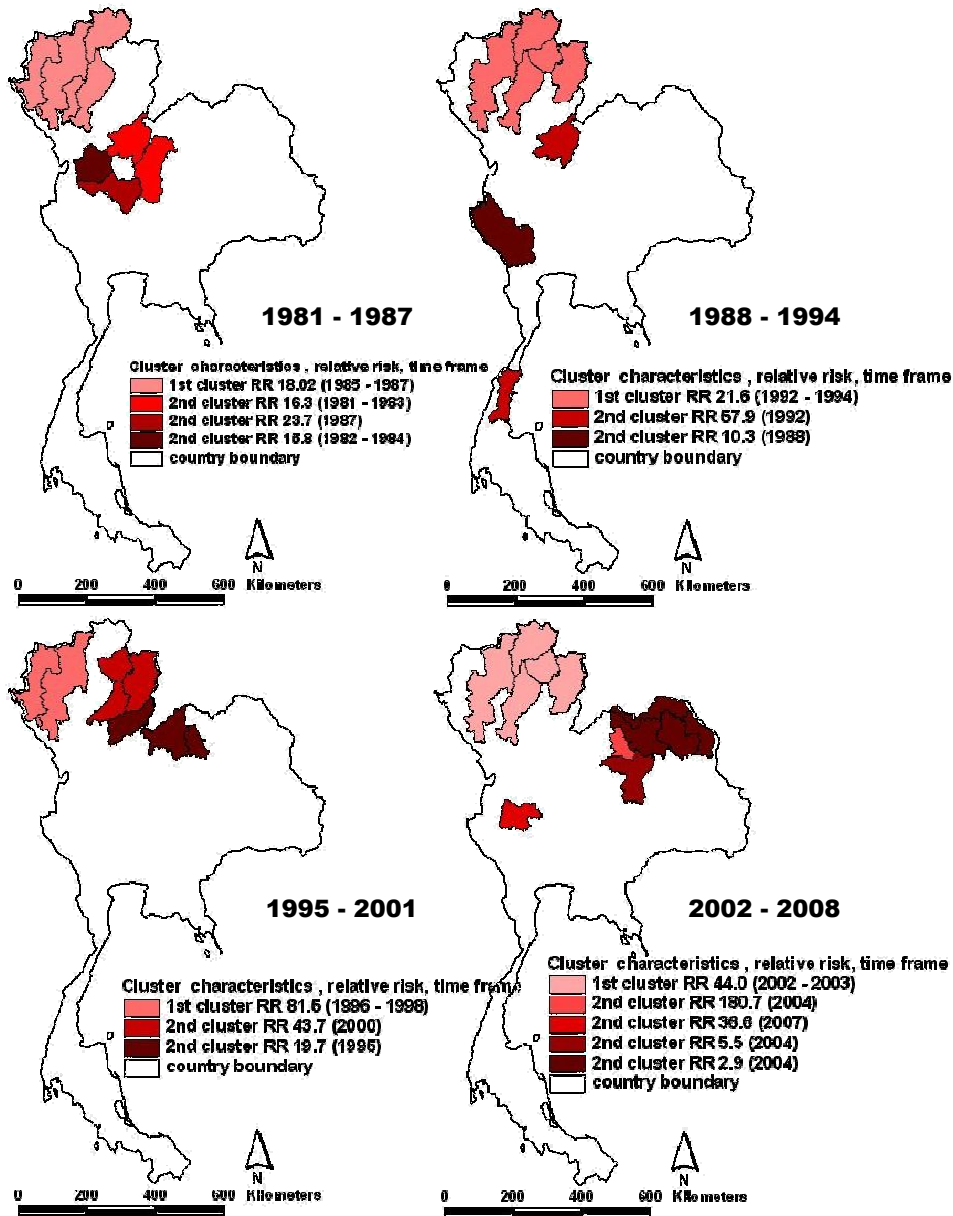
In 1981 – 1988, three secondary clusters encompassing four provinces were identified. All of them are in the Northern Region, two clusters extended over two years. Between 1988 – 1994, two secondary clusters were identified in a year time frame, one was in the Northern Region and another was in the Southern Region. The relative risks within the clusters

were 57.9 and 10.3 and in 1995 – 2001, two secondary clusters were detected. The first in 2005, encompassed two provinces with a relative risk of 19.7. The cluster radius included a province in the Northern Region with trichinellosis cases and two provinces in the Northeastern Region where they were no reported case. Another secondary cluster was detected in 2000. It contained two northernmost provinces, Nan and Phayao Provinces. In 2002 – 2008, four secondary clusters were detected. Three of them encompassed six provinces in Northeastern Region and occurring in 2004. In 2007 a secondary cluster was detected in Uthai Thani in the Northern region. After the period 1981 – 1987, none of the secondary clusters was detected over a year temporal and none of the clusters detecting outside Northern Region repeated in the same province.

Table 2-2 Cluster distribution of human trichinellosis in Thailand by the space-time scan statistic in the period 1981 – 2008

Data years	Cluster type (1 ^o most likely, 2 ^o secondary) and areas	Cluster years	Cases	Expected	Relative risk	<i>P</i> value
1981 - 1987	1 ^o Chiang Mai, Chiang Rai, Mae Hong Son, Lampang, Lamphun	1985 - 1987	804	67.6	18.0	0.001
1981 - 1987	2 ^o Phetchabun, Phitsanulok	1981 - 1983	384	28.1	16.3	0.001
1981 - 1987	2 ^o Nakhon Sawan	1987	148	6.7	23.7	0.001
1981 - 1987	2 ^o Kamphaeng Phet	1982 - 1984	165	11.2	15.8	0.001
1988 - 1994	1 ^o Chiang Rai, Phayao, Chiang Mai, Nan, Lampang	1992 - 1994	639	50.2	21.6	0.001
1988 - 1994	2 ^o Chumphon	1992	84	1.5	57.9	0.001
1988 - 1994	2 ^o Kanchanaburi	1988	25	2.5	10.3	0.001
1995 - 2001	1 ^o Mae Hong Son, Chiang Mai	1996 - 1998	466	11.5	81.5	0.001
1995 - 2001	2 ^o Nan, Phrae, Phayao	2000	121	3.2	43.7	0.001
1995 - 2001	2 ^o Loei, Nong Bua Lam Phu, Uttaradit	1995	62	3.4	19.7	0.001
2002 - 2008	1 ^o Chiang Rai, Phayao, Chiang Mai, Nan, Lampang	2002 - 2003	404	17.5	44.0	0.001
2002 - 2008	2 ^o Nong Bua Lam Phu	2004	141	0.9	180.7	0.001
2002 - 2008	2 ^o Uthai Thani	2007	22	0.6	36.6	0.001
2002 - 2008	2 ^o Khon Kean	2004	18	3.3	5.5	0.002
2002 - 2008	2 ^o Sakon Nakhon, Udon Thani, Nakhon Phanom, Nong Khai	2004	23	8.0	2.9	0.03

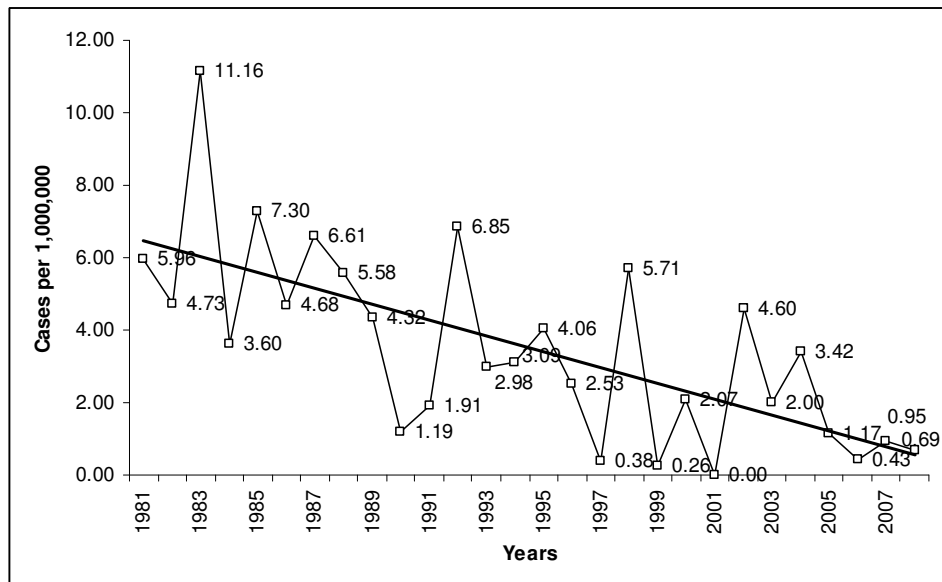
Figure 2-13 Spatial and temporal distribution of the identified clusters of human trichinellosis with significantly high in the incidence, using the maximum cluster size $\leq 10\%$ of the total population and the maximum temporal window of 50% of the time period, Thailand 1981 – 2008



2.4.5 Time trend of cases of human trichinellosis

The annual cumulative incidence of human trichinellosis in Thailand during 1981 – 2008 varied from 0 – 11.6 per 1,000,000 (median = 3.3 per 1,000,000). The p value from a Shapiro-Wilk hypothesis test for the normality of the annual cumulative incidence data was greater ($p = 0.094$) than the critical value, 0.05. The null hypothesis (H_0) that data come from a normally distributed population is accepted. It was appropriate to construct a linear regression model to predict the changing in the cumulative incidence over time. The fitted model had a negative regression coefficient ($\beta = -0.22$). The F statistic for the regression showing a highly significant decreasing trend ($F_{(1, 26)} = 21.88$; $p < 0.001$).

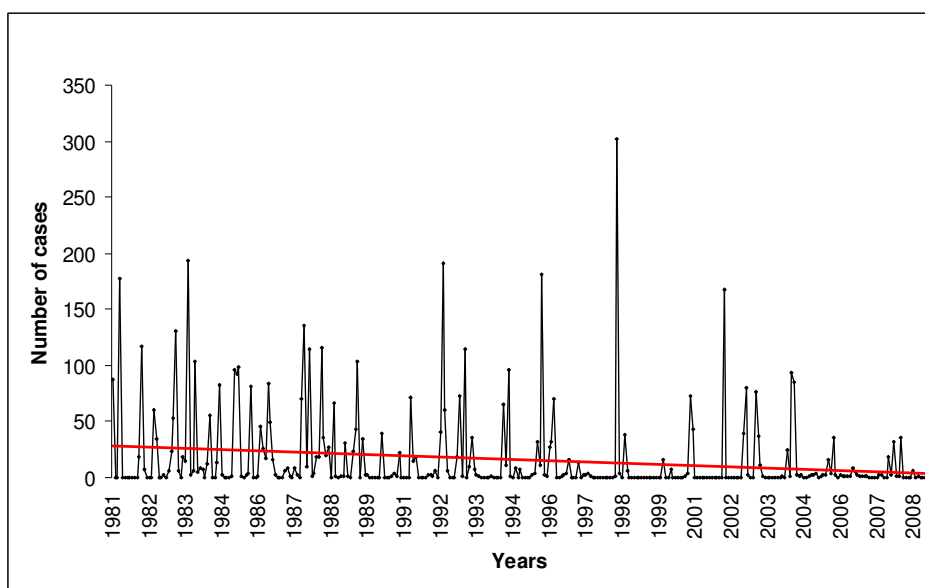
Figure 2-14 Annual cumulative incidence of human trichinellosis (per 1,000,000) in Thailand between the period 1981 – 2008



2.4.6 Monthly trend and seasonal variations of human trichinellosis

Between 1981 – 2008, the number of reported cases by month and the linear negative trend generated by the equation $y = -0.072x + 28.363$ is shown in Figure 2-15. A slight but significant decrease in reported cases of trichinellosis was observed ($F_{(1, 334)}=12.37, p < 0.0005$).

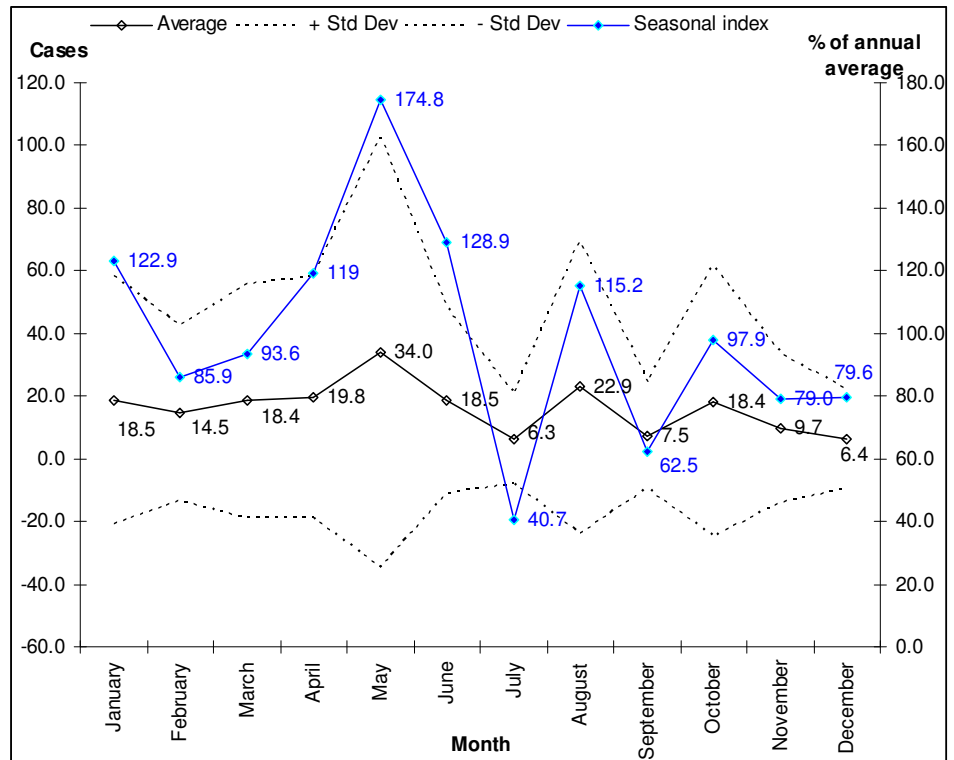
Figure 2-15 Monthly time series of trichinellosis cases in Thailand and trend, 1981-2008



Each month, trichinellosis cases varied from zero to 302. Average monthly cases between the period 1981 – 2008 varied from 6.3 to 34.0 cases, the month with highest cases was May while the month with the lowest cases was July (Figure 2-16). The monthly indices of trichinellosis shows how each monthly value relates to the annual average median number of cases equal to 100. For the years 1981 – 2008, the months of January, April, May, June and August have values higher than the average median value. Based on the seasonal index, 122.9% of the average monthly cases occurred in January, 119% of the average monthly cases occurred in April, 174.8% of average monthly cases occurred in

May, 128.9% average monthly cases occurred in June and 115.2% average monthly cases occurred in August. High seasonal indices were observed in every season. The lowest seasonal index occurred in July, with 40.7% of average monthly cases occurred in this month (Figure 2-16).

Figure 2-16 Monthly average with ± 1 standard deviation and the seasonal index for human trichinellosis in Thailand between the period 1981 and 2008



2.4.7 Gender of trichinellosis cases

For the 22 years for which gender data were available, there were 3,976 human trichinellosis cases consisting of 2,453 male cases and 1,523 female cases. The sex ratio of cases in each year varied from 0.9:1 to 3.5:1. Males outnumbered female cases in every year except 1995 when the male: female ratio was 0.9:1. The χ^2 values that measured how closely the observed number of male and female cases fit with that expected numbers (sex ratio 1:1) were large ($\chi^2_{df=1} = 4.0 - 48.89$, $p < 0.05$) in sixteen out of 22 years, indicating the deviation of observed cases from

expected cases. The number of male cases was substantial higher than the number of female cases in those years (see Appendix 4).

2.4.8 Age-specific trichinellosis incidence rates

Age-specific incidence rates of human trichinellosis in Thailand and the total number of cases in each age group for 28 years are shown in Table 2-3. The highest number of trichinellosis cases and the highest incidence rate was observed in the 25 – 34 years old age group (n= 1,415; *I* = 159.48 per 1,000,000 person-year). The incidence rates of all age groups except children (0 – 14 years old) and the elderly (over 64 years old) were higher than the crude incidence rate (3.34 per 1,000,000 person-year).

Table 2-3 Age-specific incidence rates of human trichinellosis (per 1,000,000 person- years) in Thailand between 1981 and 2008

Age group	Cases ^a	Person- year	Rates (1981 – 2008)	Annual rates
0 – 14	699	18,703,629	37.37	1.33
15 – 24	964	9,935,414	97.03	3.47
25 – 34	1,415	8,872,665	159.48	5.70
35 – 44	1,111	7,811,488	142.23	5.08
45 – 54	668	6,063,032	110.18	3.93
55 – 64	393	3,608,532	108.91	3.89
≥65	195	3,266,066	59.70	2.13
Total	5,445	58,260,852	93.46	3.34

^a Total number of cases in each age group, of which their age were known

2.4.9 Time trend in age specific trichinellosis incidence rates

Time trend in age-specific incidence rate of human trichinellosis in Thailand in 1981 – 1987, 1988 – 1994, 1995 – 2001 and 2002 – 2008, are shown in Figure 2-17. Between 1981 – 1994, trichinellosis incidence rates sharply decreased in all age groups. The rates continued to decrease until 2001 among individual of all age groups except child (0 – 14 years old) and elderly (over 64 years old) in which the incidence rates were steady from 1988 until 2008. The lowest incidence rate over time was in age group 0 – 14. Rate ratios of human trichinellosis in different age groups compared with age group 0 – 14 in four time periods are shown in Figure 2-18, the highest rate ratio belonged to age group 25 – 34 in 1981 – 1987 and age group 35 – 44 thereafter. From 1995 the rate ratios in age group 0 – 34 were smaller than those in age – group 35 – 64. Between 1981 – 2008, there was no difference in the average rate from one age-group to another based on the F test ($F_{df=6}=1.60, p = 0.19$). There was significant difference in the average rate from one period of time to another ($F_{df=3}=10.44, p = 0.0001$).

Figure 2-17: Time trend in age-specific incidence rates of human trichinellosis in Thailand between 1981 and 2008. Age-specific incidence rates were compared in four time-period, 1981 – 1987, 1999 – 1994, 1995 – 2001 and 2002 – 2008

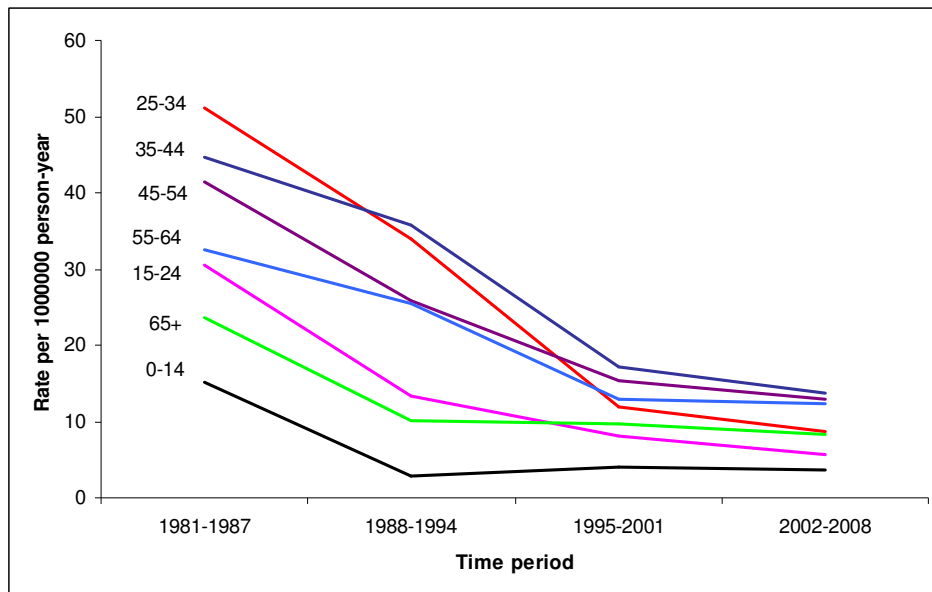
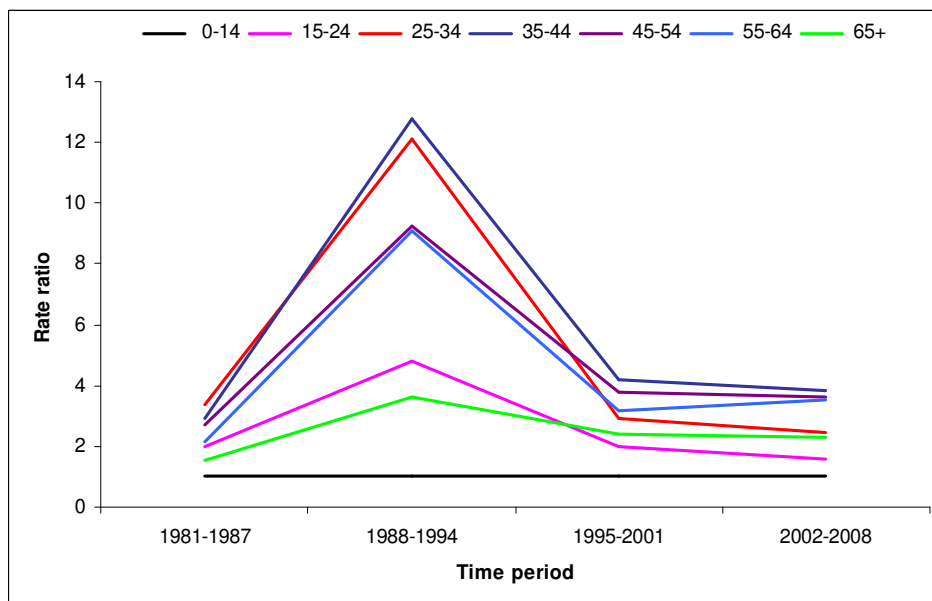


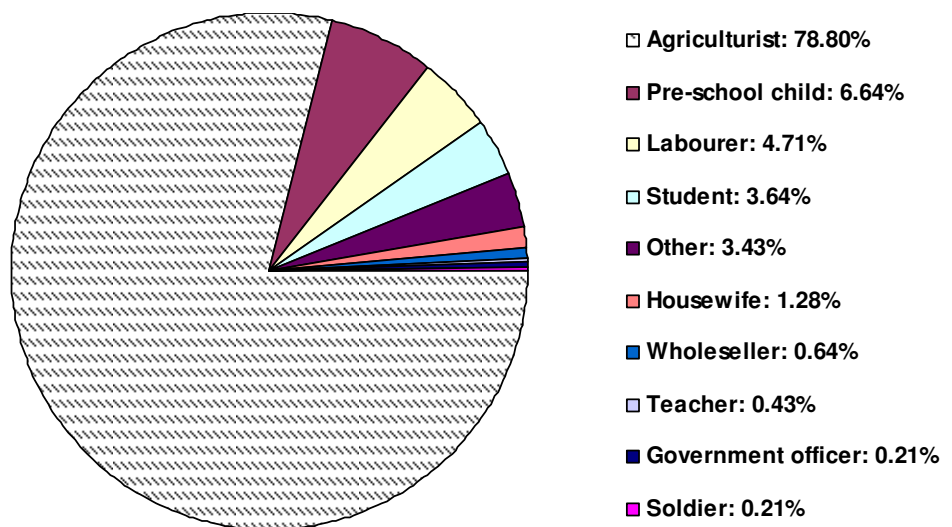
Figure 2-18 Time trend in rate in different age group of human trichinellosis in Thailand between 1981 – 2008: using age group 0 – 14 as a baseline



2.4.10 Occupation, residential area and ethnicity of trichinellosis cases

Data for the occupations for each case and for their area of residence were available from January 2003 – August 2008. The occupations of 469 cases are shown in Figure 2-19. The majority of cases were among agriculturists. Pre-school children and students accounted for about 10 percent. The remainder were labourers, housewives and others. Ninety-eight percent of cases lived in the rural areas and 2% lived in the urban areas. Little data were available for ethnicity. Those that mentioned ethnicity indicated the patients belonged to Thai ethnic groups and to Chinese and Thai hill tribe ethnic groups (Karen, Akha, Yao and Lesu).

Figure 2-19 Occupations of trichinellosis cases in Thailand during 2003 – 2008



2.4.11 Sources of trichinellosis outbreaks

One-hundred-and-six outbreaks were described in the report text. Potential sources were cited for 91 of the outbreaks. Of the fifteen outbreaks for which potential sources were not described all occurred in

the Northern Region. This region contained the majority of the outbreaks that occurred in Thailand during 1981-2008.

Overall, in the Northern Region, the potential sources of outbreaks were described in the reports as meats from local pigs and wild boars. A few outbreaks were associated with smuggled meats. Unlike the outbreaks in Northern Region, the major potential sources of outbreaks in the other regions were the meat of wild boar and other wild animals (barking deer, monitor lizard and turtle).

Table 2-4 Potential sources of 106 human trichinellosis outbreaks in Thailand during 1981-2008

Region	Total	Species and source of <i>Trichinella</i> infected meat						
		Local			Smuggled			No data
		Pig	Wild boar	Other	Pig	Wild boar	Other	
North	98	55	26	-	1	1	-	15
Northeast	2	1 ^a	-	-	-	-	1 ^b	-
Central	3	1	1	1 ^c	-	-	-	-
South	3	-	2	1 ^d	-	-	-	-
Total	106	57	29	2	1	1	1	15

^a infected in Northern region, ^b barking deer meat, ^c monitor lizard, ^d turtle

The suspected contaminated meat from pigs originated exclusively from home slaughtered animals. This home-slaughtered meat was mostly distributed to the affected individuals when it was sold or shared among neighbours within the villages (Figure 2-20). Suspected meat from wild animals was also believed to be sold or shared among neighbours, sold to other villages and also sold in markets.

Figure 2-20 Potential sources and distribution routes of suspected meat in Thailand, 1981-2008

Species and source of animal	Distribution of meats
<p style="text-align: center;"><u>Domestic pig</u></p> <p>Local</p> <ul style="list-style-type: none"> ▪ Reared in a village ▪ Acquired from other village <p>Smuggled (seldom)</p>	<p>Sold to neighbours</p> <p>Shared among neighbourhoods</p> <p>Sold in the market (seldom)</p> <p>Shared raw meat</p> <ul style="list-style-type: none"> ▪ Wedding ceremony ▪ Funeral ceremony ▪ New Year ceremony ▪ Ritual events
<p style="text-align: center;"><u>Wild boar & other wildlife</u></p> <p>Local</p> <ul style="list-style-type: none"> ▪ Subsistence hunting <p>Smuggled (seldom)</p>	

2.5. Discussion

Between 1981 and 2008 at least 106 outbreaks of trichinellosis, with 5,455 cases and 25 deaths occurred in Thailand. The disease occurred in every region and affected 31 provinces. The majority of cases and affected provinces were in the Northern Region. Both domestic pigs and wildlife, particularly wild boar, were major sources of infection in the Northern Region. Wild boar and other wildlife were major sources of infection in other regions.

2.5.1 Spatial distribution of human trichinellosis

The number of trichinellosis affected provinces between 1981 and 2008 was three times higher than between 1962 – 1983. Between 1962 – 1983 there were 15 affected provinces outside the Northern Region compared with only one affected province outside the Northern Region between 1962 – 1983 (Dissamarn & Indrakamhang, 1985). The public health

system in Thailand has improved considerably in recent years (Bureau of Policy and Strategy, 2006), both in terms of disease detection and treatment and access to health care services. This may have contributed to the improved detection of trichinellosis in new areas in 1981 – 2008. Another factor contributing to the apparent spread of trichinellosis is the migration and internal travel of people from areas that have a culture of eating raw meat to new areas. At least three trichinellosis outbreaks outside the Northern Region involved migrants and travellers. Two outbreaks occurred in the Northeast Region and a further one occurred in the Southern Region (Bureau of Epidemiology, 1991; Jongwutiwes *et al.*, 1998).

Population movements, either national or international, as factors contributing to trichinellosis outbreaks have been reported from several countries for example China, the USA, Israel and Germany (McAuley *et al.*, 1992; Graves *et al.*, 1996; Levy *et al.*, 2001; Hefer *et al.*, 2004; Cui *et al.*, 2006b; Jansen *et al.*, 2008). In China, people migration and increased importation of pork products from central to western areas of the country introduced *Trichinella* and the occurrence of trichinellosis in the latter areas. In USA and Israel, trichinellosis outbreaks occurred among migrants from Southeast Asia, Israel in particular where several outbreaks occurred among migrant workers from Thailand who consumed tradition raw foods made from wild boar. During 1996 – 2006 in Germany, mean annual rate of trichinellosis among immigrants from Southeast European Countries was 0.3 cases/100,000 compared with 0.01 cases/100,000 in German population.

Human trichinellosis in Thailand is non-randomly distributed in different provinces as has been known (Dissamarn & Indrakamhang, 1985; Khamboonruang, 1991). The incidence rates of trichinellosis between 1981 and 2008 in the five northernmost provinces were at least four times higher than that in other provinces. Clusters of trichinellosis with

significantly high incidence were detected in Northern Region, the northernmost provinces in particular, over the period of study. Both raw meat consumption practice and presence of trichinellosis in domestic pigs were major reasons to be responsible for high incidence of trichinellosis in this region (Dissamarn & Indrakamhang, 1985). It is important to note that custom of raw meat consumption prevails in the North and the Northeastern Regions of Thailand. Thus, the absence of trichinellosis cases attributed to raw pork in the Northeastern Region supported that raw meat consumption and prevalence of trichinellosis in pigs play equal role in the epidemiology of trichinellosis in Thailand. In addition, it may be inferred further that trichinellosis in domestic pigs in the Northeastern Region of Thailand was rare or pigs in this region present negligible level of risk to public health in terms of *Trichinella* transmission. This evidence underpins the need to control trichinellosis in domestic pigs in the Northern Region in order to effectively prevent infection in humans.

Geographical variations of human trichinellosis have been also described in Romania (Blaga *et al.*, 2007). However, the major factor that best explained high incidence of trichinellosis in humans there, was raw meat consumption. High incidence of human trichinellosis was found in Transylvania County where a custom of raw meat consumption prevails among those in the population of German origin. The significantly high incidence rate has been observed in this county despite the fact that prevalence of trichinellosis in pigs in this area were about a half lower in home-slaughtered pigs and eight times lower in abattoir-slaughtered pigs when compared with other counties (Blaga *et al.*, 2007).

The excess risk maps of trichinellosis in the seven year period from 1981 – 2008 demonstrated that the magnitude of trichinellosis occurred in the northernmost province always exceeded that occurred in other provinces nationwide. These areas may be addressed as trichinellosis high risk areas in Thailand both in the past and the present. The main geographical

character of the five northernmost provinces of Thailand is mountain covered with forest. It provides a good habitat for wildlife, natural reservoir host of *Trichinella*. In addition, the largest proportion of hill tribe ethnic groups that traditionally practiced free-range scavenging pigs rearing is contained in these provinces. The majority of them live in mountain and forest areas. Therefore, the likelihood that *Trichinella* transmit between wildlife and domestic pigs as well as within domestic pig population is high. The combinations of these factors perpetuate *Trichinella* in domestic pigs, the major source of human trichinellosis.

In recent years (2002 – 2008), the occurrence of trichinellosis with the magnitude comparable to northernmost provinces was observed. It indicated that although trichinellosis occurs outside Northern Region is sporadic, large outbreaks may occur. Thus, nationwide public education to raise awareness of trichinellosis is still required to be in placed.

2.5.2 Temporal patterns and trend of human trichinellosis

Trichinellosis is not a common disease in Thailand in terms of its frequency of occurrence. However, when it does occur infections usually involved a large number of cases. This is mainly due to the Thai tradition of sharing/consuming trichinellosis risk food at social gathering events such as New Year, wedding ceremony and others (Khamboonruang, 1991; Kaewpitoon *et al.*, 2006). Several social gathering events take place throughout the year. Therefore, trichinellosis in Thailand has no particular seasonal pattern. In other countries for example China, Bulgaria and Argentina a seasonal pattern of human trichinellosis does exist (Wang *et al.*, 1998; Ribicich *et al.*, 2005; Kurdova-Mintcheva *et al.*, 2009). Trichinellosis in these countries is associated with winter months of the year. Trichinellosis in China is related to the winter festivals (Wang *et al.*, 1998) while in Bulgaria it is related to the hunting season

and mass home-slaughter of pigs (Kurdova-Mintcheva *et al.*, 2009) and in Argentina outbreaks are related to the mass home-slaughter of pigs (Ribicich *et al.*, 2005).

Despite an absence of significant seasonal pattern of trichinellosis in Thailand, this study demonstrated that a high number of trichinellosis cases were reported in particular months of the year. On average more trichinellosis cases occurred in Thailand in January, April – June and August when compared to other months. New Year festivals may relate to higher number of trichinellosis cases in the first half of the year. This is of different ethnic background, celebrate New Year festivals in different months from December to April. Mass home-slaughtering of pigs has been observed during New Year in a highland village in Nan Province, Northern Region (Nakai, 2009). The other event that explains the greater number of cases particularly in May and June is the start of field crops planting season in May. This time of the year provides opportunity for farmers to hunt wild boar in the farmland as well as sharing of food during communal work and hence may also associate with large outbreaks similar to that occur during New Year celebrations.

Recently trichinellosis incidence in Romania, China and Argentina has been observed to have dramatically increased (Wang & Cui, 2001a; Ribicich *et al.*, 2005; Neghina *et al.*, 2010). In China and Argentina the infections have been recorded in historically trichinellosis-free area (Liu & Boireau, 2002; Costantino *et al.*, 2009). The reasons for those changes involved economic (economic effluent in China and economic crisis in others countries), or socio-economic factors (Romania and Argentina). In Southeast Asia, Laos and Vietnam, has seen trichinellosis as emerging or re-emerging disease in recent years (Sayasone *et al.*, 2006; Barennes *et al.*, 2008; Vu Thi *et al.*, 2009). An improvement in the detection of disease could partly explain for the high reported incidence of trichinellosis there (Barennes *et al.*, 2008; Taylor *et al.*, 2009). In contrast

to the countries mentioned above, the USA has experienced a decreasing trend of trichinellosis (Moorhead *et al.*, 1999) during the past decades. This trend has been attributed to the reduction of trichinellosis in domestic pigs (Roy *et al.*, 2003). This study showed that the incidence of human trichinellosis in Thailand has in accordance with that occurred in USA. The incidence was observed to gradually decrease in the period 1981 – 2008. Because the surveillance system has not changed, this decreasing trend is a true reflection of the trichinellosis status of Thailand. The decline could be explained by three factors: the pig production systems in Thailand have altered to systems with a low or negligible risk of trichinellosis, there is a decreased trichinellosis prevalence in pigs in high risk areas due to better production practices or possible reduction in consumption of raw meat as a result of continuing public education.

Over the last two decades pig production in Thailand has changed from a backyard production system to a commercial production system. The commercial production system supplies about 80% of the pork for domestic consumption (Cameron, 2000). This system employs good production practices that prevent *Trichinella* contamination in the pig food chain. Pigs production under such systems have a negligible risk of trichinellosis (Gamble *et al.*, 2001). National and regional surveys for trichinellosis in commercial pig farms, (using both the ELISA and the digestion methods), did not detect any infected pigs (Vitoorakool & Vitoorakool, 2005; Indrakamhang *et al.*, 2006; Panichabhonse, 2006; Vitoorakool *et al.*, 2007).

The majority of human trichinellosis cases in Thailand have been historically related to consumption of hill tribe pigs (Dissamarn & Indrakamhang, 1985). These pigs are raised in highland areas by Thai hill tribe ethnic groups. The hill tribe pigs are at risk of getting *Trichinella* infection from domestic and wildlife sources because of poor husbandry

practices. Young (1962) reported that the pigs of the Thai hill tribes were raised in substantial pens or allowed to roam free for food around the villages (Young, 1962). According to Nakai (2008), some improvements in pig husbandry practices were observed in a highland Thai hill tribe village. Pigs were fed with agricultural products and natural plants and most were kept in a semi-confined condition (although some pigs were still allowed to roam free).

Food-borne diseases remain a major public health problem but safe food education has been carried out. Public education and health promotion is believed to be altering public perception (especially in the younger generation) (National Statistical Office, 2005). It may be responsible for the decreasing in incidence of trichinellosis among population of all age groups in Thailand. From 1988 until 2008, a decrease in incidence rate of trichinellosis was observed in individuals aged 15 – 64 years old. It has been accepted that opisthorchiasis, a liver fluke infestation, in Thailand was also successfully controlled because of the extensive improvement of the health care system and the socio-economic development besides the availability of anthelmintic chemotherapy (Jongsuksuntigul & Imsomboon, 2003).

2.5.3 Potential sources of human trichinellosis

Based on the reported data, the potential sources of human trichinellosis in Thailand have not changed. These are the domestic pigs reared by the hill tribes and wild boar in the Northern Region and wild boar or other wildlife in other regions. The role of the hill tribe pigs as the potential sources of trichinellosis in Northern Region as well as the negligible risk of trichinellosis in other domestic pigs is supported by a number of studies. Vitoorakool *et al.* (1989) detected antibodies against *Trichinella* infection in 82 out of 456 hill tribe pigs from three provinces in the

Northern Region (Chiang Mai, Chiang Rai and Nan Provinces). During the period 1967 – 1983, 19 out of some 1.3 millions pigs at municipal abattoirs in the Northern Region were positive for *Trichinella* larvae when using the trichinoscopic method and all of the infected animals were hill tribe pigs (Dissamarn & Indrakamhang, 1985). There were also surveys carried out using the ELISA or the ELISA and the digestion method to determine trichinellosis prevalence in domestic pigs outside the Northern Region. These surveys did not detect any trichinellosis-positive pigs (Indrakamhang *et al.*, 2006; Panichabhonse, 2006). Pozio (1998, 2009) mentioned that once a *Trichinella* infection occurs in a population of domestic pigs, it can be perpetuated for many years within the neighbouring environment because of the scavenging and eating habits of pigs. These explanations and the surveillance information for Thailand suggest that *Trichinella* infection is established in the domestic pig population only in the Northern Region.

Many species of wildlife in Thailand are protected from hunting by law. In addition, most wildlife habitats in the country were made into wildlife protection areas in the 1960s (Department of National Parks Wildlife and Plant Conservation, 2009). These regulations restrict opportunities for any trichinellosis surveys of wildlife in Thailand. The presence of trichinellosis in wildlife is indicated through wildlife associated human trichinellosis. Through the process of outbreak investigation the species of wildlife reservoirs and *Trichinella* can be confirmed (Jongwutiwes *et al.*, 1998; Khumjui *et al.*, 2008). Wildlife increases in importance in countries where trichinellosis incidence in domestic pigs has declined or is absent for example the USA and Canada (Roy *et al.*, 2003; McIntyre *et al.*, 2007). Data on implicated meat may fill the gap from limit prevalence study of trichinellosis in wildlife. This data should be included in the surveillance system for monitoring the trend of animal reservoir hosts in Thailand.

2.5.4 Characteristics of trichinellosis

cases

Trichinellosis was observed almost exclusively in agricultural areas in Thailand. The occupations and residential areas for the trichinellosis cases occurring in Thailand were correlated for this current study. About 80% of the cases were agriculturists and 98% of the cases lived in rural locations. These were contrast markedly with a survey in China and Romania. In these countries cases were not involved in agriculture occupation and they were in urban areas. Cases that occurring during the period 1992 – 1996 in China were mainly in workers, officers and merchants (Wang *et al.*, 1998). Trichinellosis cases reported from Romania have been of people predominantly (70%) living in urban areas and the majority of them were labourers (Neghina *et al.*, 2010). An origin of implicated meat there and in Thailand may be different. In Thailand, it is in the rural areas where pigs are reared for the owner's own use and to be home-slaughtered or where wildlife could be obtained for consumption. Hence, the characteristics of cases both their residential location and occupations were closely related to the origin of implicated meat. This specific characteristics of trichinellosis cases and trichinellosis high risk areas in Thailand similar to that in Papua New Guinea, Canada and Greenland where significantly high prevalence of trichinellosis seropositivity or trichinellosis cases were observed among population who lived in close proximity to hunting areas or in hunting communities (MacLean *et al.*, 1989; Proulx *et al.*, 2002; Owen *et al.*, 2005; Moller, 2007). Because of the awareness of trichinellosis, communities in trichinellosis high risk areas in Canada have been participated in pre-consumption testing programme. In this area where walrus are the main source of *Trichinella* in humans, harvested walrus are tested for *Trichinella* prior to consumption (Proulx *et al.*, 2002). The finding in Thailand supported specific preventive measures to be implemented in the rural endemic areas.

Between 1981 and 2008, trichinellosis in Thailand was biased towards male cases, suggesting that raw meat consumption habit was more common in men than in women. Raw meat consumption may be linked to alcohol consumption which is common in Thai men during social gatherings. The association between raw meat consumption and alcohol consumption is believed to occur in Thailand (Anon, 2007; Kusolsuk *et al.*, 2010). A survey showed that alcohol consumption among Thai men is about 51.0% compared with only 8.8% among women (National Statistical Office, 2007). In other countries, Owen *et al.* (2005) investigated the prevalence of trichinellosis in the hunting villages in Papua New Guinea and associated the higher sero-prevalence of trichinellosis in men than in women with hunting activity. Surveillance data from the U.S.A. and Spain from 1990 – 2007 also showed that trichinellosis affected men more than women. Males accounted for more than 55% of the cases reported (55 – 72%) (Moorhead *et al.*, 1999; Roy *et al.*, 2003; Ambrosioni *et al.*, 2006; Kennedy *et al.*, 2009). In contrast, surveillance data from Romania showed trichinellosis affected both genders almost equally, with 53% of cases being in male patients (Neghina *et al.*, 2009). A study in semi-rural area in Mexico demonstrated that being female was at higher risk of trichinellosis seropositivity than male (de-la-Rosa *et al.*, 1998). However, they mentioned that the result was in disagreement with an earlier observation study in Mexico in which among cadavers examined, the prevalence was higher in men than in women. Reason for gender discrepancy in the prevalence of trichinellosis was not explained in these reports.

In the present study, in terms of age, people between 25 and 64 were most affected by trichinellosis. The highest annual trichinellosis incidence rates over the study period were in individuals in the age group 25 – 34. From 1988 – 2008, the highest annual incidence rate was among people of 35 – 44 years of age. The table below shows trichinellosis most

affected age groups in the USA, Spain and Romania. Among these countries Romania has the highest incidence rate of trichinellosis (51.0 per 1,000,000; 1980 – 2004) with the youngest age group most highly affected with trichinellosis. For the situation in Thailand, some reasons that may explain why the highest incidence has moved up from 25 – 34 to 35 – 44 years are: an increased awareness especially among young generations from public health education (National Statistical Office, 2005) or migration of the young working-age from rural areas (Charsombut, 1981). A study by Punpuing (2004), based on the longitudinal survey of Kanchanaburi Demographic Surveillance System in Kanchanaburi Province in the Central Region, reported that the proportion of migrants at ages 15-29 was the highest compared with those of other age groups.

Year	Country	Most trichinellosis-affected age	Reference
1991 – 1996	USA	31 – 45	(Moorhead <i>et al.</i> , 1999)
1997 – 2001	USA	30 – 39	(Roy <i>et al.</i> , 2003)
2002 – 2007	USA	40 – 49	(Kennedy <i>et al.</i> , 2009)
1994 – 2003	Spain	32 (mean)	(Ambrosioni <i>et al.</i> , 2006)
1990 – 2004	Romania	20 – 29	(Neghina <i>et al.</i> , 2009)

2.6. Limitations

It has been believed that reported cases of trichinellosis are an underestimate of the actual cases (Takahashi *et al.*, 2000; Wang *et al.*, 2006b; Kaewpitoon *et al.*, 2008). There are two issues that may be responsible for underestimation, first, surveillance systems may lack coverage, secondly, it may not be possible to detect light or subclinical cases. Underestimates of cases due to limited surveillance may explain a lack of case finding in Thailand, particularly in the 1980s and earlier. For example, trichinellosis cases attributed to dog meat consumption in north-eastern provinces (Chitchang *et al.*, 1985) were not recorded in the surveillance system. This has not been an important issue since the 1990s due to improvements in health care systems as well as the surveillance

system (Bureau of Epidemiology, 2005; Bureau of Policy and Strategy, 2006). Nonetheless, there is still concern on the sensitivity of the surveillance system to detect light or subclinical cases in Thailand, similar to that in other countries. Isolated cases may be diagnosed as other diseases with similar symptoms such as flu or rheumatism. Trichinellosis may not be correctly diagnosed in the first visit particularly in non-endemic areas.

2.7. Summary

The ecosystem in Thailand (with about a half of the land areas covered with forest in the Northern Region and about 30% of forest nationwide) supports the maintenance of wild animals which in turn maintains the circulation of *Trichinella* spp. in the sylvatic cycle. Coupled with a custom of raw meat consumption among Thai subgroup populations, trichinellosis continues to occur in indigenous Thais. Historically trichinellosis infection in Thailand was linked to consumption of domestic pigs reared by hill tribes with poor management systems. It was believed that the ecosystem of the hill tribal habitations and the poor pig management practices results in the perpetuation of *Trichinella* in domestic pigs in the highland areas (Khamboonruang, 1991). This retrospective investigation of human trichinellosis surveillance data in Thailand between the period 1981 – 2008 shows that:

1. Trichinellosis in Thailand has decreased in incidence. The overall annual incidence rate of trichinellosis was 3.37 per 1,000,000 person/year, the highest annual incidence rate was in the Northern Region (16.85 per 1,000,000 person-year).
2. The incidence rates were highest in the five northernmost provinces including Mae Hong Son, Nan, Chiang Rai, Chiang Mai and Phayao Provinces (943.67 – 2254.61 per 1,000,000 person/year at risk over 28 years). The clusters of human trichinellosis have remained in these provinces up to 2008. The occurrences outside these areas were sporadic and rarely exceed

the magnitude that has been observed in the five northernmost provinces.

3. More trichinellosis cases were observed in January, April, May and August than other months of the year.
4. The trichinellosis cases were more biased to men with a male to female ratio between 0.9 : 1 to 3.5 : 1. In term of age of the trichinellosis cases, the highest annual incidence rate occurred among the individuals aged between 25 – 34 years old (4.70 per 1,000,000 person-year). The highest annual incidence rates were observed among the individuals aged 25 – 54 years between 1981 – 1987 and among the individuals aged 35 – 44 years old between 1988 – 2008.
5. The majority of cases were agriculturists who live in rural areas. The highest number of the outbreaks linked to the domestic pig as the source of infection.

Between 1981 – 2008, human trichinellosis in Thailand has significantly decreased in the magnitude but it has not changed in the spatial patterns. Trichinellosis is likely to continue occurring in the Northern Region, unless additional control measures target specifically in high risk area are put in place. Next chapters investigate the epidemiological characteristics of trichinellosis in one of the trichinellosis endemic foci, Nan Province in the Northern Region in order to

- (i) determine the prevalence and identify risk factors of trichinellosis in domestic pigs
- (ii) identify risk factors of human trichinellosis
- (iii) identify factors influencing trichinellosis through knowledge, attitudes and practices towards trichinellosis in various ethnic communities.

3. Chapter 3 - Characterisation of factors influencing swine trichinellosis in Nan Province, Northern Region, Thailand

3.1. Study aims

This study investigated pig holdings, domestic pigs and wildlife in Nan Province in the Northern Region of Thailand in order to:

- (i) characterise the nature of pig holdings.
- (ii) estimate the seroprevalence of trichinellosis in pigs.
- (iii) characterise sero-positive pigs.
- (iv) identify the risk factors that lead to trichinellosis in pigs
- (v) estimate the prevalence of trichinellosis in wild animals caught for human consumption.

3.2. Introduction

Pigs are infected with *Trichinella* only through the consumption of food contaminated with the *Trichinella* larvae. Thus, there are two key factors that can lead to *Trichinella* infection in pigs. First, production and management practices in place allow the exposure of pigs to foods containing *Trichinella*. These include allowing pigs to ingest *Trichinella* through various means, such as garbage or leftover food, cannibalism, tail biting, wildlife carcasses, live wildlife, rat carcasses and keeping pigs that free-range or scavenge. The second consideration is the infection rates of trichinellosis present in the animal reservoir to which the pigs could be exposed. Regardless of the type of management practices, if the prevalence of trichinellosis in any reservoir hosts to which the pigs may be exposed is very low or absent then risk of swine trichinellosis is also low or negligible (Karn *et al.*, 2008).

In Thailand, persistent foci of human trichinellosis attributed to pigs have remained in the five northernmost provinces of the country including Mae Hong Son, Nan, Chiang Rai, Chiang Mai and Phayao Provinces (Chapter 2). Historically, swine trichinellosis in Thailand has been related to pigs reared in the highland areas of the Northern Region, known as the 'hill-tribe pigs'. The infection rates of trichinellosis in hill-tribe pigs during the period 1970 – 1990 were estimated between 11.4% - 32.3% (Dissamarn & Aranyakananda, 1975), compared with 0.001% for the regional rate (Dissamarn & Indrakamhang, 1985). Poor management practices, and scavenging in particular, have been associated with *Trichinella* infection among these hill-tribe pigs. The transmission cycle of *Trichinella* from wildlife to pigs has been mentioned in the proposed epidemiology of the disease (Khamboonruang, 1991) but the levels of trichinellosis in wildlife have never been investigated. A recent survey of trichinellosis in hill-tribe pigs did not find any infected animals (Vitoorakool *et al.*, 2007). However, the disease in humans, although exhibiting a decreasing trend in incidence, continues to be seen. To understand more about the status of swine trichinellosis in Thailand this study investigated the two key factors believed to be critical to there being *Trichinella* infection in pigs, namely the production and management practices of domestic pigs and the infection rate of trichinellosis in wildlife, with emphasis on wildlife caught for local consumption.

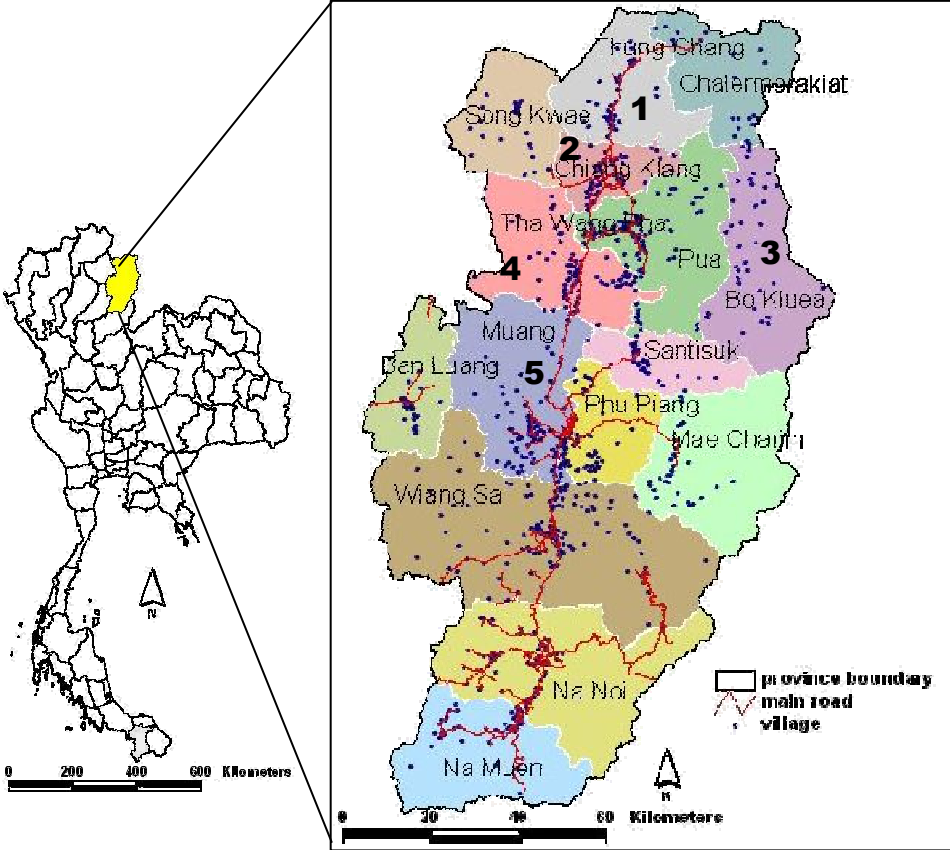
3.2.1 Description of Nan Province

Nan Province is one of the five northernmost provinces and is located in the Northern Region, about 668 km to the north of Bangkok. The province lies between the coordinates 18° 46' 04.07" N and 100° 46' 56.74" E, comprises an area of 11,472 km² and shares an international boundary with Laos in the north and the northeast.

The main geographical character of Nan Province is mountains covered with forest providing a good habitat for wildlife. Approximately 40% of Nan Province is within the Luang Pra Bang and Phi Pan Nam Mountains ranges which are about 600 – 1,200 metres above sea level. Eight-six percent of the province's area is composed of land with a slope of more than 30 degrees. The breakdown on land composition in Nan Province, by area, gives 47.9% mountain and forest, 39.3% degraded forest, 12.2% agriculture and 0.6% residential (Ministry of Interior, 2006).

The climate of Nan Province reflects the typical climate of the upper Thai mainland, with three seasons: the rainy season, winter and summer. The mean temperature across the year is 25.49°C (ranging from 7.7 to 38.4 °C). The annual rainfall recorded at the Nan meteorological station is about 1,200 mm. The province is mostly dry throughout the winter, with only 1-3 days of sparse rainfall (Thai Meteorological Department, 2005). Heavy rainfall can cause landslides and disrupt transportation to some remote villages.

Figure 3-1 Geographical location of Nan Province and its administrative areas; the seroprevalence study of trichinellosis in pigs were undertaken in (1) Thung Chang, (2) Chiang Klang, (3) Bo Kluea, (4) Tha Wang Pha and (5) Muang Districts



3.2.1.1. Administrative areas and human population in Nan Province

The administrative areas of Nan Province are divided into 15 districts (Figure 3-1), 98 Tambon and 889 villages (Nan Provincial Operation Centre, 2007). There are eight municipalities in Nan Province, the majority of which are rural areas. According to the criteria defined by the Ministry of Interior (Chapter 2) 178 villages can be classified as being highland areas with the rest being lowland areas.

In 2006 Nan Province had a population of 464,262, living in 136,039 households (Table 3-1), with an average population density of 40.5 per

km². The population in the highland areas, estimated from the Highland Communities' Census in 2002 and the registered population in Nan Province in 2006, accounted for 19.6 percent of the total population. There were 90,787 inhabitants and 15,706 households in the highland areas of Nan Province. These highland populations comprise six Thai hill tribe ethnicities plus Thai and Thai Luea. These populations can be subdivided as follows: Lua (41,772), Hmong (25,058), Yao (10,684), Khmu (7,468), Mlabri (120), Lahu (20), Thai (4,431) and Thai Luea (1,234), (Department of Social Development and Welfare, 2002). Thus the hill tribes (Lua, Hmong, Yao, Khmu, Mlabri and Lahu) accounted for 18 % of the total population of Nan Province.

Table 3-1 Number of administrative area and statistic of population and households in Nan Province in 2006

Districts	Tambon	Villages	Population	Households
1. Muang	10	109	80,863	27,551
2. Mae Cha Rim	5	38	14,500	3,791
3. Ban Luang	4	26	12,138	3,467
4. Na Noi	7	68	29,968	8,457
5. Pua	12	105	64,585	17,240
6. Tha Wang Pha	10	91	51,551	14,279
7. Wiang Sa	17	127	66,934	20,280
8. Thung Chang	4	40	15,184	4,341
9. Chiang Klang	6	60	27,053	7,794
10. Na Muen	4	48	15,000	4,008
11. Santisuk	3	31	15,667	4,251
12. Bo Kluea	4	39	14,387	3,932
13. Song Kwae	3	25	11,584	2,959
14. Phu Piang	7	60	35,668	11,135
15. Chalermprakiat	2	22	9,180	2,554
Overall	98	889	464,262	136,039

Source: Ministry of Interior, Thailand (2006)

3.2.1.2. Economic and agricultural systems in Nan Province

Economically, Nan Province is ranked among the poorest provinces in the Northern Region. Annual per capita income of the population in 2006 was 37,743 Thai Baht (about US\$1,177). This is lower than both the national-level (119,759 Thai Baht: US\$3,735) and the regional-level (57,914 Thai Baht: US\$1,806) (Office of the National Economic and Social Development Board). Agriculture is the major activity in the province, and field crops are the most important agricultural products. In 2006 the agricultural holdings in Nan Province were primarily utilised for cultivation of field crops, predominantly rice (49.2%), maize (35.9%), soya bean (7.7%) and peanut (2.7%). Permanent crops, rubber plantations (4.45%) and coconut (0.08%), account for less than five percent of the agricultural holdings.

Livestock production in Nan Province includes dairy cows, beef cattle, buffaloes, pigs, goats, sheep, ducks and chickens (Table 3-2). Meat of cattle, pigs and poultry including eggs, produced in the province fall far below local demand. Thus, live animals, meat and eggs are imported from other provinces. In 2007 28,904 pigs, 3,960 beef cattle, 4,155 buffaloes, 14,814,198 eggs and 2,065,476 kg of chicken meat were imported to meet the annual consumption in the province (Nan Provincial Livestock Office, 2006-2007).

Table 3-2 Number of livestock in Nan Province in 2006

Species	Number
Dairy cows	42
Cattle	72,144
Buffaloes	15,274
Pigs	56,714
Goats	1,154
Sheep	346
Ducks	1,8341
Chicken	428,081

Source: Department of Livestock Development (2006)

The pig production systems in Nan Province are primarily small-scale. A small proportion of pigs are produced in the small commercial farms with a holding size of less than 500 pigs. In 2006 15,273 pigs were reared in breeding farms with a holding size of 10 – 50 pigs and 1,658 pigs were reared in fattening farms with a holding size of 50 – 500 pigs (Department of Livestock development, 2006). Pig farming is present in every district. In 2006 some 56,714 pigs and 7,604 holdings in total were reported in Nan Province (Table 3-3). Eleven pig holdings have already been certified by Nan Provincial Livestock Office as having attained the ‘Farm Standard’ (Nan Provincial Livestock Office, 2008).

There are innumerable small pigs or pig-and-cattle slaughterhouses distributed through each district in the province. At least 222 slaughterhouses were recorded in a survey by the Nan Provincial Livestock Office in 2006. However, only six of them were certified by the Nan Provincial Livestock Office as attaining a standard appropriate for a ‘Standard Abattoir’. These are located in Muang, Pua, Wiang Sa, Thung Chang, Chiang Klang and Phu Piang Districts (Nan Provincial Livestock Office, 2006) and all received live animals both from commercial farms in Nan Province and imported from other provinces. The majority of the rest of the small slaughterhouses slaughter both

animals that are kept on a farm and animals that are collected from local small holdings in the province (A. Thorkee personal communication).

Table 3-3 Pig population and holdings in Nan Province, 2006

Districts	Number of pig	Number of holdings
1. Muang	4,728	633
2. Mae Cha Rim	799	125
3. Ban Luang	971	383
4. Na Noi	1,471	280
5. Pua	21,467	1,684
6. Tha Wang Pha	6,627	1,175
7. Wiang Sa	4,808	532
8. Thung Chang	1,453	284
9. Chiang Klang	4,172	352
10. Na Muen	258	32
11. Santisuk	no data	no data
12. Bo Kluea	1,226	350
13. Song Kwae	1,353	462
14. Phu Piang	4,795	306
15. Chalermprakiat	2,586	1,006
Overall	56,714	7,604

Source: Department of Livestock Development (2006)

3.2.1.3. Human trichinellosis and some food-borne diseases in Nan Province

Trichinellosis and other food-borne diseases are common in Nan Province. Poor sanitation results in a higher parasitic infection rate among the people living in remote areas close to the Thai-Laotian border than that in other areas of the province (Waikagul *et al.*, 2006; Waikagul *et al.*, 2002). Between 1981 and 2008 there were 641 reported cases of trichinellosis in Nan Province. The incidence rate over this period was 1486.66 per 1,000,000 person-years (Chapter 2). This incidence rate was, after Mae Hong Son Province, the second highest in the country. Cases were reported almost every year in Nan Province with the exception of 1987, 1989 – 1990, 1993 – 1994, 1999 and 2001. A median number of 4.5 reported cases per year can be estimated (inter quartile range (IQR) 39.25, range 0 – 126) (Bureau of Epidemiology, 1981-2008).

Besides trichinellosis, particularly in rural and the border areas, helminth infections are observed at a significantly high level in Nan Province than

nationally. In 2001, Waikagul *et al* (2002) examined 1,010 faecal samples of primary school children in Nan Province using the formalin-ether sedimentation technique. This demonstrated a helminth infection rate of 60.0%. This infection rate was about three times higher than that estimated infection rate of children and adults at the national level (Waikagul *et al.*, 2002). Uncommon food-borne parasitic infections in Thailand are also recorded. For example pulmonary paragonimiasis (a food-borne parasitic disease caused by the *Paragonimus* fluke that lives in cavities in the lungs or other viscera of mammals) was detected in two children who lived in districts near the Thai-Laotian border (Watthanakulpanich *et al.*, 2005). Food poisoning through bacterial infection or food-associated toxins has also been reported in a large-scale outbreaks. For example, outbreaks of botulism occurred in 2000 and 2006 due to the consumption of contaminated home-canned bamboo shoots. The first outbreak caused illness in 13 villagers and caused two fatalities (Swaddiwudhipong & Wongwatcharapaiboon, 2000). The second outbreak caused illness in 209 villagers, of whom 134 were hospitalized, with 42 requiring mechanical ventilation (Ungchusak *et al.*, 2007). From 2007 to 2009 there were 17 reported cases of *Streptococcus suis* infection, a bacterial infection, associated with the consumption of raw pork (Bureau of Epidemiology, 2007-2009).

3.3. Materials and Methods

3.3.1 Selected study areas

A survey was undertaken to determine the *Trichinella* infection rate in wildlife over the whole area of Nan Province. Meanwhile, a study in pigs focused on the districts in Nan Province that had reported cases of human trichinellosis between 2003 and 2006. Five districts were selected: Thung Chang, Chiang Klang, Bo Kluea, Tha Wang Pha and Muang District (Figure 3-1). The districts contained 337 villages. Twenty-seven villages were in the municipality area where there were no pig holdings and thus

were excluded from the sampling frame. Among the 310 villages included in the study, 99 villages were classified as being in highland areas and 211 villages in lowland areas.

3.3.2 Sampling method and sample size determination

Random sampling (Thrusfield, 2007) was used for the survey of pig holdings and seroprevalence of trichinellosis in pigs. Convenience sampling (Thrusfield, 2007) was used to collect samples from wild animals.

3.3.2.1. Stratified single-stage cluster sampling

Samples were selected by stratified single-stage cluster sampling for the survey of pig holdings and seroprevalence survey of trichinellosis in pigs. The study areas were divided into two strata: highland and lowland areas. Each area contains clusters of villages. Villages were primary sampling units. For the pig-holding survey, all pig holdings in a village were observed. For the survey of trichinellosis in pigs, all pigs aged ≥ 2 months old in a village were observation units.

Sample size was estimated with the major aim of determining trichinellosis seroprevalence in pigs. It was calculated based on one-stage cluster sampling for determining the numbers of sample need to estimate a seroprevalence of trichinellosis in pigs with 90% confidence level (Thrusfield, 2007).

$$g = \frac{1.64^2 (nV_C + P_{\text{exp}} [1 - P_{\text{exp}}])}{nd^2}$$

Where

g = number of clusters to be sampled;

n = predicted average number of animals per cluster;

P_{exp} = expected prevalence

d = desired absolute precision;

V_c = between cluster variance.

$$g_{adj} = \frac{G \times g}{G + g}$$

Where

g_{adj} = adjusted number of clusters to be sampled;

G = total number of clusters in the population

To determine sample size for one-stage cluster sampling the predicted number of animals per cluster, expected prevalence and between cluster variance are required. The actual figures of these values were not known and were assumed as follows: the predicted number of pigs per village was estimated as 20 by dividing the pig population in the province in 2006 by the total number of villages in 2006. The expected prevalence was 2% based on the average prevalence of trichinellosis seroepidemiological surveys in northern Thailand between 1989 and 2002 (Nontasut *et al.*, 1999; Wijit *et al.*, 2002). The between-cluster variance was estimated as 0.001. It was the estimated of variance of trichinellosis prevalence in pigs in 15 villages in northern Thailand (Nontasut *et al.*, 1999; Wijit *et al.*, 2002) multiplied by the estimated design effects of 2.0 (Otte & Gumm, 1997). Based on these assumptions, an expected prevalence of 2% was to be estimated with a desired absolute precision of $\pm 1\%$, and the predicted average number of pigs per cluster was 20. Fifty-three clusters including 1,060 pigs in total were required.

The villages (primary sampling units) were selected from a list of villages in highland and lowland areas. A list of all of the villages in the

highland and lowland areas in Thung Chang, Chiang Klang, Bo Kluea, Tha Wang Pha and Muang Districts was used as the sampling frame. Villages were selected randomly by simple random sampling within each stratum using a random number table (Cannon & Roe, 1982).

Equal numbers of villages were selected from the highland areas and the lowland areas. Ten additional villages were selected in advance from each area to replace any sampled villages with no pig holdings.

Every household in the selected villages and every pig aged ≥ 2 months old were observed in the pig holding survey and seroprevalence survey, respectively. Sampling pigs aged ≥ 2 months old increased the chance of detecting *Trichinella* in pigs. *Trichinella* infection rates in piglets can be low because (i) they are primarily fed on milk and (ii) if infection does occur, it takes about 14 to 42 days to detect sero-conversion by ELISA test (Nockler *et al.*, 2005).

3.3.2.2. Convenience sampling

Convenience sampling, was chosen to sample wild animal specimens. Thrusfield (2007) has described convenience sampling as collection of easily-accessible sampling units. For this reason they may not always be truly representative of the population and a study using convenience sampling method may therefore produce biased estimates (Thrusfield, 2007). Sample of wildlife specimens in Thailand inevitably employs this sampling method because there is limited information on the wildlife populations. Hunting of wildlife is prohibited in most forest areas across Thailand but despite this fact hunting is commonly practised in rural communities. This is a possible portal for collecting samples and screening for *Trichinella* infection. Based on convenience sampling, any meat samples of wild animals that were found and killed in Nan Province by the villagers who agreed to submit samples for *Trichinella* identification were included in this study.

each in two villages in Muang District. They were not subsequently included in the respective data-set. Questionnaires were revised and simplified according to comments from the enumerators. The parameters of interest in the pig holding survey included owner demography, holding location, pig production and management (Box 3-1). The parameters of interest in the trichinellosis seroprevalence survey included pig owner ethnicity, holding information and individual pig information (Appendix 5& Appendix 6).

Every pig holding site was visited on the first visit to observe the environment of the pig holding and to calculate the geographical coordinates and altitude of the holdings. Coordinates of the holding site and altitude were obtained using a handheld GPS device (*eTrex* Garmin International Inc, USA). Cartesian coordinates were recorded for each holding location, and the altitude was recorded in metres.

Box 3-1 Specific parameters included in the questionnaire for the pig holding survey in the selected five districts in Nan Province

- Demography of holding owner: ethnicity, religion, main occupation
- Altitude where the holding is located
- Total number of pigs in the holding
- Total number of pigs in each category: boar, sow, piglet, fattening, replacement
- Main purpose of production
- Main source of pigs
- Pigs' breed
- Management: feed, vaccination, de-worming, dead-pig management, rodent control

Box 3-2 Specific parameters included in the questionnaire for the survey of trichinellosis seroprevalence in pigs in the selected five districts in Nan Province

Owner's ethnicity: Hmong, Khmu, Lua, Thai, Yao

Holding information:

- Co-ordinates and altitude where the holding is located
- Type of area (highland vs. lowland)
- Location of pig holding (residential area vs. farmland)
- Type of holding ('Farm Standard' yes vs. 'Farm Standard' no)
- Home slaughter of pigs occurs (yes vs. no)
- Total number of pigs on the holding
- Dead pig carcass is buried immediately when it is found (yes vs. no)
- Rodent control (yes vs. no)

Information specific to individual pigs:

- Pig's breed (native-breed vs. improved-breed)
- Category of pig: boar, sow, piglet, fattening, replacement
- Pig's age in month
- Source of pigs (breed in a holding vs. acquired)
- Estimated age in months when the pig was acquired into the holding
- Main purpose of production (sell vs. owner's own use)
- Total confine (yes vs. no)
- Pig food contains household leftover (yes vs. no)
- Pig was de-wormed (yes vs. no)
- Expose to wild animals (yes vs. no)
- Expose to other domestic animals (yes vs. no)

3.3.3.2. Blood collection from pigs

About 5 ml of venous blood was collected from each pig at the holding. At the same time, its demographic characteristics, production and management information were collected during a face-to-face interview with the pig owner (see Appendix 7). The blood sample and questionnaire for each pig were labelled with the same number. A disposable 18 gauge x 1.5 inches needle and 10 ml vacutainer, Monovette (Sarstedt Inc, USA), were used to collect blood. The blood sample was left for about 3-4 hours at ambient temperature (15-25 °C). At the District or Provincial Livestock Office, sera were transferred to a 2.5 ml. tube, labelled, and stored at -20°C. Sera were kept at the Nan Provincial Livestock Office before transferred weekly in a cool box to the Upper Northern Veterinary Research and Development Centre in Lampang Province.

3.3.3.3. Wild-animal sampling

Muscle samples from wild animals were collected from animals that were killed and used for consumption in the villages in Nan Province. Samples were obtained from villagers voluntarily from March 2008 to March 2009, inclusive.

In February 2008, during a monthly assembly at the Nan Province Livestock Office, the author informed all the district veterinarians in Nan Province about the study and gave out the instructions for meat sample collection. Later, in the month during a monthly assembly at the District Hall, the district veterinarians gave similar information to village heads and trained-volunteer veterinarians in each district across Nan Province.

Muscle specimens, either from the diaphragm or other sites, including hind limb, fore limb, intercostals, masseter and tongue were collected from wild mammal species. For reptiles, tail muscle was collected. Fist-sized muscle specimens were collected from each carcass and kept in a

clean plastic bag labelled with the common name of the animal species, site of collected muscle, the place where the animal was found and the date when the animal was killed. On the day of collection each specimen was placed in a freezer at -4°C at the District Livestock Office.

Between March 2008 and March 2009, samples were collected by the village heads or trained-volunteer veterinarians. These were transferred to the District Veterinary Office. The Provincial Veterinary Office acted as a centre for sample collection, receiving the samples from every District Veterinary Office. Each sample was checked for quality by observing its colour, the quantity of meat and the associated information. These were then transferred on a weekly basis to the Upper Northern Veterinary Research and Development Centre in Lampang Province in the Northern Region for *Trichinella* identification.

3.3.4 Laboratory investigations

The specimens from pigs and wild animals were submitted for laboratory investigation at the Upper Northern Veterinary Research and Development Centre in Lampang Province. Examination for *Trichinella* infection was carried out by staff at the centre using standard procedures that are recommended by the National Institute of Animal Health, Department of Livestock Development, Thailand. These tests comply with recommended guidelines issued by the World Organization of Animal Health (OIE, 2008). Pig sera were examined for antibodies against *Trichinella* infection using an ELISA test. Meat samples from wild animals were examined for *Trichinella* larvae using both the trichinoscopic and the digestion methods.

3.3.4.1. ELISA test

Each serum sample was tested for the presence of antibodies against *Trichinella* infection with a commercial ELISA kit (SafePath Laboratories, Carlsbad, California, USA). The test uses a *T. spiralis*

excretory/secretory (ES) antigen. The diagnostic sensitivity and specificity of the test are 98.0% and 99.0%, respectively. Sera were tested at a 1:200 dilution and followed the procedures recommended by the manufacturer. The ELISA optical density (OD) value ≥ 0.30 was the cut-off value for determining *Trichinella*-positive. Sera with an ELISA OD below 0.30 were considered negative for *Trichinella* infection. ELISA kits have been used in previous prevalence studies of trichinellosis in pigs in Thailand (Vitoorakool & Vitoorakool, 2005; Vitoorakool *et al.*, 2007). ELISA ODs from ELISA kit control sera and the field negative and positive control sera obtained from *Trichinella*-free pigs and *Trichinella*-infected pigs in Thailand are shown (Table 3-5). The field negative control sera were obtained from cross-bred pigs (Large white X Landrace) free from *Trichinella* infection (Chaichanapunpol *et al.*, 1988). The field positive control sera were obtained from 25 day-old cross-bred pigs (Large white X Landrace) orally infected with *Trichinella* larvae.

Table 3-5 ELISA optical densities of ELISA kit (SafePath) control sera and field positive and negative sera at the Upper Northern Veterinary Research and Development Centre, Lampang Province, Thailand

Type of sera	ELISA OD	Interpretation
- Test positive control	0.87	Positive
- Test negative control	0.04	Negative
- Field positive sera	0.92	Positive
- Field negative sera	0.05	Negative

Source: Vitoorakool and Vitoorakool (2005)(Vitoorakool & Vitoorakool, 2005)

3.3.4.2. Trichinoscopic method

The trichinoscopic method was performed according to the standard laboratory procedure for veterinary diagnosis used by the National Institute of Animal Health, Department of Livestock Development, Thailand. The procedure complies with the method recommended by the World Organization of Animal Health (OIE, 2008) (<http://www.oie.int/international-standard-setting/terrestrial-manual/access-online>).

3.3.4.3. Artificial digestion method

The artificial digestion method was performed according to the standard laboratory procedure for veterinary diagnosis used by the National Institute of Animal Health, Department of Livestock Development, Thailand. The procedure complies with the method recommended by the World Organization of Animal Health (OIE, 2008) (<http://www.oie.int/international-standard-setting/terrestrial-manual/access-online>). Samples from each animal were examined individually using at least 30 grams of muscle tissue.

3.3.5 Statistical analysis

Estimates of Normally-distributed continuous parameters obtained from the holding survey and trichinellosis seroprevalence survey in pigs were expressed as their weighted means with associated 95% confidence intervals. Binomial parameters were expressed as weighted percentages with associated 95% confidence intervals. The relationships between two variables were assessed by Rao and Scott χ^2 test (Rao & Scott, 1984), F statistic, using the two-by-two table analysis. Degree of freedom equals to the number of primary sampling units, minus the number of strata. The relationships between each continuous variable and binary variable were

assessed by adjusted Wald χ^2 test using binary logistic regression analysis in Stata (Statacorp LP, College Station, TX, USA).

3.3.5.1. Weighting

The sampling method employed in this study led to unequal probabilities of selection because there were different sampling fractions of pig holdings and pigs in highland relative to lowland survey data. Therefore, the survey data needed to be weighted in the analysis in order to compensate for the unequal sampling fractions. Sample weight is the inverse of the selection probability; that is, the sampling fraction interpreted at the level of the individual animal in the population. In this study two weights were used, the first weight (W_1) adjusted for uneven village sampling fractions. The second weight, (W_2) adjusted for the uneven sampling fractions of pigs, relative to pig census data. The 2006 census of pig holdings and pigs in Thung Chang, Chiang Klang, Bo Kluea, Tha Wang Pha and Muang Districts were used as the population composition.

$$W_1 = \frac{M_i}{M}$$

$$W_2 = \frac{N}{N_i}$$

Where M_i is the total villages in stratum i and M is the selected villages in stratum i , N is the population (holdings/pigs) distribution by strata based on census data of pig holding/ pigs in 2006 in stratum i and N_i is the sample distribution of holdings/pigs in stratum i (Lee & Forthofer, 2006).

The analysis was also adjusted by a finite population correction factor (fpc). The finite population correction factor is an adjustment factor applied to sampling without replacement where sampling fractions are greater than 10%. Derivations of adjustment factors for holding survey

data and pig survey data are shown in Table 3-6 and Table 3-7, and the formulae for calculating weights and the finite population correction are:

$$fpc = \frac{n-h}{N-h}$$

Where $n-h$ is the number of units sampled from stratum h and $N-h$ is the total number of units in the population belonging to stratum h (Statacorp LP, College Station, TX, USA).

Table 3-6 Derivation of weights and adjustment factor: pig holding survey in Thung Chang, Chiang Klang, Bo Kluea, Tha Wang Pha and Muang District, Nan Province

Strata	Number of villages		Number of holdings		Weights		<i>fpc</i> (2)/(1)
	Total (1)	Sample (2)	Total ^a (3)	Sample (4)	W ₁ (1)/(2)	W ₂ (3)/(4)	
Highland	99	29	1,295/2866	446/607	3.41	0.61	0.29
Lowland	211	32	1,571/2866	161/607	6.59	2.07	0.15
Overall	310	61	2866	607			

^a Census data of pig holdings in 2006 (Nan Provincial Livestock Office, 2006)

Table 3-7 Derivation of weights and adjustment factor: seroprevalence survey of trichinellosis in pigs in Thung Chang, Chiang Klang, Bo Kluea, Tha Wang Pha and Muang District, Nan Province

Strata	Number of villages		Distribution		Weights		<i>fpc</i> (2)/(1)
	Total (1)	Sample (2)	Population ^a (3)	Sample (4)	W ₁ (1)/(2)	W ₂ (3)/(4)	
Highland	99	28	6,101/19,183	721/1,196	3.54	0.53	0.28
Lowland	211	33	13,082/19,183	475/1,196	6.39	1.72	0.16
Overall	310	61	19,183	1,196			

^a Census data of pig in 2006 (Nan Provincial Livestock Office, 2006)

3.3.5.2. Characteristics of pig holding production and management

Point estimates of weighted means and weighted percentages of the overall and area-specific estimates for each parameter of interest, and 95% confidence intervals were reported. Analyses were conducted using Stata (Statacorp LP, College Station, TX, USA). Rao and Scott χ^2 test (Rao & Scott, 1984), F statistic, was used to assess the association between production and management characteristics and the areas where the holdings were located. Degree of freedom equals to the number of primary sampling units, minus the number of strata. The F statistic with significance at 5% level was used to determine whether there was statistically significant relationship (Statacorp LP, College Station, TX, USA).

3.3.5.3. Estimate of prevalence of trichinellosis in pigs

Test prevalence (P^T) and true prevalence (P) (Thrusfield, 2007) of trichinellosis in pigs were estimated. The weighted proportion estimates with 95% confidence interval of the overall and stratum-specific trichinellosis seroprevalence were computed by the Stata procedure svy (Statacorp LP, College Station, TX, USA). The true prevalence (P) (Thrusfield, 2007), based on the ELISA test performance of 98% sensitivity and 99% specificity, was calculated in Survey Toolbox version 1.0 beta (Cameron, 1999). The formulae for calculating test prevalence and true prevalence are (Thrusfield, 2007):

$$P^T = \frac{\text{number of individual having a disease at a point in time}}{\text{number of individual in the population at risk at that point in time}}$$

$$P = \frac{P^T + \text{specificity} - 100\%}{\text{sensitivity} + \text{specificity} - 100\%}$$

From this study, the estimated village prevalence of trichinellosis was used to calculate between-cluster variance (V_c). Between-cluster variance is the variation expected between clusters if all animals in the cluster were sampled (Thrusfield, 2007). This estimate is required to determine sample size in a survey using cluster sampling.

$$V_c = c \left\{ \frac{K_1 c V}{T^2 (c-1)} - \frac{K_2 \hat{P}(1-\hat{P})}{T} \right\}$$

Where c = number of cluster in the sample

T = total number of animals sampled

$K_1 = (C-c)/C$, where C = number of clusters in the population

$K_2 = (N-T)/N$, where N = total number of animals in the population

$$V = \hat{P}^2 (\sum n^2) - 2\hat{P}(\sum nm) + (\sum m^2)$$

Where \hat{P} = sample estimate of overall prevalence

n = number of animals sampled in each cluster

m = number of diseased animals sampled in each cluster

3.3.5.4. Factors associated with swine trichinellosis in Nan Province

To identify factors that associated with swine trichinellosis in Nan Province, the relationship between demographic, production and management characteristics of individual pigs (**Error! Reference source not found.**) and trichinellosis was measuring by the odds ratio (OR) (Thrusfield, 2007). The associations were assessed by adjusted Wald χ^2 test using the binary logistic regression analysis. The Stata procedure svy was used to fit the multivariable model (Statacorp LP, College Station, TX, USA).

Logistic regression is a way of performing a regression analysis when the dependent variable is categorical *i.e.* diseased/non-diseased. It is the method most commonly used for the analysis of dependent (outcome) variables when there are two or more independent (exposure) variables (Kirkwood & Sterne, 2003). Whether the association between independent variables and dependent variable occurs is measured based on the odds of an event. Odds of event are defined as the probability that event does occur divided by the probability that it does not occur (Kirkwood & Sterne, 2003). The general form of logistic regression equation with several independent variables is (Kirkwood & Sterne, 2003):

$$\log \text{odds of outcome} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_p x_p$$

Where the quantity $\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_p x_p$ is known as the linear predictor of the log odds of the outcome, given the particular value of the p exposure variables x_1 to x_p and the β 's are the regression coefficient associated with the p exposure variables (Kirkwood & Sterne, 2003). The logistic regression coefficients show the change (increase when $\beta > 0$, decrease when $\beta < 0$) in the predicted log odds of having the outcome of interest for a one-unit change in the independent variables.

By taking the exponential of both sides of the regression equation as given above, the equation can be rewritten as:

$$\text{odds of outcome} = \exp(\beta_0) \times \exp(\beta_1 x_1) \times \exp(\beta_2 x_2) \times \dots \times \exp(\beta_p x_p)$$

The goal of logistic regression is to find the best fitting and biologically reasonable model to describe the relationship between the dependent variable or the outcome of interest and a set of independent variables (potential risk factors) (Noordhuizen *et al.*, 2001). The steps for logistic

regression analysis have been described for example by Hosmer and Lemeshow (2000), Kirkwood and Sterne (2003) and Noordhuizen et al (2001).

Logistic regression models were built using stepwise backward removal (Noordhuizen *et al.*, 2001). All variables associated with trichinellosis with p values < 0.25 were included in the models (Noordhuizen *et al.*, 2001). If two variables were highly correlated one of them was omitted from the model (Noordhuizen *et al.*, 2001). The model building strategies and methods for logistic regression followed the guidelines of Hosmer and Lemeshow (2000) and Noordhuizen *et al.* (2001). Following fitting of the multivariable model, any variables not selected for the original multivariable model were added back into the model to see whether they made an important change in the coefficient (β) of the variables present in the model. A relative change in β of variables in the model of at least 25% (when the β is larger than 0.40 or smaller than -0.40) or at least 0.1 absolute change (when the β is between -0.40 and 0.40) was considered significant and the variable was considered as a confounder (Noordhuizen *et al.*, 2001). Factors are called confounders when they are: related to the disease and also are related to the exposure factor under study and they are not intermediate steps in the causal path between the exposure factor and the disease (Noordhuizen *et al.*, 2001). The process of deleting, refitting, and verifying continued until all of the important variables were included in the model. The variables included in the model were based on two criteria (i) each variable is significantly associated with the outcome with p value < 0.05 (ii) an estimated coefficient of the model with the variable is significantly different than the coefficient from the model without the variable, based on the likelihood ratio test (Kirkwood & Sterne, 2003). Finally, the interactions among the variables in the model were checked. Interaction in an epidemiological context is defined as a quantitative interdependence between two or more factors, such that the frequency of disease when

two or more factors are present is either in excess of or less than that expected from the combined effects of each factor (Thrusfield, 2007). The likelihood ratio test for interaction was used to test for the null hypothesis that there is no interaction ($P > 0.05$) by comparing the log likelihoods obtained from models that include interaction terms and the model exclude the interaction terms. According to Noordhuizen *et al.* (2001) only biologically meaningful interactions should be tested.

3.3.5.5. Swine trichinellosis cluster detection

Geographical clusters of trichinellosis in pigs were detected using spatial scan statistics. The spatial scan statistic uses a circular moving window that goes from one geographical coordinate to another positioned across the study area, increasing its size from zero to a maximum size specified by the user (Kulldoff, 1997). The spatial scan statistic compares observed and expected number of events (*i.e.* cases) inside and outside the scan window to detect clusters that are least likely to have occurred by chance. For each location and size of the scanning window, the null hypothesis is that the risk within and outside the window is not different and the alternative hypothesis is that there is an elevated risk within the window as compared to outside (Kulldoff, 1997). The statistical significance for each cluster is obtained through Monte Carlo hypothesis testing where results of the likelihood function are compared for a large number of random replications of the dataset generated under the null hypothesis (Dwass, 1957). The p value is obtained by comparing the rank (R) of the maximum likelihood from the real data set with the maximum likelihoods from the random data sets. The p value represent: $R / (1 + \text{number of simulation})$. In SaTScan, the number of replications must be at least 999 to ensure excellent power for all types of data sets (Kulldoff, 2009).

$$\left(\frac{c}{n}\right)^c \left(\frac{n-c}{n}\right)^{n-c} \left(\frac{C-c}{N-n}\right)^{C-c} \left(\frac{(N-n)-(C-c)}{N-n}\right)^{(N-n)-(C-c)} I()$$

Where

C = total number of cases;

c = observed number of cases;

n = total number of cases and controls in the window;

N = combined total number of cases and controls in the data set

I() = an indicator function, when SaTScan is set to scan for cluster with high rate, I() is equal to 1 when the window has more cases than expected under the null-hypothesis, and 0 otherwise

Cluster analysis was performed based on the Bernoulli model (Kulldoff, 1997). Calculations were conducted using spatial scan statistic, SatScan™8.0 (Kulldoff & Information Management Service, 2009). Pigs that tested positive for trichinellosis were defined as cases, and pigs that tested negative for trichinellosis were defined as control. Cases and controls were aggregated at the holding coordinates; they constituting the population as a whole. The coordinates of each holding represented the centre of each circular window with the maximum radius including 50% of pig population in the data set. By performing the spatial analysis in SaTScan, a circular window with prior defined maximum size was initiated at the coordinates of the pig holdings. For each circular window the numbers of cases inside and outside the window are noted, together with the expected number of cases reflecting the population at risk. On the basis of these numbers, the likelihood is calculated for each window. The window with the maximum likelihood, and with more than its expected number of cases, is denoted the most likely cluster. This study is set to generate 999 simulations, the smallest recommended number of simulation (Kulldoff, 2009). Clusters with the maximum likelihood rank with in highest 5% of the maximum likelihoods from the random data sets was considered significant cluster ($p < 0.05$).

3.3.5.6. Estimated prevalence of *Trichinella* infection and characteristics of wild animal samples

Prevalence of trichinellosis in each wild animal species was estimated from the proportion of the test-positive animals. Samples characteristics were described spatially and temporally according to the place and time where they were caught.

3.4. Results

3.4.1 Pig holding production and management characteristics

The holding survey was carried out in a total of 61 villages. Because some number of holdings were closed down when the price of live pigs dropped before the survey had taken place, an additional two highland village and five lowland villages were sampled. As a result, the survey included 29 highland villages and 32 lowland villages. In each village the number of pig holdings was ascertained by asking the village head. According to the village heads there were 784 holdings. They comprised 601 holdings in highland villages and 183 holdings in lowland villages.

Six hundred and seven holdings were interviewed, the remainder not being available to interview because the holding owners were not at home on the day of survey. The interviewed holdings represented of 77% of potential respondents. Of these respondents 446 were in highland areas and 161 were in lowland areas. Thus, the interviewed holdings represented 74% of holdings in highland and 88% of holdings in lowland areas.

3.4.1.1. Occupation, ethnicity and religion of pig holding owner

The survey showed that, overall, almost every pig holding owner was an agriculturist. Agriculturalists accounted for 98.89% (95%CI 96.99 – 99.59) of the owners in highland areas and 96.88% (95%CI 93.28 – 98.59) of the owners in lowland areas. The rest were merchants, labourers, and government or non-government officers.

Up to 59.67% (95%CI 50.68 – 68.06) of overall pig holdings belonged to Thai owners. The remainder belonged to Yao (18.43%, 95%CI 10.21 –

31.01), Lua (14.09%, 95%CI 7.75 – 24.26), Hmong (6.99%, 95%CI 3.18 – 14.69), and Khmu (0.81%, 95%CI 0.14 – 4.44), respectively. Pig holdings in highland areas predominantly belonged to Yao (32.51%, 95%CI 17.81 – 51.72) and Lua (28.92%, 95%CI 14.85 – 48.70). The holdings in lowland areas were mostly owned by Thai (91.30%, 95%CI 68.68 – 98.05).

About 94.7% (95%CI 90.96 – 96.98) of the total pig holding owners and 100% of those in the lowland, practised Buddhism. Approximately 1.62% (95%CI 0.42 – 6.03) and 3.65% (95%CI 1.92 – 6.80) of the pig holding owners in the highland practised Christianity and animism respectively.

3.4.1.2. Pig holding characteristics

3.4.1.2.1 Altitude of the holding

On average the pig holdings over the whole study area were located at an altitude 361.4 metres above sea level (median = 322, range 195 – 1228). The average altitude of holdings in the highland areas was 492.7 metres above sea level (median = 383, range 238 – 1228) and the average altitude of the holdings in lowland areas was 253.2 metres above sea level (median = 229, range 195 – 742).

3.4.1.2.2 Holding size

Pig holdings over the entire study area had an average of 6.6 pigs (median = 3, range 1 – 51). The holding size in the highland areas was 4.0 pigs (median = 3, range 1 – 30) and the holding size in the lowland areas was 8.7 pigs (median = 5, range 1 – 51). The holding size differed significantly between highland and lowland areas ($F_{(1,59)} = 4.22$, $P = 0.04$).

3.4.1.2.3 Pig breed

The majority of holdings reared either native-breed pigs or improved-breed pigs. About 5% (4.96%, 95%CI 2.91 – 8.34) of the holdings reared both native-breed and improved-breed pigs and wild boar. Overall,

native-breed pigs were reared by the majority of holdings (57.8%, 95%CI 50.16 – 65.08). However, the majority of native-breed pigs were reared in the highland holdings (95.52%, 95%CI 92.56 – 97.33). Almost all of the improved-breed pigs were reared in the lowland holdings (66.46%, 95%CI 50.60 – 79.31). The pig breed profiles in the highland and the lowland holdings differed significantly ($F_{(1.91,112.88)} = 108.03$, $P < 0.001$).

3.4.1.2.4 Production scheme

The production of pig holdings in the study was split equally between production for capital earning and production for the owner's own use. However, this differed significantly between the highland areas and the lowland areas ($F_{(1, 59)} 61.27$, $P < 0.001$). While the production in highland areas was mainly for the owner's own use (88.57%, 95%CI 6.34 – 19.75), the production in lowland areas was mainly for capital earning (83.23%, 95%CI 67.61 – 92.19).

3.4.1.3. Pig holding management characteristics

3.4.1.3.1 Sources of pig

The sources of pigs reared in the holdings included being bred in the holding, purchasing from local market or from a mobile seller, purchasing from other holdings, or the receipt of pigs as a gift (Table 3-8). Purchasing pigs from other holdings and breeding of pigs in a holding were common to both the highland and lowland areas. There were no differences in the way that pigs were acquired in the holdings between the highland and lowland areas other than that the acquisition of pigs as a gift was observed more among the highland holdings than among those in the lowland areas.

Table 3-8 The overall and stratum-specific percentages of pig holdings regarding the sources of pigs (n = 588)

Source of pigs	Overall	Highland	Lowland
Breed in a holding	194	149	45
Percentage	31.81	34.02	30.00
(95%CI)	(24.63 – 39.98)	(21.49 – 40.16)	(22.64 – 47.59)
Purchase from market or mobile seller	106	73	33
Percentage	19.59	16.67	22.00
(95%CI)	(14.01 – 26.71)	(11.31 – 23.88)	(11.36 – 34.03)
Purchase from other holdings	254	184	70
Percentage	44.56	42.01	46.67
(95%CI)	(36.70 – 52.70)	(33.81 – 50.68)	(34.23 – 59.54)
Gift	34	32	2
Percentage	4.03	7.30	1.33
(95%CI)	(2.13 – 7.51)	(3.50 – 14.64)	(0.37 – 4.64)

3.4.1.3.2 Pig feed

Several kinds of food were used to feed the pigs in the areas under study (Table 3-9). About 3.8% of holdings used only agriculture products e.g. rice bran, broken rice, fermented rice, soya bean, maize and leaves. About 14.1% of the holding used only commercial feed-grain. Cooked household leftovers were used in about 35.8% of the overall holdings. Though the majority of the overall holdings (46.30%) used un-cooked household leftovers, the use of un-cooked household leftovers was more common in the highland areas (79.18%, 95%CI 72.49 – 84.58) when compared with the lowland areas (19.21%, 95%CI 10.95 – 31.48). On the other hand, cooked household leftovers were used by only 14.87% (95%CI 10.49 – 20.66) of holdings in the highland areas compared with 52.98% (95% 41.73 – 63.93) in the lowland areas. Commercial feed-grain was used by 0.69% (95%CI 0.12 – 3.85) of the holdings in the highland holdings compared with 25.17% (95%CI 16.52 – 36.36) of

holdings in the lowlands. Overall there was a significant difference between the types of pig feed used in the highland and lowland areas ($F_{(2.58, 149.62)} 40.42, P < 0.001$)

Table 3-9 The overall and stratum-specific percentages of pig holdings regarding the type of pig feed (n = 588)

Pig feed	Overall	Highland	Lowland
Agricultural product	27	23	4
Percentage	3.83	5.26	2.65
(95%CI)	(2.33 – 6.24)	(2.89 – 9.40)	(1.10 – 6.22)
Grain	41	3	38
Percentage	14.10	0.69	25.17
(95%CI)	(9.45 – 20.54)	(0.12 – 3.85)	(16.52 – 36.36)
Heated leftover	145	65	80
Percentage	35.76	14.87	52.98
(95%CI)	(29.47 – 42.59)	(10.49 – 20.66)	(41.73 – 63.93)
Un-heated leftover	375	346	29
Percentage	46.30	79.18	19.21
(95%CI)	(40.16 – 52.56)	(72.49 – 84.58)	(10.95 – 31.48)

3.4.1.3.3 De-worming, vaccination, rodent control and dead pig carcass disposal

Approximately 59% of the holdings in the study had their pigs de-wormed, 27% of the holdings vaccinated pigs with at least one vaccine (the most common being Classical Swine Fever vaccine), 95% of the holdings buried dead pig carcasses immediately after they were found but only 5% of holdings had any rodent control regime in place.

De-worming and vaccination were less commonly practised among holdings in highland areas compared with the holdings in lowlands. The difference in the proportions of these two management practices between the two regions were highly significant: ($F_{(1, 59)} 26.14, P < 0.001$) and ($F_{(1, 59)} 156.30, P < 0.001$, respectively). About 38% of holdings in the highland areas had de-wormed pigs compared with 76% of the holdings in the lowland areas. Only 2.47% of the highland holdings

vaccinated pigs compared with up to 47.20% of holdings in the lowland areas.

Although rodent control was not commonly practised in either area, the percentage of holdings practising rodent control in the lowland areas was significantly greater than that in the highland areas ($F_{(1,59)} 13.82$, $P < 0.001$). In almost all of the holdings in highland and lowland areas, dead pig carcasses were reported to be disposed of properly by burial immediately when they were found. About 4% reported that dead pig carcasses were consumed; whereas a little under 1% stated that they were left on the ground.

Table 3-10 The overall and stratum-specific percentages of pig holdings regarding the type of management regime

Management	Overall	Highland	Lowland
De-worm (n = 607)	292	169	123
Percentage	59.00	37.89	76.40
(95%CI)	(52.06 – 65.59)	(29.93 – 46.56)	(64.63 – 85.15)
Vaccination (n = 607)	87	11	76
Percentage	26.99	2.47	47.20
(95%CI)	(21.40 – 33.42)	(1.28 – 4.71)	(36.59 – 58.08)
Buried dead pig carcasses (n = 265)	252	178	74
Percentage	95.01	95.18	94.87
(95%CI)	(90.78 – 97.36)	(91.51 – 97.32)	(89.33 – 98.19)
Rodent control (n = 607)	17	4	13
Percentage	4.83	0.90	8.07
(95%CI)	(2.71 – 8.47)	(0.24 – 3.29)	(4.32 – 14.59)

3.4.2 Swine trichinellosis seroprevalence

The seroprevalence survey was conducted in the same villages as the pig holding survey. Due to personal risk, following two fatal accidents on the unfinished hill road to it, one previously-sampled village in the highlands was removed from the study. Twenty-eight highland villages remained in the study, including one village that could be accessed on foot about one kilometre up-hill on an un-surfaced road. Because of the closure of some pig holdings during the survey period, an additional lowland village was added to maintain sufficient pig numbers in the analyses.

The survey was carried out between January and March 2008. Blood was collected in holdings where owners had given prior permission for the seroprevalence study and where there were eligible pigs available at the time of blood collection. Blood samples, together with pig demographic data and management characteristics, were collected from pigs in 456 holdings. One thousand one hundred and ninety-six pig sera samples were collected in the sampled villages (Table 3-11), 721 samples were from pigs in the highland areas and 475 samples were from pigs in the lowlands. On average 2.2 and 3.6 samples were collected from each holding in the highland and lowland areas respectively. Based on data from the household survey, 58% of the holdings were included in the seroprevalence survey. They represented 54% of holdings in the highland and 71% of holdings in the lowlands. The non-respondents included a small number of owners who did not agree to blood sampling, owners who were not accessible, the holding only had piglets at the time of survey or the holdings were temporarily empty because the pigs had been slaughtered during the New Year Festival or other events.

Table 3-11 Characteristics of pig samples obtained during a trichinellosis seroprevalence survey in Thung Chang, Chiang Klang, Bo Kluea, Tha Wang Pha and Muang Districts in Nan Province between January 2008 – March 2008

Districts	Number of samples					
	Highland		Lowland		Total	
	Pigs	Holdings	Pigs	Holdings	Pigs	Holdings
Muang	261	99	109	27	370	120
Tha Wang Pha	124	62	289	83	413	142
Thung Chang	203	96	30	4	233	99
Chiang Klang	18	16	47	18	65	33
Bo Kluea	115	62	0	0	115	62
Total	721	335	475	132	1,196	467

3.4.2.1. Characteristic of trichinellosis sero-positive pigs

Seventeen of 1,196 sera samples had an ELISA Optical Density (OD) over the cut-off value of 0.30. These were therefore identified as trichinosis sero-positive. The OD in trichinosis sero-positive samples ranged from 0.41 – 1.54. Ten samples had an OD \leq 0.50 and five samples had an OD between 0.51 and 0.70. Two samples had an OD of 1.29 and 1.54 respectively.

One of the trichinellosis sero-positive pigs found in the lowland areas was a four-month old pig purchased from the Thai-Laotian border market about a week before the blood sample was taken. Its serum OD was the highest (1.54) among the sero-positive pigs. None of them could be followed up with confirmation of the presence of *Trichinella* larvae and no further test was used to confirm the infection. The trichinellosis sero-positive pigs were aged in the range 4 – 40 months (median = 24). They were reared in the holding with a mean holding size of 6.7 pigs (median = 4, range 2 – 40). The average altitude of the holding's location was 282.3 metres above sea level (median = 286, range 170 – 424).

3.4.2.2. Estimation of swine trichinosis seroprevalence

The overall trichinellosis seroprevalence from the ELISA tested positive samples was 1.05% (95% CI 0.59 – 1.65) (Table 3-12). The area-specific trichinellosis seroprevalence was 1.94% (95% CI 0.58 – 3.30) in the highland areas and 0.63% (95% CI 0.007-1.26) in the lowland areas. The seroprevalence of swine trichinellosis in these two areas was not significantly different at 5% level ($F_{(1, 59)} 3.82, P = 0.06$).

The true trichinellosis seroprevalence estimated at 95% confidence for the overall areas, adjusted using values of 98% sensitivity and 99% specificity of the ELISA test, was 0.05% (95% CI 0.00 – 0.35%). The true trichinellosis seroprevalence specific for the areas estimated with 95% confidence was 0.97% (95%CI 0.46-1.48) in the highland areas and 0.0 – 0.35% in the lowland areas.

Table 3-12 Overall and area-specific swine trichinellosis seroprevalence estimated from 1,196 pigs sampled in Thung Chang, Chiang Klang, Bo Kluea, Tha Wang Pha and Muang District in Nan Province between January 2008 – March 2008

Prevalence	Highland n=14		Lowland n=3		Total n=17	
	Percent	95% CI	Percent	95% CI	Percent	95% CI
Apparent	1.94	0.96 – 3.88	0.63	0.23 – 1.69	1.05	0.59 – 1.87
True	0.97	0.46 – 1.48	0.0	0.00 – 0.36	0.05	0.00 – 0.35

3.4.2.3. Estimation of swine trichinosis seroprevalence at village and holding level

Swine trichinellosis seroprevalence at village and holding level is shown in Table 3-13. The village sero-positive rate was 13.11% (8/61). The infection rates within each village varied from 0.0 – 7.4% (see Appendix 8), the between-cluster variance was estimated at 0.00018. The proportion of holdings with swine trichinellosis sero-positive pigs was

3.64% (17/467). Seventeen swine trichinosis sero-positive cases were identified in separate holdings in which 1 – 10 pigs in a holding aged ≥ 2 months old were examined. The infection rate within a holding was estimated at 10 – 100% (1/10 – 1/1).

Table 3-13 Estimation of swine trichinellosis seroprevalence at village and holding levels

Level	Number examined ^a	Number positive ^b	Prevalence (%)
Village	61	8	13.11
Holding	467	17	3.64

^a Number of village/holding that pigs were examined for trichinellosis

^b Number of village/holding that trichinellosis sero-positive pigs were found

3.4.3 Descriptive of trichinellosis sero-positive pigs and factors associated with trichinellosis

The average age of trichinellosis sero-positive pigs observed was 22.3 months, which was significantly higher than the average age of 12.4 months in trichinellosis sero-negative pigs. Three parameters - the age when pigs were acquired into holdings, the holding size and the altitude where holding was located - were not significantly different between the sero-positive and sero-negative pigs at 5% level (Table 3-14).

Table 3-14 Estimated means for pigs' age, age when pigs were acquired, holding size and altitude where holding was located for trichinellosis sero-positive pigs and trichinellosis sero-negative pigs

Parameter	Sero-positive pigs	Sero-negative pigs	<i>p</i> -value ^a
	Mean (95%CI)	Mean (95%CI)	
Age (months)	22.31 (15.84 – 28.78)	12.36 (11.14 – 13.58)	<0.001
Acquired age (months)	3.12 (2.69 – 3.56)	3.84 (1.40 – 6.29)	0.95
Holding size	11.53 (8.07 – 15.00)	9.16 (0.78 – 17.54)	0.69
Altitude (metres)	260.86 (232.04 – 289.68)	311.18 (282.68 – 339.68)	0.13

^a *p*- value was evaluated from the *F* statistic with 1 and 59 degree of freedom ($F_{(1, 59)}$)

Detail of the management characteristics of trichinellosis sero-positive pigs and the *p*- value from the Rao-Scott χ^2 test in two-by-two table analysis of the association between management characteristics and swine trichinellosis is shown in Table 3-15. Among the management characteristics which could be associated with trichinellosis in pigs, only two of them were significant with the *p*- value of the *F* test <0.05. These two management characteristics were confinement and de-worming. Estimated prevalence of trichinellosis in free-scavenging pigs was 3.45% compared with estimated prevalence of 0.74% in totally confined pigs. Meanwhile, estimated prevalence of trichinellosis was 0.42% in pigs that had been de-wormed compared with 2.35% in pigs that had never been de-wormed.

Table 3-15 Detail of the management characteristics of trichinellosis sero-positive pigs and the *p*-value from the two-by-two table analysis for the association between management characteristics and swine trichinellosis

Management regimes	Total	Sero-positive pigs			<i>p</i> -value ^a
		Number	Percent	95% CI	
Free-scavenging					
- Yes	252	9	3.45	1.84 – 6.35	0.0004
- No	944	8	0.74	0.36 – 1.52	
Fed household leftovers					
- Yes	844	15	1.38	0.68 – 2.76	0.30
- No	352	2	0.64	0.18 – 2.29	
Exposed to live wildlife					
- Yes	59	0	0		0.58
- No	1,137	17	1.10	0.62 – 1.96	
Exposed to live domestic animals					
- Yes	1,195	17	1.05	0.59 – 1.87	0.91
- No	1	0	0		
De-worming					
- Yes	591	2	0.42	0.12 – 1.49	0.01
- No	615	15	2.35	1.21 – 4.52	

Rodent control					
- Yes	46	0	0	0	0.65
- No	1,150	17	1.11	0.63 – 1.96	
Buried dead pig carcasses					
- Yes	1,137	17	0		0.76
- No	59	0	1.08	0.61 – 1.91	
'Farm Standard'					
- Yes	40	0	0	0	0.68
- No	1,156	17	1.11	0.63 – 1.96	
Acquisition of pigs					
- Born in holding	684	12	1.06	0.55 – 2.00	0.98
- Acquired	512	5	1.04	0.43 – 2.48	

^a *P* value was evaluated from the *F* statistic with 1 and 59 degree of freedom ($F_{(1, 59)}$)

Trichinellosis seroprevalence in male pigs, native-breed pig and boar and sow was higher than female pigs, improved-breed pigs and fattening and replacement pigs, however, the difference between the two groups was not significant at 5% level (Table 3-16).

Table 3-16 Detail characteristics of trichinellosis sero-positive pigs and the *P* value from the two-by-two table analysis for the association between characteristics of pigs and swine trichinellosis

Parameter	Total	Sero-positive pigs			<i>p</i> -value ^a
		Number	Percent	95% CI	
Sex					
- Male	396	8	1.48	0.69 – 3.16	0.31
- Female	800	9	0.86	0.38 – 1.90	
Breed					
- Native	795	15	1.69	0.84 – 3.35	0.11
- Improved	401	2	0.52	0.14 – 1.89	
Category					
- Boar & sow	385	8	1.83	0.78 – 4.22	0.06
- Fattening & replacement	811	9	0.71	0.35 – 1.42	

^a *p*-value was evaluated from the *F* statistic with 1 and 59 degree of freedom ($F_{(1, 59)}$)

The seroprevalence of trichinellosis in pigs belonging to the Thai hill tribes was about 2.12% compared with 0.61% in pigs belonging to the Thai ethnic group. Similarly, the seroprevalence of trichinellosis was greater in pigs that were reared for the owner's own use compared with pigs that were reared for sale and the difference between the two groups was significant. However, the seroprevalence of trichinellosis in pigs reared in the residential areas was not different from pigs reared in the farmland areas. Seroprevalence of trichinellosis in pigs reared for home slaughter was greater than the seroprevalence in pigs that were not to be slaughtered at home, but this difference was not significant at 5% level (Table 3-17).

Table 3-17 Detail of holding characteristics of trichinellosis sero-positive pigs and the *p*- value from the two-by-two table analysis for the association between holding characteristics and swine trichinellosis

Holding characteristics	Total	Sero-positive pigs			<i>p</i> -value ^a
		Number	Percent	95% CI	
Owner ethnicity					
Thai hill tribes	621	14	2.12	1.08 – 4.13	0.03
Thai	575	3	0.61	0.23 – 1.62	
Production scheme					
Sell	581	3	0.61	0.23 – 1.63	0.03
Consumption	615	14	2.11	1.09 – 4.07	
Farm location					
Resident area	864	14	1.02	0.50 – 2.05	0.92
Farmland	332	3	1.09	0.37 – 3.18	
Home-slaughtering pig					
Yes	649	14	1.78	0.91 – 3.48	0.09
No	547	3	0.66	0.24 – 1.79	

^a *p*- value was evaluated from the *F* statistic with 1 and 59 degree of freedom ($F_{(1, 59)}$)

3.4.4 Potential factors associated with trichinellosis in pigs in Nan Province

The odds ratios obtained from the logistic regression model to describe factors associated with trichinellosis in pigs in Nan Province suggested that there were two significant factors: free-scavenging and older-aged pigs. The odds of trichinellosis in pigs that were not totally confined, in other words free-range scavenging pigs, was estimated to be 2.96 times larger than the odds for pigs that were totally confined. The confidence interval estimate suggests that odds for free-scavenging pigs could be as little as 1.20 or as much as 7.31 times greater than the odds for totally confined pigs. The odds ratio for trichinellosis increases 1.02 times with each one month increase in age of the pigs, with a 95% confidence interval (1.00 – 1.04). Pigs that had never been de-wormed were approximately 5.21 times as likely to be trichinellosis cases as pigs that

had been de-wormed and the odds could be as much as 30.53 times greater or as little as 0.89 times smaller with 95% confidence. The difference between two groups was not significant.

Table 3-18 Factors associated with swine trichinellosis, controlling for areas and de-worming among 1,196 pigs aged ≥ 2 months old from a survey in five districts in Nan Province

Variables	Odds ratio	95%CI	<i>p</i> -value
Free-range scavenging	2.96	1.20 – 7.31	0.02
Age (month)	1.02 ^a	1.00 – 1.04	0.02
No de-worming	5.21	0.89 – 30.53	0.07
Highland areas	0.52	0.11 – 2.43	0.40

^a Odds ratio reflect a 1-month change in age

3.4.5 Spatial distribution and a cluster of swine trichinellosis

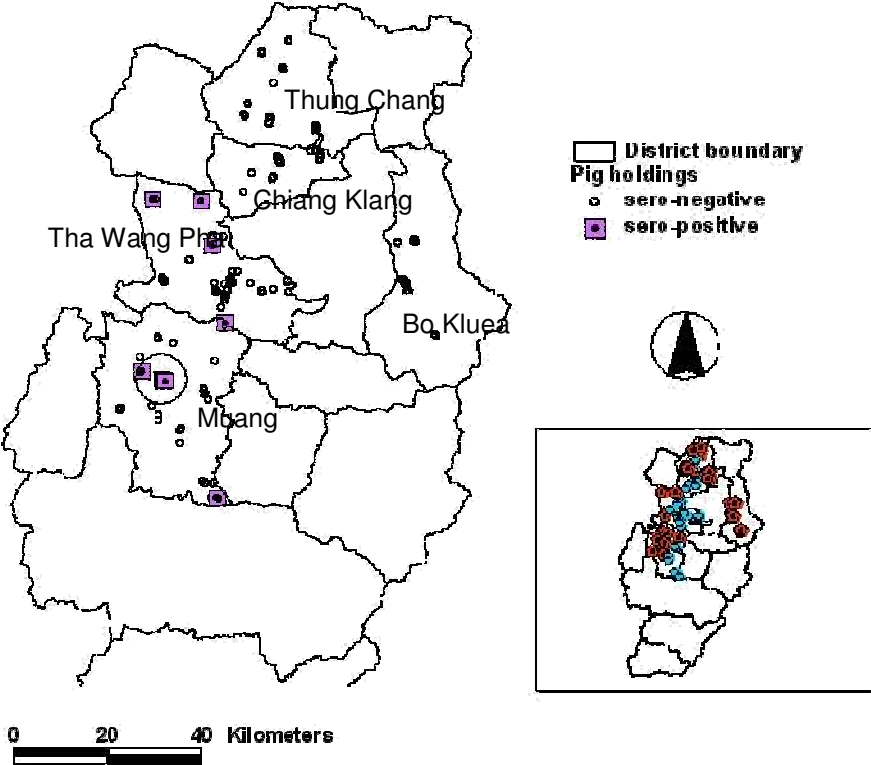
Of the 61 villages and 467 holding examined, the sero-positive holdings were found in eight villages. These were situated in the Muang and Tha Wang Pha Districts. The geographical distribution of the holdings that pigs were examined for trichinellosis and the holding with sero-positive pigs is shown in Figure 3-2.

In Muang District 10 cases were identified, of which nine cases were identified in three adjacent highland villages occupied by the Yao ethnic group. One case was identified in a lowland village occupied by the Thai ethnic group.

Seven cases were identified in Tha Wang Pha District. Four cases were identified in a village in which the Hmong ethnic group lived, two cases were identified in two separate villages in which the Thai ethnic group lived, and another case was identified in a village in which the Yao ethnic group.

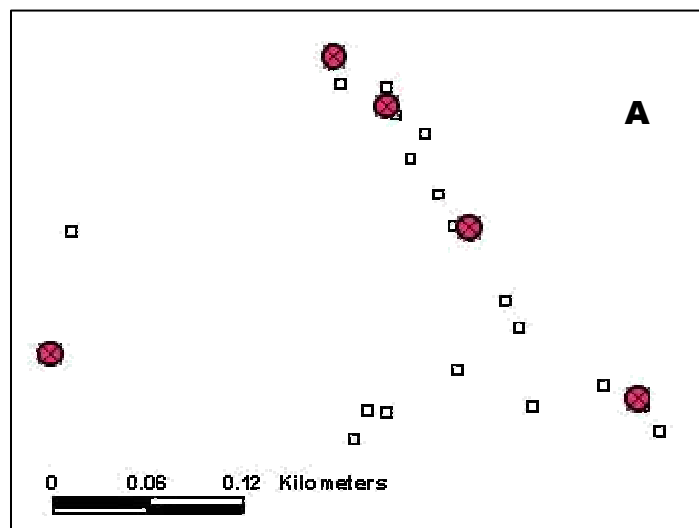
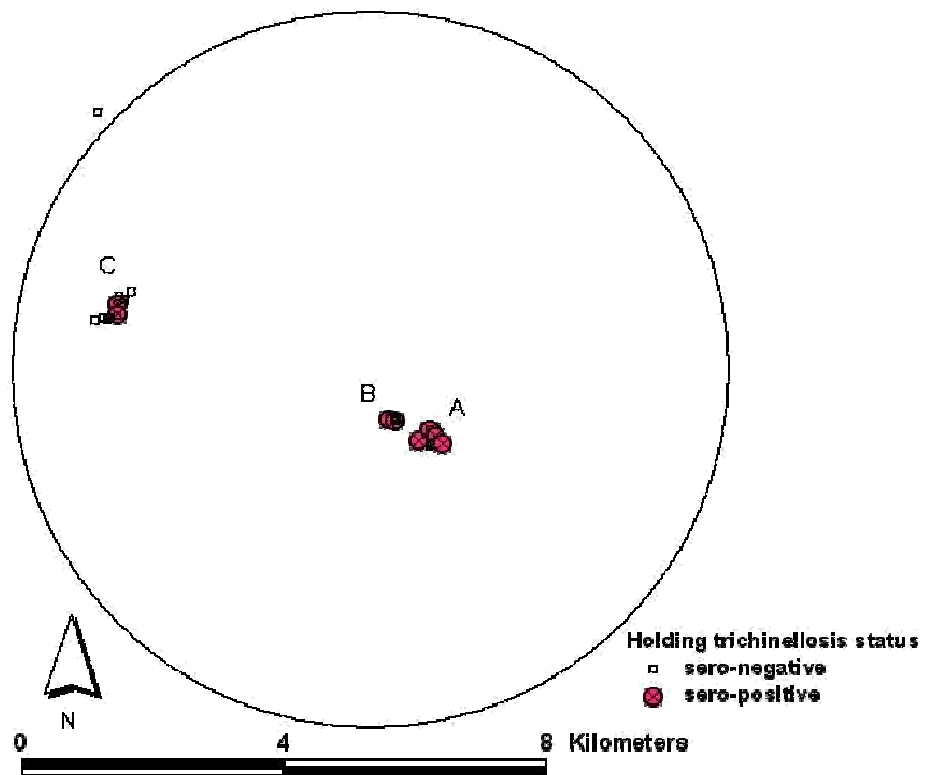
Using the spatial scan statistic test based on a Bernoulli model with the maximum window including 50% of population, a most-likely cluster of swine trichinellosis ($p = 0.02$) was detected (Figure 3-2). The cluster encompassed the areas of three villages in Muang District, including nine trichinellosis sero-positive cases among the 139 pigs. The number of observed cases was 4.55 times higher than the expected number of cases. The risk of swine trichinellosis within the cluster was 8.54 times the risk outside this cluster. In a village within the identified cluster, pig holdings were located near to one other or attached to one another as shown in Figure 3-3. The holdings with the trichinellosis sero-positive pigs were located within about 300 metres of each other (Figure 3-3).

Figure 3-2 Geographic distribution of pig holdings from a trichinellosis seroprevalence survey in the five districts of Nan Province in 2008



Note: A circle surrounds the significant cluster of swine trichinellosis. Inset shows the distribution of sampled highland villages (red) and lowland villages (blue).

Figure 3-3 Swine trichinellosis most-likely cluster encompasses three villages A, B and C. Inset shows the distribution of six trichinellosis cases in village A. Pig holdings gathered in close proximity and some holdings, including the two holdings with trichinellosis sero-positive pigs, attached to one another



3.4.6 *Trichinella* infection in wild animals

Ninety-seven samples of muscle from wild animals were obtained between March 2008 and March 2009. They comprised of samples from seven species of mammal and one reptile. The mammalian species were two omnivores: wild boar (*Sus scrofa*) and bear (*Ursus* spp.); one carnivore: civet (*Paradoxorus hermaphroditus*) and four herbivores: barking deer (*Muntiacus* spp.), Sambar deer (*Cervus unicolor*), pangolin (*Manis* spp.) and wild rabbit (*Sylvilagus* spp.). The single reptile species was a monitor lizard (*Varanus* spp.). The most common species obtained in the study were wild boar (n = 53; 54.6%), barking deer (n = 26; 26.8%) and monitor lizard (n = 11; 11.3%). Samples of wild boar were obtained every month with the exception of February and December. Net total samples from the other species were obtained during 10 months of the survey period (Table 3-19). These samples were obtained from 12 of 15 districts in Nan Province. The total number of samples submitted from each district varied from 1 – 23. Muscle samples from wild boar were obtained from all of the 12 districts that submitted samples. Samples of the barking deer and monitor lizard were obtained from seven and five districts respectively. Each sample was examined individually for *Trichinella* larvae by both the trichinoscopic method and the digestion method. *Trichinella* larvae were not found in any of the samples examined (Table 3-20).

Table 3-19 Monthly distribution of wild animals caught for consumption and meat specimens were submitted by voluntary for *Trichinella* identification between March 2008 - March 2009 in Nan Province

Species	Number of meat samples													
	2008							2009						
	March	April	May	June	July	August	September	October	November	December	January	February	March	
Wild boars	2	2	6	1	9	15	11	1	1	-	3	-	2	
Other	7	7	8	9	1	2	1	5	1	-	3	-	-	
Overall	9	9	14	10	10	17	12	6	2	-	6	-	2	

Table 3-20 District-wise distribution of wildlife sample and *Trichinella* in wild animals caught for consumption in Nan Province between March 2008 – March 2009

Species ^a	District (number of samples)	Total number of sample examined	Number of positive samples
Wild boar (<i>Sus scrofa</i>)	Bo Kluea (13), Chiang Klang (2), Chalermprakiat (1), Mae Charim (3), Muang (5), Na Muen (1), Na Noi (6), Phu Piang (3), Song Kwae (1), Tha Wang Pha (3), Thung Chang (7), Wiang Sa (8)	53	0
Barking deer (<i>Muntiacus muntjak</i>)	Bo Kluea (10), Chalermprakiat (1), Mae Charim (9), Muang (1), Na Noi (1), Phu Piang (2), Tha Wang Pha (2)	26	0
Sambar deer (<i>Cervus unicolor</i>)	Mae Charim (1), Tha Wang Pha (1)	2	0
Bear (<i>Ursus</i> spp.)	Thung Chang (1)	1	0
Civet (<i>Paradoxurus hermaphroditus</i>)	Na Muen (1), Phu Piang (1)	2	0
Wild rabbit (<i>Sylvilagus</i> spp.)	Muang (1)	1	0
Pangolin (<i>Manis</i> spp.)	Phu Piang (1)	1	0
Monitor lizard (<i>Varanus</i> spp.)	Chalermprakiat (2), Muang (2), Phu Piang (5), Tha Wang Pha (1), Wiang Sa (1)	11	0
Overall		97	0

^a The scientific names of the animals were cited from published work on wildlife in Thailand (Lekagul & McNeely, 1977), and only the genus was used when there were more than one species in the genus and the exact species was uncertain: bear, wild rabbit, pangolin and monitor lizard

3.5. Discussion

The risk of swine trichinellosis on a farm that takes measures to ensure that *Trichinella* larvae are absent in the pig food chain is negligible (Gamble *et al.*, 2001). Thus the production and management practices that allow the exposure of pigs to foods containing *Trichinella* larvae are associated with trichinellosis in pigs. This study in human trichinellosis-endemic foci revealed pig production and management characteristics in the affected areas and their association with swine trichinellosis.

3.5.1 Pig production systems in Nan Province

Pig production is recognized as being a major source of family income, a supplementary source of funds for particular purposes, a savings bank (Steinfeld, 1998) or a cultural and tradition (Lemke *et al.* (2002) cited in (Huynh *et al.*, 2007)). Pig production in the study areas of Nan Province predominantly fulfilled the three last purposes. This was based on holding size, type of food, pig breed and management and use. The median holding size in the study areas was three pigs per holding, in which pigs of all ages were included. Over a half (57.80%) of the holdings reared native-breed pigs. The majority of holdings used household leftover food to feed the pigs. The use of de-wormers and vaccines in the holdings was observed in only 59.0% and 26.99% of the entire holdings respectively. Lastly, about half of the holdings reared pigs mainly for the owner's own use. Nakai (2009) observed the consumption of pigs in a village included in this study for a two years. He found that about 78% of consumption directly related to ritual events such as ancestor worship, cures for disease, New Year celebrations, marriages, funerals and Christmas festivities.

Intensive medium-scale or large-scale pig production - which is the norm in Thailand nowadays (Cameron, 2000) - was hardly observed in this study area in Nan Province. Instead, it was dominated by small scale holdings (A small farm with 1-100 animals raised per year (FAO/OIE/World Bank, 2010)). Among the holdings' management practices and production schemes within the holdings in the two different areas, the highland and lowland differed significantly in various aspects. Highland production was mainly for the owner's own use, native-breed pigs predominated, pigs were not commonly vaccinated or de-wormed and some pigs were observed free-ranged scavenging. They were fed with un-cooked household leftovers in about 78% of the holdings. Only about 38% of holdings de-wormed their pigs with an even smaller proportion of the holdings vaccinating their pigs (2.5%). In contrast, scavenging pigs were rarely observed in the lowland areas, instead, improved-breed pigs were used for breeding, sound management practices and inputs such as commercial feed-grain, de-worming, and vaccination were used within the majority of holdings. The production was predominantly for capital earning.

During the 1970s and earlier, the majority of pigs raised in the highland areas in Thailand were free-range scavengers (Visitpanich & Falvey, 1980). This was similar to pig production of the same period in the mountainous areas of North-west Vietnam (Lemke & Zarate, 2008). Pig production in the highlands nowadays has have improved compared with historical husbandry practices. Although some scavenging pigs were observed during this field study, they were not wholly free- roaming reliant on self-supported feeding. Observation revealed that all farmers had at least provided simply-constructed pens and food for their pigs. This was conforms with the study performed by Nakai (2009) although he did add that pig feed given by farmers was not sufficient for pigs during the rainy season. In addition to providing pens and food for pigs, about 15% of the pig owners in the highlands also used cooked

household leftovers to feed their pigs. Although not widely practised, some of them had managed to de-worm or vaccinate their pigs. What had not changed was the use of native-breed pigs in the production. Almost all of the holdings in the highland areas reared native pigs. The live improved-breed pigs were bought into the village only for consumption, they were not used for breeding (Nakai, 2009).

Lemke and Zarate (2008) investigated the dynamics and development trends of smallholder pig production systems in the marginalized mountainous areas of North-west Vietnam. They found a rapid transition process from subsistence to increasingly market-oriented production since the 1980s. In these areas in recent years pigs were being permanently penned and their feed was usually cooked; these being management practices that can prevent *Trichinella* transmission to pigs. These changes in production methods such as have occurred in Vietnam, were not clearly present in the highland areas in this study. Here the aim of pig production was still for subsistence purposes with no motivation or incentive to improve the production practices.

3.5.2 Trichinellosis seroprevalence in pig

This study documents 17 trichinellosis sero-positive pigs from 1,196 sampled pigs, thus indicating that swine trichinellosis is still occurring in the Northern Region of Thailand. In the areas studied during this current work swine trichinellosis occurred with a true prevalence estimated as 0.05% with 95% confidence intervals (0.0 – 0.55%) or the tested prevalence of 1.05% (95%CI 0.59 – 1.87). The screening of swine trichinellosis in northern Thailand in 2005 and 2006 failed to detect any infection, but this was likely to be the result of an insufficient sample size. In those surveys 215 serum samples from improved-breed pigs from 43 commercial farms (five pigs per farm) in northern provinces (Vitoorakool & Vitoorakool, 2005) and 330 serum samples from pigs in the upper northern part of Thailand were screened (Vitoorakool *et al.*,

2007). The last survey included 173 improved-breed pig and 32 native-breed pigs in the lowland areas and 125 native-breed pigs in the highland areas.

When the prevalence of swine trichinellosis in the pig population is generally low, any survey for surveillance purposes will require an appropriate sample size in order to determine or detect infection. In the USA a survey assuming 0.05% prevalence required a sample of 4,078 pigs (Gamble *et al.*, 1999). Within-herd prevalence of 0.05% was estimated (Beal, 1983). Thus to detect infection with 0.95 probability, almost every animal in herds needs to be tested, and all animals need to be tested in herds with less than 21 pigs. In practice, however, there is a need to direct the test towards animals at greatest risk *i.e.* breeding pigs. In this current study the holdings were small in size. They would hold a minimal number of breeding pigs, often one or two though sometimes there would be no breeding pigs on the holding. Thus all pigs ≥ 2 months old were tested.

Highland areas in the Northern Region of Thailand have been considered the high risk areas of swine trichinellosis. Dissamarn and Chai-Anan (1970) used the digestion method to examine 70 pig carcasses in 15 highland villages of Chiang Mai, Chiang Rai, Phrae and Nan Provinces and identified eight infected pigs (11.4%). Vitoorakul *et al* (1989) performed the ELISA test using crude antigen to examine 220 pig sera from highland areas located in Nan, Chiang rai and Chiang Mai Provinces in Northern Region and found 71 (32.3%) trichinellosis seropositive cases (Vitoorakool *et al.*, 1989). The digestion method has a high specificity but low sensitivity. The sensitivity of the digestion method has been defined as 43.4% compared with the ELISA test (Beck *et al.*, 2005a). A further limitation is that the ELISA test using crude antigen can cross-react with other parasitic infections and thus yield a number of false positive results, and hence, resulting in low specificity

(Rapic *et al.*, 1986). This current prevalence survey found 14 trichinellosis sero-positive pigs from 721 sampled pigs in the highland areas. The true prevalence estimated as 0.97% with 95% confidence intervals (0.46 – 1.48) or the test prevalence of 1.94% with 95% confidence interval (0.96 – 3.88), was much lower than that detected in the past. Regardless of the difference between the tests that have been used to determine prevalence, the ELISA test using ES antigen performed on samples in this study yield high sensitivity and specificity (Gamble *et al.*, 1988). Thus the results indicating a decrease in the prevalence of swine trichinellosis in the highland areas over time can be regarded as reliable. Improvements in pig management practices could have been responsible for the observed changes.

Swine trichinellosis prevalence outside the highland areas has been considered to be low. The abattoir surveillance programme for trichinellosis in pigs in the Northern Region between 1967 - 1983 using the trichinoscopic method found only 19 *Trichinella*-infected pigs among 1,286,754 animals; and all of the positives were from highland areas (Dissamarn & Indrakamhang, 1985). This study found that swine trichinellosis prevalence outside the highland areas was lower when compared with the prevalence in the highland areas but the difference was not significant. This finding confirmed that trichinellosis was not absent in the lowland areas. Swine trichinellosis prevalence in the lowland areas from this study was estimated with 95% confidence level as between >0.0 – 0.36%. Acquisition of infected-pigs by the holdings was one of the reasons for finding swine trichinellosis in the lowland holdings. Sources of infected pigs can be indigenous pigs or pigs purchased from the Thai-Laotian border market, the latter being observed in this study. Although little is known about swine trichinellosis prevalence, there are reports of human trichinellosis outbreaks attributed to the consumption of Laos' pork in Laos (Sayasone *et al.*, 2006; Barennes *et al.*, 2008) and in Vietnam (Phan (1997) cited in Vu Thi *et*

al., 2009). Based on this evidence, live pigs or pork from Laos posed a quantifiable risk of *Trichinella* transmission to the Thai people in border provinces who consume raw pork. The public should be made aware of this problem, and screening of live pigs or pork from Laos should be considered. Certain trichinellosis outbreaks in northern Thailand were attributed to these infected-pigs (Bureau of Epidemiology, 1981-2008).

The prevalence of swine trichinellosis estimated from this study differed from that observed in trichinellosis endemic areas in Vietnam and China. In Vietnam 206 of 1,035 (19.9%) free-roaming pigs tested positive by the ELISA technique (Vu Thi *et al.*, 2009). Seroprevalence of swine trichinellosis in China varies from 1.63% to 15.21% (Wang & Cui, 2001b; Cui *et al.*, 2006a). Assuming that the sensitivity and the specificity of the tests employed in these surveys are comparable to the tests used in this study, the magnitude of swine trichinellosis prevalence in the trichinellosis endemic areas in Thailand was the lowest of these three countries. This difference may relate to the difference in types of pig production systems between countries and the pig population density within the production system. Pig production on a large scale usually operates with level of high bio-security to safeguard the pigs from various pathogens. This serves to prevent *Trichinella* transmission to pigs. Of these three countries Thailand had the smallest pig population and, in contrast to China and Vietnam, its production systems are dominated by intensive medium- and large-scale production in contrast to China and Vietnam (Cameron, 2000; Huynh *et al.*, 2007).

3.5.3 Cluster of swine trichinellosis

A significant cluster of swine trichinellosis was identified in the survey dataset. It encompassed three adjacent Yao villages. Trichinellosis is not, by nature, a highly infectious disease. The infection rate within the one herd was only 0.05% and the herd prevalence identified in USA pig farms was 6.4% (Gamble *et al.*, 1999). In this survey, sero-positive pigs

were identified in 13.1% of villages and among 3.6% of holdings. The cluster reflected the poor management practiced across those villages within the cluster. A cluster may also relate to the persistence of the source of infection in the area. These three villages not only maintained free-ranging scavenging pigs but also slaughtered pigs (and potentially wildlife for food) at home. Pigs were reared in close proximity and therefore shared contaminated food. The aggregation of these factors would explain why the cluster was identified in these three villages.

Khamboonruang (1991) classified the *Trichinella* transmission cycle in Thailand as a sylvatic cycle where *Trichinella* is transmitted from wild animals to pigs or humans. This study suggested that there is also the potential presence of a domestic cycle in which *Trichinella* is transmitted from pig-to-pig. This transmission cycle would occur when infected-pigs were home-slaughtered with the meat waste being ingested by scavenging pigs. In the Yao and Hmong communities, where home-slaughtering of pigs prevails and scavenging pigs are observed, trichinellosis sero-positive pigs were identified more than one cases.

3.5.4 Risk factors and characteristics of swine trichinellosis sero-positive animals

From the two-by-two table analysis the age of pigs, free-ranging scavenging, pigs not being de-wormed, pigs belonging to hill-tribe ethnic owners and pigs produced for the owner's own use had higher trichinellosis seroprevalence than groups of pigs without these characteristics. The last three characteristics were predominantly found in the highland areas. Therefore, when taking the difference between areas into account in the analysis for an association with trichinellosis, only older-aged pigs and free-ranging scavenging pigs were significantly associated with trichinellosis in pigs in the area.

In this study, the age of trichinellosis sero-positive pig (mean = 22.31 months; 95% CI 15.84 – 28.78) was significantly higher than the trichinellosis sero-negative pigs (mean = 12.36 months; 95%CI 11.14 – 13.58). The odds ratio for trichinellosis increases 1.02 times with each one month increase in the age of the pigs, with a 95% confidence interval (1.00 – 1.04). This finding was in accordance with fact and findings in other settings. *Trichinella* infect animals of all age with the chance of infection is increasing as the animal ages. For this reason surveys for swine trichinellosis in pigs usually bias the sample toward the older pigs, such as boars and sows (Gamble *et al.*, 1999; Appleyard *et al.*, 2002; Schuppers *et al.*, 2009). The study in Vietnam found the infection rate among sero-positive pigs was significantly higher in pigs older than eight months (Vu Thi *et al.*, 2009). This study in Vietnam also sampled pigs younger than two months and identified a 1.1% sero-positivity against *Trichinella* infection. The proportion of sero-positive pigs in the group of pigs aged under two months, and the group of pigs aged between 2 – 8 months did not differ significantly (Vu Thi *et al.*, 2009). As it was considered that the risk of infection in young pigs and the possibility to detect a resultant sero-conversion was low, the current study did not sample pigs younger than two months of age. The youngest sero-positive pig detected in this study was a 4-month old pig purchased from the Thai-Laotian market. All other sero-positive pigs were indigenous pigs aged from 12 to 40 months. Monroy (2001) investigated the risk factors for swine trichinellosis by examining pigs at the slaughterhouses that had originated from commercial farms and backyard farms. They associated swine trichinellosis risk with pigs from backyard farm aged from 7 to 12 months. All of these reports confirm that the infection rate for *Trichinella* in pigs is low up to the age about 7 – 8 months. Hence it was justified for this study to exclude pig younger than two months. However, following the finding from the study in Vietnam that infection occurs in pigs very early in life, it is recommended that pigs of all age should be kept in bio-secure confinement in order to prevent *Trichinella* infection.

The prevalence of trichinellosis was significantly high in scavenging pigs (3.45%; 95%CI 1.35 – 5.58) compared with the trichinellosis prevalence in totally confine pigs (0.74%; 95%CI 0.2 – 1.27). The odds of trichinellosis in free-ranging scavenging pigs, was estimated to be 2.96 times larger than the odds for pigs that were totally confined and the confidence interval estimate suggested that odds for free-ranging scavenging pigs could be as little as 1.20 or as much as 7.31 times as great as the odds for totally confined pigs. Free-ranging allows pigs to be exposed to food contaminated with *Trichinella* and therefore is likely to be the key factor facilitating *Trichinella* transmission to pigs. Free-ranging scavenging pigs may have access to carcasses (including wild animals, rats and other domestic animals such as dog and cats that may have been left on the ground), food waste scraps in garbage and meat waste by-products that are left on the ground following home-slaughtering of pigs and other wild animals. In highland areas home-slaughtering of pigs and wild animals was a common practice: 649 of 1,196 (54.3%) sampled pigs in this study were subjected to home-slaughtering. Meat scraps left on the ground were potential sources of *Trichinella* for scavenging pigs. Scavenging pigs that eat infected tissues poses a risk of *Trichinella* infection to both pigs and other potential reservoir hosts. As a consequence, the results of this study were no surprise. They strongly confirmed that scavenging was the most important risk-associated management practice related to swine trichinellosis in the highland areas. A study in highland areas dominated by the Hmong ethnic group in Vietnam by Vu Thi *et al.* (2009) found high prevalence of swine trichinellosis antibodies in pigs in areas where they were allowed to roam free or kept in simply-constructed pens. In China there are two management practices associated with swine trichinellosis. One was the outdoor rearing of pigs in mountainous areas similar to that seen in Thailand and Vietnam. The second was the use of

leftovers from restaurants being fed to pigs reared in small farms in suburban areas (Cui *et al.*, 2006a).

This study found that pigs that had never been de-wormed were approximately 5.21 times as likely to be trichinellosis cases as pigs that had been de-wormed and the odds could be as much as 30.53 times greater or as little as 0.89 times smaller with 95% confidence. The difference between two groups was not significant at the 5% level but it was at 10% level. Successful use of anthelmintic prophylaxis and treatment to control trichinellosis in pigs has been reported from both China and Romania (Olteanu, 2001; Wang & Cui, 2001b). An experiment in restricted areas in China to prevent trichinellosis in pigs by adding an anthelmintic (albendazole) to pig feed found that the prevalence of trichinellosis in that study area decreased from 32.2% to 0.12% (Wang & Cui, 2001b). In Romania, where trichinellosis was wide spread in commercial farms, the administration of anthelmintic showed some positive results in the control of trichinellosis in pigs (Olteanu, 2001). Although anthelmintics can prevent *Trichinella* infection in pigs, the drugs have to be administered in pig feed over a long period of time. There is a potential for drug residues in the meat and because the side effects of such drug residues in pork are not known, and so this method has not been used widely as a preventive measure for trichinellosis in pigs (Liu & Boireau, 2002).

Gamble *et al.* (1999) investigated the association between feed type, rodent infestation, access to wildlife and wildlife carcasses and access to swine carcasses with swine trichinellosis in USA . Only pigs with access to wildlife carcasses had an increased risk of trichinellosis ($p = 0.01$). None of these factors associated with trichinellosis in pigs in Nan Province. Although the use of household leftovers was a common practice in the study areas, there was no association with restaurant leftovers: one of the risk factors for swine trichinellosis identified in

China (Cui *et al.*, 2006a). In general the majority of holdings in the study areas did not have any barrier to prevent the access of domestic animals and wildlife and a rodent control regime was not in place. However, wild carnivores that are known to be important wildlife reservoir hosts for *Trichinella* elsewhere (van der Giessen, 2001; Malakauskas *et al.*, 2007; Oivanen & Oksanen, 2009) are not abundant in the pig holdings in Thailand. Therefore, access to live wildlife did not have an effect on the epidemiology of swine trichinellosis in this study. Other factors, such as access to dead carcasses of dogs and cats that have been left on the ground, may be more important. Based on previous studies of potential animal reservoir hosts in Thailand in dogs and rats, *Trichinella* infection in dogs were identified in trichinellosis endemic and non-endemic areas, while *Trichinella* infection in rats were identified only in some of the trichinellosis outbreak related areas. Two surveys of dog meat samples, using the digestion method, identified *Trichinella* infection rates of 1.66% (7/421) (Srikitjakarn *et al.*, 1981) and 2.42% (3/124) (Chaimanee *et al.*, 2002). An environmental study in area where a human trichinellosis outbreak had occurred identified *Trichinella* infection in 8 of 15 dogs and one of three cats (Chalermchaikit *et al.*, 1982). Environmental studies on two separate human trichinellosis outbreak areas identified *Trichinella* larvae in one of 12 rats (Dissamarn & Chai-Anan, 1970) and one of 21 rats (Wijit *et al.*, 2002). However, a survey in a municipality in Chiang Mai Province in northern Thailand did not find any *Trichinella* larvae in 1,075 rats (Khamboonruang *et al.*, 1976). Additionally a survey carried out in two highland villages in Chiang Mai Province found none of the 155 rats had *Trichinella* larvae (Yamaguchi (1979) cited in (Dissamarn & Indrakamhang, 1985)). Finally an investigation, using the digestion method, of 201 rodents trapped around pig farms in several parts of Thailand also revealed that none of the rats carried *Trichinella* larvae (Panichabhonse, 2006). Based on this evidence, the role of rat as a reservoir of *Trichinella* to pigs can be considered low, particularly outside trichinellosis endemic areas.

Factors such as sex and category of pig (breeder and fattening) were not associated with trichinellosis in this study. The finding on the gender of the pig agreed with studies in Vietnam and Mexico (Monroy *et al.*, 2001; Vu Thi *et al.*, 2009). About an equal number of female and male trichinellosis sero-positive pigs were identified and there was no significant difference prevalence between the genders. There was a report of a difference in prevalence of trichinellosis between genders in the rat. A survey in Finland found the prevalence and intensity (number of larvae) of infection in female rats was greater than in male rats (Mikkonen *et al.*, 2005). It was believed that the effects of reproduction in the female rats, which are often pregnant and weaning, decrease their immunity against the parasites (Mikkonen *et al.*, 2005). There is no published information investigating the difference in the biological characteristics and *Trichinella* infection in male and female pigs. Additionally, whether pigs were breeders or fattening pig did not significantly affect swine trichinellosis in this study. The prevalence of trichinellosis was slightly higher in the breeder group but this could be anticipated as the breeder pig is usually older than a fattening pig. In areas, particularly the highland areas in this study, where the pig-rearing is dominated by native-breed pigs, these pigs were given insufficient food, as observed by Nakai (2009), were slow growing and were kept without a time limit. Under this breeding regime, risk of *Trichinella* infection accumulates as production time increase, hence, the prevalence of swine trichinellosis in breeders and fattening pigs did not differ significantly in this study. Shortening production time was one of the explanations for a decrease in prevalence of swine trichinellosis in China (Cui *et al.*, 2006a).

3.5.5 *Trichinella* infection in wild animals

Muscle samples from wild boar, bear, monitor lizard, barking deer, sambar deer, civet, wild rabbit and pangolin were examined for *Trichinella* infection using the trichinoscopic and the digestion methods. None of the 97 samples examined had *Trichinella* larvae. The absence of test-positive samples could be due to an insufficient sample size, the quality of meat specimens and the sensitivity of the test.

Pozio and Rossi (2008) mentioned that the prevalence of *Trichinella* infection in wildlife is generally very low, therefore, large sample sizes may be required to detect infected animals. For example, surveys in Italy, Spain and Sweden between 1985 – 2003 found prevalence of trichinellosis in wild boar at 0% (n = 1,508) (Rossi & Dini, 1990), 0.3% (n = 29,333) (Perez-Martin *et al.*, 2000) and 0.05% (n = 8,000) (Pozio *et al.*, 2004), respectively. If the infection rate in the wildlife population is high, a small number of samples can yield positive results. A study in Argentina identified *Trichinella* larvae in three of only 12 wild boar samples examined (Ribicich *et al.*, 2010). In the same study 169 wild animal samples were examined. Infection was detected in nine of 66 rats (*Rattus norvegicus*), one puma (*Puma concolor*) and three of 19 armadillos (*Chaetophractus villosus*) (Ribicich *et al.*, 2010). The 53 wild boar samples examined in Nan Province were much more than four times the quantity examined in Argentina, implying that the infection rate in wild boar in Nan Province is not as high as can be found in Argentina.

In terms of the quality of samples, the predilection site for *Trichinella* specific for each animal species plays an important role in determining which muscles to sample. This study obtained samples from various sites of muscles provided by villagers. In order to compensate for the limitation that sample may not always have been from muscles from predilection sites the test was performed on at least 30g of muscle from

each individual animal. It has been recommended to increase the amount of tissues sampled in order to maximize the chances of detecting infection in wild animals when (i) samples were not obtained from predilection sites and (ii) the infection level was thought to be low (Gamble *et al.*, 2000; Dupouy-Camet & Murrell, 2007). Pozio and Rossi (2008) recommended that the minimum amount of muscle that should be tested from each animal, irrespective of the species, is $\geq 10\text{g}$ from preferential muscles and $\geq 20\text{g}$ from other muscles. Prevalence surveys of trichinellosis in wildlife that have been conducted until recently have been based on specimens of meat from animals using the digestion method as the standard diagnostic tool (Gamble *et al.*, 2000). False negative results using the digestion method occur when the larval burden is low *i.e.* < 1 larvae per gram of muscle sample (Venturiello *et al.*, 1998). Some studies have shown that the digestion method can fail to detect *Trichinella* even when the larvae burden in muscle is ≥ 6 larvae per gram (Beck *et al.*, 2005b). False negative results obtained by the digestion method performed on meat samples of wild boar have been recorded. Frey *et al.* (2009) examined 1,458 wild boar meat samples using the digestion method, (a 10g muscle sample per individual animal), and did not detect larvae in any of the samples. However 57 of these samples tested positive by the ELISA test and three samples were positive by Western Blot (Frey *et al.*, 2009a). Despite its limitation, the digestion method is suitable for individual meat samples; it is cheap and is a standardized method that has been in place in many laboratories, in part as it is the method that has been recommended by the OIE (OIE, 2008).

Various wildlife species are taken for food in Nan Province and subsistence hunting for wild animals occurs throughout the year. These wild animals are slaughtered at home, like the domestic pigs, and were therefore another potential source of *Trichinella* for free-ranging scavenging pigs. The results of this investigation indicate that the trichinellosis prevalence in wild animals that are caught for human

consumption in Nan Province may be low, although the actual extent can not be determined. The role of wildlife in transmitting *Trichinella* to pigs in the study areas thus may be no higher than the transmission from the infected pigs there. Regardless of the low infection rate of *Trichinella* in these wildlife species, they are natural hosts of *Trichinella*. There are always risks that these animals are infected with *Trichinella* and transmit the parasite to domestic pigs and humans. In the community where people depend on subsistence hunting they should be advised to properly discard the waste from wildlife carcasses and consume only cooked meat in order to prevent infection.

3.6. Advantages and limitations

This study was able to include the marginalized pig population with the findings providing useful baseline information for future surveys or evaluations of the disease situation. Prevalence studies of trichinellosis in pigs have been mainly conducted through the examination of carcasses at slaughterhouses. This is appropriate where most pigs are destined to be slaughtered at the slaughterhouse. In many settings, include Thailand, home-slaughtering of pigs still occurs in rural areas, with the pigs having been reared for the owner's own used and to be mostly consumed in the community. This study, therefore, investigated the seroprevalence in pigs by collecting samples at the farm-holding, thus including all types of pig production in the study areas. Using this approach the findings demonstrated the prevalence of *Trichinella* infection in the overall pig population of the study areas without bias. This enabled comparison of the trichinellosis prevalence in groups of pigs which have been believed to have had greater and smaller risks of trichinellosis. By using the cluster sampling method and collecting from every pig aged ≥ 2 months in the cluster (village), the between-cluster variance can be estimated. This value can be used to determine sample size using cluster sampling that may probably take place in the future. Additionally, the information

on prevalence obtained from this random sampling survey could be used as baseline data to evaluate disease status and trends in the future.

The limitations of this study include the sampling method, generalization of the findings and the sensitivity of the laboratory test. Sample selection was biased towards pigs in the highland areas. Therefore the analysis of the survey data required compensation for differentiation in sampling rates, making the analysis complicated and cumbersome. It would have been desirable to use another sampling approach *i.e.* cluster sampling proportional to population size, if pig population data had been available. The study areas were selected because they were known as persistent trichinellosis foci and. Thus, they held unique characteristics in terms of pig population demography and the production systems not present elsewhere in Thailand. Therefore, the findings are applicable to the study areas or those areas, such as in the northernmost provinces in Thailand, with a similar environment. In terms of the testing method used to test for *Trichinella* infection in wildlife samples, it is accepted that samples could not always be obtained from the predilection sites. As the digestion method has low sensitivity, the use of higher sensitivity tests, such as using the ELISA test on meat juice, would increase the chance of detecting evidence for infection in wildlife. This study was not able to collect meat samples in order to confirm the presence of *Trichinella* larvae in sero-positive pigs. Sero-positivity was not additionally confirmed using other tests with a proven superior performance, such as the Western Blot.

3.7. Summary

Nan Province is one of the five northernmost provinces in Thailand that have been considered as the endemic areas of trichinellosis where the major source of human infections was the domestic pigs. The production and management practices which allow the exposure of pigs to food

containing *Trichinella* larvae are associated with trichinellosis in pigs. These were investigated in this study.

Pig production in Nan Province is dominated by small-scale production. Within this production system in two areas, namely the highland and lowland areas, pigs were managed differently and produced for different purposes. The production in the highland areas was for the owner's own use and the management practices were considered poorer when compared with those in the lowland areas. However, there were some improvements in husbandry practices. Every holding provided pens and food to pigs, although some of the holdings still allowed pigs to roam free. The introduction of such management practices to the pig production in the highland areas has resulted in a decrease in the prevalence of swine trichinellosis.

Swine trichinellosis has still occurred in domestic pigs in the study areas. The seroprevalence in the highland areas was higher than those of the lowland area but the difference was not significant. In the lowland areas the introduction of infected pigs from neighbouring countries into the holding was observed. Scavenging by the pigs was the only management practice significantly associated with swine trichinellosis. The seroprevalence was significantly higher in older pigs than in young pigs. A significant cluster of swine trichinellosis was detected in the highland area in three Yao villages. Scavenging by the pigs and the home-slaughtering of pigs were commonly practiced in these areas. The pig-to-pig transmission cycle of *Trichinella*, where scavenging pigs become infected from consumption meat scraps left on the ground during home-slaughtering of pigs is proposed for the transmission and maintenance of swine trichinellosis in the study area.

Many wild animal species were taken for food by subsistence hunters, which occurred throughout the year. This study examined 97 meat

samples from wild animals caught for consumption in Nan Province but failed to identify *Trichinella* larvae in any of the samples screened. Although the wildlife sample size was small and certainly did not reflect the true prevalence of trichinellosis because of convenience sampling, some conclusions could be drawn. The results indicated the likelihood of a low prevalence of trichinellosis at least in wild boar, barking deer and monitor lizard from which 53, 26 and 11 samples respectively, were screened. Thus the contribution of wildlife trichinellosis on swine trichinellosis prevalence in the study areas may not be higher than the effect caused by the infected pigs known to already exist in the area.

Overall, the prevalence of swine trichinellosis in the endemic areas in Thailand was substantially lower when compared with other countries where the disease is found. This can be attributed to two major factors: (i) 80% of pig production in Thailand is in intensive medium-scale and large-scale production systems that are managed with practices that present a negligible risk of *Trichinella* transmission, coupled with sound management practices within the small scale pig production that too limit the spread of the disease and (ii) baseline husbandry management practices in the swine trichinellosis high risk areas have been gradually improved, already resulting in a decrease in swine trichinellosis prevalence.

4. Chapter 4 - Case-control study of trichinellosis risk factors in Nan and Chiang Rai Province, Northern Thailand

4.1. Study aims

The work in this chapter aimed to (i) identify the risk factors for human trichinellosis in trichinellosis foci in Thailand and (ii) to determine changes in trichinellosis risk-associated behaviour of individuals that have experienced infection. A case-control study was undertaken to explore the exposure to risk factors, in place prior to the infection occurring in both cases and the controls, to assess different magnitudes of possible risk-associated behaviour practised by those individuals. In addition, trichinellosis case risk-associated behaviour was examined a year after infection in these cases and was compared to the controls to determine the changes in trichinellosis risk-associated behaviour post infection.

4.2. Introduction

Although the incidence of human trichinellosis has decreased in Thailand endemic foci have been maintained in rural communities in the Northern Region, particularly in the five northernmost provinces. Domestic pigs were responsible for the majority of outbreaks that occurred between 1981 and 2008 (see Chapter 2) but we observed that the seroprevalence of trichinellosis in pigs in Nan Province, one of the trichinellosis endemic areas in Thailand, has decreased from what has been observed in recent decades (see Chapter 3). This is likely to have been brought about through improvements in pig management practices resulting in a collateral benefit in prevention of transmission of *Trichinella*.

This chapter extends the study of animal reservoirs of *Trichinella* infection to a study of human behaviour to obtain insights into factors influencing *Trichinella* transmission in the endemic areas in Thailand. The behaviour of individuals regarding raw-meat consumption, their household characteristics and their knowledge in relation to food-borne disease infection was investigated in association with trichinellosis infection. Consumption of raw-meat is the primary cause of trichinellosis. The custom of raw-meat consumption continues in the trichinellosis endemic areas of northern Thailand. Additional behaviour patterns have been proposed as factors contributing to infection in the human population in endemic areas of Thailand. These include consumption of alcohol and festivals or ceremonies that are associated with eating raw-meat. People in these areas believe that lime-juice added to meat dishes is sufficient to kill parasites and microbes and so meat is consumed raw without heating or cooking. In addition, the custom of raw meat consumption (despite knowledge of infection risk) was also investigated. A previous study in Chiang Mai Province in the Northern Region of Thailand showed that buying pork from neighbours was a risk factor of trichinellosis (Mahannop *et al.*, 1999). In other cases specific sources of meat and particular characteristics of individual consumers were identified as risk factors. A study of risk factors in trichinellosis foci in the semi-rural areas of Mexico identified being female and the consumption of a sausage-like pork product made of blood and meat as a key risk factor in 48 factors that were investigated, that included social, economic, demographic characteristics, hygiene and dietary practices (de-la-Rosa *et al.*, 1998). In Papua New Guinea, sero-positivity for trichinellosis was significantly higher in males than in females, was shown to increase with age, in areas nearest to hunting areas and in people that had consumed raw or undercooked wild-boar meat and also in individuals that had reported pain in their muscle joints and limbs (Owen *et al.*, 2005).

4.3. Materials and methods

4.3.1 Study sites

The study was conducted in Nan and Chiang Rai Provinces, trichinellosis-endemic areas in Thailand. Between 2003 - 2006 there were 149 reported cases of trichinellosis in these provinces. The study sites comprised five villages where trichinellosis outbreaks had occurred between 2003 and 2005. Villages were selected as during the previous outbreaks, active surveillance and follow up of cases had taken place and individual case data were available. Four villages were in Nan Province and one village was in Chiang Rai Province. The villages were 4 – 6 km² in size with populations in each village ranging between 339 and 841 people. All the villages were in areas classified as being rural, and the majority of the population comprised Thai ethnic groups. Two of the villages in Nan Province were in highland areas, with the remainder being in lowland areas. Villages are located between 5 and 22 km from the district centres, and between 13 and 133 km from the provincial centres. None of the villages were located on the main road but all had easy access to primary health care centres and primary schools, and had electrical and piped-water supplies.

4.3.2 Case-control study

The case and control groups were selected on disease status, with cases being defined as those having been diagnosed with trichinellosis, while the controls were individuals that had not been diagnosed with trichinellosis. Cases and controls were matched by age and gender. The two groups were compared in terms of exposure to possible risk factors (Jekel *et al.*, 2007).

In this study 24 risk factors relating to individual households, behaviour, and knowledge associated with trichinellosis were investigated. As this

was a retrospective study of already existing trichinellosis cases, there was no delay to setting up the study and no direct test costs. Controls were recruited from the same neighbourhood as the cases there was a saving on travel time and costs. Age and gender were controlled during the study design, by matching a case and control pair according to confounding factors. In this study, since the outbreak event had occurred more than one year previously it allowed determination of the prevalence of trichinellosis risk-associated behaviour in individuals after the trichinellosis outbreak in the affected communities by comparing with trichinellosis risk associated behaviour in the cases and the controls.

4.3.2.1. Trichinellosis case definition

A trichinellosis case is defined by the Bureau of Epidemiology, Ministry of Public Health, Thailand (2002) as a patient who fulfils two of the three following criteria:

- (1) *Clinical manifestations*: a patient presents with at least one of the signs or symptoms compatible with trichinellosis, such as periorbital oedema, myalgia, and fever,
- (2) *Positive examination*: a patient tests positive for evidence of recent *Trichinella* infection based on one of the following laboratory criteria: eosinophilia $\geq 10\%$, ELISA test positive or *Trichinella* larvae were identified in muscle tissue,
- (3) *Epidemiological linkage*: a patient shares an epidemiologically implicated meal or raw meat product from a confirmed trichinellosis outbreak.

4.3.2.2. Criteria for cases and controls

A case was any reported trichinellosis case in Nan or Chiang Rai Provinces during 2003-2006. Exclusion criteria were cases in children under 12 years of age (who might have been too young to recollect past exposure), cases with communication difficulties and cases that were not available at the time of study. A control was any non infected individual

residing in the same village matched for age and sex. After case selection was completed, controls were selected by simple random sampling from a list of residents of the village. The list of controls included name and address of each resident of the village with similar gender and age (a person who was born in the same calendar year) and who were not trichinellosis cases. If there was no control with the same age as a case in a village, a control of the closest age was selected.

4.3.2.3. Controlling for bias

Bias may occur during the design, execution and analysis of an epidemiological study that may invalidate results (Thrusfield, 2007). Examples of bias that may occur include bias due to confounding, measurement bias, selection bias and recall bias (Gregory, 2006; Jekel *et al.*, 2007; Thrusfield, 2007). In this study biases that were controlled for included bias due to confounding, interviewer bias and selection bias.

Confounding occurs when an extraneous variable, the confounder, accounts for an association between variables. The confounder must be a risk factor for the outcome of the study and associated with the explanatory variable (Thrusfield, 2007). Age and gender are known risk factors for trichinellosis and associated with the behavioural practice of consumption of raw meat consumption (Owen *et al.*, 2005). Males preferred raw meat more than females and older people tended to indulge more in the tradition of raw food consumption. Males had a higher in risk of becoming a trichinellosis cases compared with females and people of an older age were at higher in risk of becoming trichinellosis cases than children. These factors may be considered as confounders. To control for the effect of these two factors on the association of other explanatory variables and trichinellosis age-matched and sex-matched pairs cases and controls were selected. The analysis was conducted using the matched analysis. Other confounding factors were explored in the analysis using logistic regression (see 4.3.8.3).

Case-control studies are prone to selection bias (Jekel *et al.*, 2007). For example asymptomatic cases could be classified as controls. This can occur with trichinellosis because there have been several confirmed reports describing the presence of asymptomatic trichinellosis (Chomel *et al.*, 1993; Owen *et al.*, 2005). In this study selection bias was controlled for by selecting participants from the villages where trichinellosis cases were confirmed.

Interviewer bias may occur in studies in which subjects are interviewed or medical records are reviewed and the investigator's opinion affects the way that data were collected and interpreted (Gregory, 2006). In this study to reduce this type of potential bias, the disease status of participants was not revealed to the interviewers and the author until interviews and data coding were completed.

4.3.3 Sample size

The study was designed for matched sets of cases and controls, with one matched control per case. It was assumed that the probability of exposure among controls is 0.5. This estimate was based on the common practices of raw meat consumption in these study areas. If the true odds-ratio for disease in exposed subjects relative to unexposed subjects is 2.25, the study needed at least 101 cases with one matched control per case to be able to reject the null hypothesis that this odds ratio equals one with a power of 0.8. The type I error probability associated with the test of this null hypothesis is 0.05. The sample size calculation was performed using Win Episcopo 2.0 (Thrusfield *et al.*, 2001).

The formula for the required sample size is specified in Win Episcopo 2.0 (Thrusfield *et al.*, 2001):

$$m = \frac{\left(\frac{Z_{(\alpha)}}{2} + Z_{(\beta)} \times \sqrt{P \times (1-P)} \right)^2}{\left(P - \frac{1}{2} \right)^2}, \quad M = \frac{m}{P_e}$$

$$P = \frac{OR}{1 + OR}, \quad p_1 = \frac{p_0 \times OR}{1 + p_0 \times (OR - 1)}$$

$$p_e = p_0 \times (1 - p_1) + p_1 \times (1 - p_0)$$

Where M = number of required pairs to detect m discordant pairs

M = minimum number of required discordant pairs

$Z_{(\alpha)}$ = the value of student's t at the specified confidence level

$Z_{(\beta)}$ = the value of student's t (2-tailed) at the specified power

p_0 = expected proportion exposed in control group

p_1 = calculated proportion exposed in case group

OR = estimated odds ratio accepted as important in the study

4.3.4 Trichinellosis cases and population data

Data on individual trichinellosis cases were either stored at the Provincial Public Health Offices or at the Tambon Public Health Offices (for the administrative areas where trichinellosis outbreaks had occurred). These data were collected through collaboration with the Public Health Offices in Nan and Chiang Rai Provinces. There were 161 trichinellosis cases in total that occurred in people aged ≥ 12 years old, between 2003 and 2005 in the selected study sites. These cases were higher than the reported cases observed in the surveillance system. As previously mentioned (in Chapter 2) cases tracked from outbreak investigation, namely active investigation or active case finding, were not always reported. In this study active case investigation in a village during each outbreak ensured

that cases and controls were already identified. Population data, with the detailed demographic data (age, sex) and address of every individual in each village in the selected study sites, were obtained from District Offices or Tambon Public Health Offices and were used to select controls.

4.3.5 Selection of the possible risk factors for investigation

Twenty-four possible risk factors associated with trichinellosis were investigated. These factors were subjected to investigation based on information from a literature review, suggestions from experts and observations of the livelihoods of people in the study areas. The 24 determinants were classified into three groups: (i) household and individual characteristics (6 risk factors); (ii) individual behavioural characteristics (14 risk factors); and (iii) individual knowledge or perception (4 risk factors).

4.3.5.1. Individual risk behaviour

The risk factors related to individual behavioural characteristics comprised 14 determinants: (i) regular alcohol consumption, (ii) regular de-worming, (iii) consumption of home-slaughtered pigs, (iv) consumption of native-breed pigs, (v) consumption of wildlife, (vi) consumption of dogs, (vii) consumption of raw pork, (viii) consumption of raw beef, (ix) consumption of raw pork from wild boar, (x) consumption of raw meat from wildlife, (xi) consumption of raw dog meat, (xii) consumption of homemade raw meat, (xiii) consumption of purchased raw meat and (xiv) consumption of raw meat at a social gathering.

Raw meat consumption is the principle risk factor for trichinellosis in Thailand. Thus consumption of pork, wild boar, other wildlife besides

wild boar, beef and dog were the main factors examined in this investigation. Pork and wild boar had been previously identified as the first and second major sources of human trichinellosis in Thailand (Dissamarn & Indrakamhang, 1985). Other wildlife species have also contributed to trichinellosis outbreaks (Dissamarn & Indrakamhang, 1985). Although beef has not been reported as the source of trichinellosis outbreaks in Thailand, this meat is also commonly consumed raw and trichinellosis in cattle has previously been reported in China (Wang *et al.*, 2007). Outbreaks of human trichinellosis arising from consumption of dog meat have been reported in China and Russia (Liu & Boireau, 2002; Ozeretskovskaya *et al.*, 2005). Trichinellosis in dog meat has been reported in Thailand and China (Srikitjakarn *et al.*, 1981; Wang *et al.*, 2007) and an outbreak of trichinellosis was attributed to dog meat consumption in Thailand .

Mahannop *et al.* (1999) found that buying pork from a neighbour was a risk factor for trichinellosis in Chiang Mai Province. This may refer to pork from home-slaughtered pigs and native-breed pigs. Thus both factors were investigated in the current work.

Similarly, in Thailand, *Streptococcus suis* infections may be acquired through consumption of raw pork. The majority of *S. suis* infection cases were in individuals who drank alcohol regularly or heavily and alcohol consumption has been linked to raw meat consumption and *S. suis* infection (Suankratay *et al.*, 2004; Anon, 2007). Alcohol consumption was proposed as a risk factor of trichinellosis in this study. Regular alcohol consumption in this study is defined as consuming alcohol at least once a week.

The Northern Region of Thailand is endemic for opisthorchiasis, a food-borne disease caused by the liver fluke *Opisthorchis viverrini*. Opisthorchiasis control programmes including education, stool

examination and anthelmintic treatment, have been undertaken across the entire Northern Region since 1992 (Jongsuksuntigul & Imsomboon, 2003). This programme encourage the practice of regular deworming and result in changes in practice of raw meat consumption among people in the areas. Poor or irregular de-worming (< two times a year) may be a proxy indicator for the lack of awareness of an individual to prevent food-borne parasitic infection and this was also selected as a possible risk factor for trichinellosis.

4.3.5.2. Individual and household characteristics

The six risk factors relating to household and individual characteristics included: (i) agricultural occupation, (ii) illiteracy, (iii) a member of household going hunting, (iv) not possessing a refrigerator, (v) a family member having epilepsy and (vi) a family member having an acquired hearing-impairment.

Hunting and agricultural occupation were proposed as risk factors for trichinellosis because of the likelihood of people in rural communities obtaining wildlife meat and becoming infected when preparing and consuming meat containing *Trichinella* larvae. The evidence of high sero-positivity of trichinellosis among the hunting population has been reported in Papua New Guinea (Owen *et al.*, 2005) and there have been several reports of trichinellosis outbreaks related to hunting populations and hunters (Ancelle *et al.*, 2005; Moller *et al.*, 2005).

The level of an individual's education has found to be a risk factor that is associated with intestinal parasitic infection and blood-borne parasitic infection (Coldren *et al.*, 2006; Wani *et al.*, 2007). Therefore level of education was examined in this study for an association with trichinellosis. Zimmermann (1973) mentioned the availability of a refrigerator and freezer as one of the factors that reduced trichinellosis

prevalence in USA since freezing can deactivate some *Trichinella* species. This factor was investigated in this study.

Cysticercosis, a food-borne parasitic disease caused by *Taenia solium*, can cause an epileptic syndrome (Yeh & Wu, 2008). *Streptococcus suis* infection, a food-borne bacterial infection caused by *Streptococcus suis* type II, can cause hearing loss (Wangsomboonsiri *et al.*, 2008; Navacharoen *et al.*, 2009). Both diseases can be acquired from raw pork consumption. Thus an individual with a family member having epilepsy or an acquired hearing-impairment associated with raw pork consumption may also indicate exposure to raw pork consumption and therefore a risk of *Trichinella* infection. Although the actual prevalence of cysticercosis and *Streptococcus suis* infection in Thailand is not well defined, there have been a number of reports of cases, particularly in the Northern Region (Fongcom *et al.*, 2001; Waikagul *et al.*, 2006; Sirikulchayanonta *et al.*, 2007; Wangsomboonsiri *et al.*, 2008). The Bureau of Epidemiology established a reporting system for *Streptococcus suis* infection in 2007, in 2007, 2008 and 2009 there were 150, 230 and 158 reported cases, respectively. Five-hundred and eleven cases were in the Northern Region (Bureau of Epidemiology, 1981-2008). These numbers are considered to be an underestimation because the disease has not been included in the surveillance system and laboratory diagnosis is not widely available (Bureau of Epidemiology, 2007-2009).

4.3.5.3. Individual knowledge and perceptions

Risk factors related to an individual's knowledge or perceptions included four determinants: (i) inability to identify uncooked food, (ii) belief that alcohol can kill parasites and microbes in raw meat, (iii) belief that lime juice can kill parasites and microbes in raw meat and (iv) ignorance of food-borne diseases.

Trichinellosis occurs in rural areas in Thailand where health education programmes for food safety are not particularly well established. Public health personnel have suggested that the occurrence of trichinellosis may be associated with people who lack essential knowledge about food-borne diseases or have incorrect perceptions, e.g. when people believe that alcohol and lime juice or acidity can kill parasites and microbes in raw meat (Faichid, 2002). There is also a belief that local raw food, namely *nhaem*, *larb*, and *luh*, are essentially 'cooked' because these foods have been prepared by adding lime juice or as a result of a fermentation process (Faichid, 2002).

4.3.6 Ethical considerations

The study was conducted with the permission of the Nan Provincial Public Health Office and Chiang Rai Provincial Public Health Office to access data from individual trichinellosis cases. Verbal consent from participants was obtained before data were collected. The author acknowledged that the participants' rights to confidentiality would be protected during data collection. Participants could refuse to answer any question or withdraw from the interview at any time.

4.3.7 Data collection

All data were collected in November and December 2008. A structured questionnaire was used to interview cases and controls (see Appendix 9). For the convenience of the participants all interviews took place at the Tambon Public Health Offices or in the village assembly halls. Public health staff in the study areas contacted participants and made an appointment for the interview. Participants were informed about the study project, the interview and the participants' right to participate or to refuse. Interviewers included the author, five District Veterinarians and six Tambon public health personnel. All the interviewers were trained by

the author. None of the interviewers were aware as to whether or not participants were in the case or control group for the study.

Each participant was reminded about the trichinellosis outbreak event that had taken place in the village. They then were asked to recall their risk factors and their symptoms with reference to the time of outbreak event. Questions on the possible risk factors related to trichinellosis were asked with reference to the time within the year before the trichinellosis outbreak occurred. To determine if there were changes in individual trichinellosis risk behaviour after the trichinellosis outbreak, some questions regarding individual risk behaviour were asked with reference to the time approximately one year after the outbreak.

Questions about symptoms compatible with trichinellosis were asked with reference to the time that the trichinellosis outbreak occurred, and also one year later. Six clinical symptoms that can be easily recognised by participants were selected. The symptoms that were commonly found among the trichinellosis cases in Thailand included periorbital oedema (4.5 – 70.8%), facial oedema (29.0 - 85.7%), myalgia (59.7 – 100%), calf pain (17.9 – 73.5%), diarrhoea (8.3 – 55.1%) and vomiting (26.5 – 29.2%) (Doege *et al.*, 1969; Suriyanon & Klunklin, 1972; Limsuwan & Siriprasert, 1994; Jongwutiwes *et al.*, 1998; Khumjui *et al.*, 2008). The answers to these questions were collated to determine whether cases and controls were able to recall these past risk factors.

Questions (except for demographical data) were collected in binary format. After the interview was completed responses associated with putative risk factors were coded as 1 (present), or 0 (absent). A case was coded as 1 and a control was coded as 0. Pairs of cases and controls were numbered from 1 – n (n = 133). Then the data were entered into a database, Microsoft Office Access 2003 (Microsoft Office Access 2003. Ink), by the author and an assistant to assure correct data entry.

4.3.8 Statistical analysis

4.3.8.1. Descriptive statistics of the demographic and ethnicity of participants

Demographic data and ethnicity data for cases and controls were described using descriptive statistics; frequency and proportions. The median value was used to describe age. Percentage values were used to describe sex, ethnicity of cases and controls.

4.3.8.2. Prevalence of exposure to possible risk factors associated with trichinellosis among cases and controls

The prevalence of exposure to possible risk factors associated with trichinellosis among cases and controls was described as a proportion.

4.3.8.3. Factors associated with trichinellosis

The association between each variable and trichinellosis was assessed by measuring the odds ratio (OR) with 95% confidence intervals (Kirkwood & Sterne, 2003). McNemar's Chi-squared test or Fisher's exact test were used to test for significance in the association (at the 5% level). Calculations were performed using a two-by-two table matched analysis in Epi Info (Epi Info™ Version 3.5 2008).

$$\begin{aligned} \text{OR} &= \text{ratio of discordant pairs} \\ &= \frac{\text{number of pairs in which case exposed, control not exposed}}{\text{number of pairs in which control exposed, case not exposed}} \end{aligned}$$

Conditional logistic regression models were performed using stepwise backward removal (Noordhuizen *et al.*, 2001). All variables associated with trichinellosis with p value <0.25 were included in the models (Noordhuizen *et al.*, 2001). If two variables were highly correlated, one of them was omitted from the model (Noordhuizen *et al.*, 2001). The model building strategies and methods for logistic regression followed

the guidelines of Hosmer and Lemeshow (2000) and Noordhuizen *et al.* (2001) Following the fit of the multivariable model, any variables not selected for the original multivariable model were added back into the model to see whether it made an important change in the coefficient (β) of the variables present in the model. Relative changes in β of variables in the model of at least 25% (when β is larger than 0.40 or smaller than -0.40) or at least of 0.1 absolute change (when β is between -0.40 and 0.40) were considered significant and the variable was considered as a confounder (Noordhuizen *et al.*, 2001). Factors are called confounders when they are: related to the disease, related to the exposure factor under study and they are not an intermediate step in the causal path between the exposure factor and the disease (Noordhuizen *et al.*, 2001). The process of deleting, refitting, and verifying continued until all of the important variables were included in the model. The variables included in the model were based on two criteria (i) each variable was significantly associated with the outcome with p value <0.05 (ii) an estimated coefficient of the model with the variable significantly different from the coefficient from the model without variable based on the likelihood ratio test (Kirkwood & Sterne, 2003). Finally, the interactions among the variables in the model were checked. Interaction in an epidemiological context is defined as a quantitative interdependence between two or more factors, such that the frequency of disease when two or more factors are present is either in excess of or less than that expected from the combined effects of each factor (Thrusfield, 2007). The likelihood ratio test for interaction was used to test the null hypothesis that there is no interaction ($P>0.05$) by comparing the log likelihoods obtained from models that include interaction terms and the models that exclude the interaction terms. According to Noordhuizen *et al.* (2001) only biologically meaningful interactions should be tested.

4.3.8.4. Clinical symptoms compatible with trichinellosis

Whether there were difference in the presentation of clinical symptoms compatible with trichinellosis in cases and controls during the event of trichinellosis outbreak and about one year after trichinellosis outbreak were assessed using the odds ratio (Kirkwood & Sterner, 2003). The McNemar's Chi-squared test or Fisher's exact test were used to test for significance at the 5% level. Calculations were performed using a two-by-two table matched analysis in Epi Info (Epi Info™ Version 3.5 2008).

4.3.8.5. Practise of trichinellosis risk-associated behaviour after trichinellosis outbreak

The practise of trichinellosis risk-associated behaviour in cases for the year following a trichinellosis outbreak event in the cases was assessed relative to the practise of trichinellosis risk-associated behaviour in the controls. The magnitude of differences was determined by the odds ratio (Kirkwood & Sterner, 2003). Statistical test for different at 5% level were based on the McNemar's Chi-squared test or Fisher's exact test. Calculations were performed using a two-by-two table matched analysis in Epi Info (Epi Info™ Version 3.5 2008).

4.4. Results

Of the 161 pairs of cases and controls that were invited for interviews, 133 matched pairs were available. Reasons for participants not having interviewed included either where the selected cases or controls had died, were hospitalized for treatment of an illness or now worked or studied in other areas or had moved out of the village. The remaining 133 matched pair data sets were analysed in this study.

4.4.1 Descriptive characteristics of cases and controls

The median age of the 133 trichinellosis cases were 50 years of which 77 (57.9%) were male. The youngest case was 17 years of age and the oldest was 81 years of age. All 133 cases were matched to 133 controls by age and gender. Ages were matched to the exact year for all but 55 controls. Of these 55 controls, 26 had a 1-year age difference from their matched cases, 14 had a 2-year difference, six had a 3-year difference, four had a 4-year difference, two had a 5-year difference, one had a 7-year difference, and two had a 9-year difference. The median age of the controls was similarly 50 years. The youngest control was 17 years old and the oldest one was 79 years old. All the cases and controls were from across Thai ethnic groups.

4.4.2 Estimated prevalence of exposure to possible risk factors in cases and controls

Exposure to the possible risk factors among cases and controls the year before a trichinellosis outbreak had occurred is shown in Table 4-1. Among the cases and the controls, the most common occupation was related to agricultural work. Illiteracy rates were almost equal among

cases (27.07%) and controls (27.82%). Hunting was practised in 39.85% of the case households and 34.59% of the control households. Epileptic cases were reported in 0.75% of the case households and 4.51% of the control households. Acquired hearing-impairment cases were reported in 7.52% of the case households and 3.76% of the control households.

Regular alcohol consumption was practised by 48.12% of the cases and 36.84% of the controls. About 20% of the cases and of the controls did not practise regular de-worming. Consumption of home-slaughtered pigs was practised by more than 80% of the cases and controls, while 60.90% of the cases and 57.89% of the controls consumed pork from native-breed pigs.

Dog meat was consumed by a quarter of cases and controls while wildlife meat was consumed by up to three quarters of both cases and controls. Consumption of raw pork and raw beef was more than 70% in both cases and controls and consumption of raw wild boar was 34.59% among cases compared with 27.07% among controls. The proportions of cases consuming raw pork, beef and pork of wild boar were about 10% higher than controls and were significantly different. Less than half of the cases and controls that consumed wildlife meat consumed it raw. Raw dog meat was consumed only among 0.75% of cases and 2.25% of controls. Consumption of homemade raw food, purchased raw food and raw food at a social gathering was practised among over 70% of cases and controls.

The belief that any of the local raw food (*nhaem, larb and luh*) is 'cooked' was 2.25% among the cases. The belief that alcohol can kill parasites and microbes was 9.77% among the cases. These proportions were lower than that among the controls – which were 4.51% and 18.04%, respectively. The belief that lime juice can kill parasites and

microbes in raw meat was 17.29% in cases and about 13.53% in controls. All of the cases and controls were aware of food-borne diseases.

4.4.3 Risk factors of trichinellosis from the matched univariate analysis

Of the 24 factors being studied, six individual behavioural factors and one individual belief factor were found to be significantly associated with individuals being trichinellosis cases (see Table 4-1). Regular alcohol consumption increased the odds of individuals being trichinellosis cases compared with the controls who did not consume alcohol regularly. The odds ratio of being trichinellosis cases among individuals with regular alcohol consumption compared individual who did not consume alcohol regularly was 2.0 ($p = 0.02$). The same applies to the individuals who consumed raw pork, raw beef, raw meat of wild boar for which the odds ratios were 3.80 ($p = 0.004$), 3.71 ($P < 0.001$) and 2.77 ($p = 0.001$) respectively when compared with the controls who did not consume raw pork, raw beef and raw wildlife meat. Additionally, the odds of being a trichinellosis case among individuals who consumed homemade raw food was 3.00 times higher than that of individuals who did not consume homemade raw food ($p = 0.005$). The odds of being a trichinellosis case among individuals who consumed raw food at a social gathering was 4.60 times higher than that of individuals who did not consume raw food at a social gathering ($p < 0.001$). In contrast and perhaps counter-intuitively, the odds of being a trichinellosis case was 0.61 lower among individuals who believed that alcohol can kill parasites and microbes in raw meat when compared with the individuals who were aware that alcohol cannot kill parasites and microbes in raw meat ($p = 0.03$).

Table 4-1 Descriptive statistics of cases and controls and 1:1 matched univariate analysis of household characteristics, behavioural and perception of retrospective trichinellosis cases and age-matched, sex-matched neighbourhood controls in Nan and Chiang Rai provinces, Northern Thailand (n=133)

Possible risk factors	Total number of cases exposed to risk factor (%) ^a		Total number of controls exposed to risk factors (%) ^b		Number of discordant pairs		Odds ratio	p-value
	Exposed cases, non-exposed controls	Non-exposed cases, exposed control						
1.1 Agricultural occupation	119	(89.47)	111	(83.46)	17	9	1.89	0.12
1.2 Illiteracy	36	(27.07)	37	(27.82)	12	13	0.92	0.84
1.3 Does not have refrigerator	6	(4.51)	12	(9.02)	4	10	0.40	0.11
1.4 Family member goes hunting	53	(39.85)	46	(34.59)	35	28	1.25	0.38
1.5 Family member has epilepsy	1	(0.75)	6	(4.51)	1	6	0.17	0.06
1.6 Family member having acquired hearing-impairment	10	(7.52)	5	(3.76)	9	4	2.25	0.13
2.1 Regular alcohol consumption	64	(48.12)	49	(36.84)	30	15	2.00	0.02
2.2 Does not take regular de-worming	28	(21.05)	30	(22.56)	21	23	0.91	0.76
2.3 Consumption of home-slaughtered pigs	115	(86.47)	109	(81.95)	21	15	1.40	0.32
2.4 Consumption of native-breed pigs	81	(60.90)	77	(57.89)	20	16	1.25	0.50
2.5 Consumption of wildlife meat	102	(76.69)	95	(71.43)	19	12	1.58	0.21
2.6 Consumption of dog meat	30	(22.56)	27	(20.30)	20	17	1.18	0.62
2.7 Consumption of raw pork	124	(93.23)	110	(82.71)	19	5	3.80	0.004
2.8 Consumption of raw beef	117	(87.97)	98	(73.68)	26	7	3.71	<0.001
2.9 Consumption of raw meat of wild boar	59	(44.36)	36	(27.07)	36	13	2.77	0.001
2.10 Consumption of raw wildlife meat	46	(34.59)	37	(27.82)	24	15	1.60	0.15
2.11 Consumption of raw dog meat	1	(0.75)	3	(2.25)	1	3	0.33	0.32
2.12 Consumption of homemade raw food	120	(90.22)	104	(78.20)	24	8	3.00	0.005
2.13 Consumption of purchased raw food	98	(73.68)	96	(72.18)	21	19	1.10	0.75
2.14 Consumption of raw food at a social gathering	120	(90.22)	102	(76.69)	23	5	4.60	<0.001

3.1 Belief that local raw food is cooked	3	(2.25)	6	(4.51)	3	6	0.50	0.32
3.2 Belief that lime juice kill parasites and microbes	23	(17.29)	18	(13.53)	18	13	1.38	0.37
3.3 Belief that alcohol kill parasites and microbes	13	(9.77)	24	(18.04)	7	18	0.39	0.03
3.4 Ignorance of food-borne diseases	0	(0.0)	0	(0.0)	0	0	NA	NA

^a percentages of exposure in cases (n = 133) ; ^b percentages of exposure in controls (n = 133)

4.4.4 Risk factors of trichinellosis from the multivariate analysis

Of the 24 factors being studied for a possible association with trichinellosis, the *p*-values of the significance test for the difference in the odds of being a trichinellosis case in individuals exposed to the possible risk factors compared with those who were not exposed to the risk factors were less than 0.25 for 13 risk factors (Table 4-1). These comprised (i) having an agricultural occupation, (ii) not having a refrigerator, (iii) a member of the household having epilepsy, (iv) a member of the household having acquired a hearing-impairment, (v) regular alcohol consumption, (vi) consumption of wildlife meat, (vii) consumption of raw pork, (viii) consumption of raw beef, (ix) consumption of raw meat from wild boar, (x) consumption raw meat from wildlife, (xi) consumption of homemade raw food, (xii) consumption of raw food at a social gathering and (xiii) belief that alcohol can kill parasites and microbes in raw meat. These factors were used to build a model to explain the associations between any potential factors and human trichinellosis in Nan and Chiang Rai Provinces.

A conditional logistic regression model based on hierarchical step-wise backward elimination identified a model that included three factors significantly associated with an individual being a trichinellosis case: consumption of raw wild boar, consumption of raw meat at a social gathering and a belief that alcohol kills parasites and microbes in raw meat (Table 4-2). The odds ratio for the consumption of raw pork from wild boar suggested that individuals in the study who did consume raw wild boar pork were 2.66 times more likely to be a trichinellosis case than the individuals who did not consume raw wild boar pork. Noting the 95% confidence intervals for the population the increase in risk could be as small as 1.35 or as high as 5.25 times. Having consumed raw food at a

social gathering increased the risk of being a trichinellosis case by 3.89 times (as little as 1.43 times or as much as 10.62 times in the population with 95% confidence). The odds ratio for the individual believing that alcohol kills parasites and microbes in raw meat produced 0.36 times reduction in the risk of being a trichinellosis case (Here the confidence interval suggested that there could be a range from 0.14 – 0.91 times decreased in risk).

Table 4-2. Pair-wise matched logistic regression findings: significant risk factors associated with trichinellosis ($\alpha=0.05$)

Variables	Odds ratio	95%CI	<i>p</i> -value
Consumption raw pork of wild boar	2.66	1.35 – 5.25	0.005
Consumption raw food at a social gathering	3.89	1.43 – 10.62	0.008
Belief that alcohol kill parasites and microbes in raw meat	0.36	0.14 – 0.91	0.03

4.4.5 Clinical symptoms of trichinellosis in cases and controls during and after trichinellosis outbreaks

All of the symptoms compatible with trichinellosis, with the exception of diarrhoea, were found in the cases more than the controls with a significant difference at the 5% level (Table 4-3). Trichinellosis cases were 5.6 times more likely to report periorbital oedema than the controls ($p < 0.001$). They were also 6.75 times more likely to report facial oedema ($p < 0.001$), 2.76 times more likely to report myalgia ($p < 0.001$), 2.80 times more likely to report calf pain ($p < 0.001$) and 2.06 times more likely to report vomiting ($p = 0.01$). Trichinellosis cases were 1.65 times more likely to report having diarrhoea ($p = 0.07$) but this was not significantly different from controls.

Table 4-3. Matched univariate analysis of clinical symptoms compatible with trichinellosis experienced by cases and controls during trichinellosis outbreaks

Clinical symptoms	Number of discordant pairs		Odds ratio	<i>p</i> -value
	Cases (+) Control (-) ^a /			
	Cases (-) Control (+) ^b			
Periorbital oedema	28/5		5.60	<0.001
Facial oedema	27/4		6.75	<0.001
Myalgia	47/17		2.76	<0.001
Calf pain	56/20		2.80	<0.001
Diarrhoea	28/17		1.65	0.07
Vomiting	33/16		2.06	0.01

^a Cases were exposed to risk factors (+), control were not expose to risk factors (-)

^b Cases were not exposed to risk factors (-), controls were exposed to risk factors (+)

Table 4-4. Matched univariate analysis of clinical symptoms compatible with trichinellosis in cases and controls one year after trichinellosis outbreaks

Clinical symptoms	Number of discordant pairs		Odds ratio	<i>p</i> -value
	Cases (+) Control (-) ^a /			
	Cases (-) Control (+) ^b			
Periorbital oedema	6/1		6.00	0.13
Facial oedema	6/0		NA	NA
Myalgia	24/10		2.40	0.01
Calf pain	27/12		2.25	0.01
Diarrhoea	13/8		1.62	0.19
Vomiting	10/3		3.33	0.09

^a Cases were exposed to risk factors (+), control were not expose to risk factors (-)

^b Cases were not exposed to risk factors (-), controls were exposed to risk factors (+)

4.4.6 Trichinellosis risk-associated behaviour after trichinellosis outbreak

Data on possible trichinellosis risk-associated behaviour patterns that were practised in the year following a trichinellosis outbreak event were collected including (i) regular alcohol consumption, (ii) consumption of raw pork, (iii) consumption of raw beef, (iv) consumption of raw pork from wild boar, (v) consumption of raw dog meat and (vi) consumption of raw meat from wildlife other than wild boar. During the year after a trichinellosis outbreak took place, the practise of regular alcohol consumption and of consumption of raw meat remained higher among the trichinellosis cases compared with controls. Alcohol consumption and consumption of raw pork remained high and significantly higher in the cases than in the controls (Table 4-5).

For the year following a trichinellosis outbreak event, trichinellosis cases were 1.93 times ($p = 0.02$) and 2.50 times ($p = 0.02$) more likely to report regular alcohol consumption and consumption of raw pork than the controls respectively. Trichinellosis cases were more likely to report consumption of raw beef, raw pork from wild boar and raw meat of wildlife other than wild boar by 1.64 times ($p = 0.09$), 1.27 times ($p = 0.03$) and 1.25 times ($p = 0.26$) than the controls respectively. Consumption of raw dog meat was found in only one case but none of the control had consumed dog meat in the year following a trichinellosis outbreak event.

Table 4-5. Individual behaviour about one year following trichinellosis outbreak event

Possible risk factor	Number of discordant pairs	Odds ratio	<i>p</i> -value
	Cases (+) Control (-) ^a / Cases (-) Control (+) ^b		
Regular alcohol consumption	29/15	1.93	0.02
Consumption of raw pork	20/8	2.50	0.02
Consumption of raw beef	23/14	1.64	0.09
Consumption of raw wild boar meat	19/15	1.27	0.30
Consumption of raw wildlife meat	20/16	1.25	0.26
Consumption of raw dog meat	1/0	NA	NA

^a Cases were exposed to risk factors (+), control were not exposed to risk factors (-)

^b Cases were not exposed to risk factors (-), controls were exposed to risk factors (+)

4.5. Discussion

In this chapter attempts were made to identify the factors that associate an individual with being a trichinellosis case. Additionally it was found that cases that had experienced the disease did not necessarily change their risk-associated behaviour. The main findings and selected characteristics of trichinellosis cases, the exposure to possible risk factors and the clinical symptoms are discussed below.

4.5.1 The characteristics of trichinellosis cases

All of the cases of trichinellosis in this study were from individuals from Thai ethnic groups. There is a need to consider the ethnicity of

trichinellosis cases in Thailand as this helps in understanding the manner in which cultural differences influence *Trichinella* transmission to humans. Although there are reports of trichinellosis cases among both the Thai hill tribes and the Thai ethnic groups in Thailand (see Chapter 2), the majority of trichinellosis cases were in the Thai ethnic group (Bureau of Epidemiology, 1981-2008). This is not because members of the Thai ethnic group are the majority of the population (in fact they represent 80% population) but because it is this ethnic group that consumes the types of foods known to be the sources of *Trichinella* (Bureau of Epidemiology, 1981-2008). It was believed that raw foods were introduced to the Thai hill tribes only in the past few decades when transportation between the highlands and the lowlands had been improved (P. Chumkasian personal communication). Regardless of the fact that the pigs they reared were infected with *Trichinella* (Bureau of Epidemiology, 1981-2008), the Thai hill tribe ethnic groups in the past, through their non-consumption of high-risk foods, were at a lower risk of infection. The hill-tribe pigs however posed a risk to the Thai ethnic groups who consume such dishes. Kunstadter (1967) recorded that pigs reared from the highland areas were leashed and walked to market in the lowland areas. In that way *Trichinella* from pigs reared in the highland areas could enter the food chain of Thai ethnic groups in the lowland areas. Nowadays there is an increasing trend for the Thai hill tribes groups to acquire pigs from the lowland areas for their own consumption (Nakai, 2009) (author's observations). This trend, coupled with a decrease in prevalence of *Trichinella* infection in hill-tribe pigs, resulted in the reduction of human trichinellosis in Thailand irrespective of the possible widespread practice of raw meat consumption to Thai hill tribes.

4.5.2 Exposure to possible risk factors among cases and controls

This study chose controls from the same villages as the cases. Therefore the characteristics and behaviour of the cases and controls were comparable in many aspects. Exposure to the main risk factors, in particular the consumption of raw pork and raw beef among both the cases and controls, was substantially high. It indicates that the custom of raw meat consumption still prevails in these areas and that is why trichinellosis continues to be seen in this ethnic group. Hunting was also a common practice in these communities. Consumption of raw wildlife meat was practised in as many as 34.59% case households and in 27.82% of the control households. Both wildlife and domestic animals can be potential sources of *Trichinella* to people. The number of outbreaks attributed to wildlife estimated from known-source trichinellosis outbreaks in Thailand increased from 23.1% (12/52) during 1962 – 1983 (Dissamarn & Indrakamhang, 1985) to 36.3% (33/91) during 1981 – 2008 (Chapter 2). The most likely species leading to infection was wild boar. The investigation in the previous chapter aimed to determine the extent of *Trichinella* infection in wildlife in trichinellosis-endemic areas through examination of meat samples from animals caught for local consumption. *Trichinella* larvae were not identified in any of the samples in the work described in Chapter 3 and did not suggest a high infection rate of *Trichinella* infection in wildlife in Thailand. Although no infection was found in wildlife it can still not be discarded as a risk factor for infection because outbreak investigations have shown the potential of wildlife, in particular wild boar, as a source of *Trichinella* transmission to humans in Thailand.

There is one particular area in the Northeastern Region of Thailand where dog meat is consumed and the meat is sold in a market (Srikitjakarn *et al.*, 1981). Consumption of dog meat in these study areas

was high. The reports from the participants here showed that dog meat consumption is widespread. A survey investigating *Trichinella* infection in dog meat sold in the markets in the Northeastern Region found an infection rate of 1.66% (n = 421) (Srikitjakarn *et al.*, 1981) while a survey in the Northern Region found an infection rate of 2.42% (n = 124) (Chaimanee *et al.*, 2002). Based on these reports, there is a risk of *Trichinella* transmission from dogs to people in these communities. However dog meat is not commonly eaten raw when compared with pork, beef and wildlife meat. This was confirmed by the responses of the participants of this study and explains why human trichinellosis caused by the consumption of dog meat rarely occurs in Thailand. In the past five decades there has been only one outbreak attributed to raw dog meat consumption. This occurred in Chaiyaphum Province in the Northeastern Region (Chitchang *et al.*, 1985). The report was not included in the surveillance data probably because the institute that carried out the investigation did not belong to the Ministry of Public Health and thus it was not recorded through the official channels.

The risk of trichinellosis associated with the type of meat that is eaten raw, rather than the high level of infection rates of *Trichinella* in the meat has been shown through the meat consumption habits of the Inuit (native people residing in northern Canada). The Inuit commonly consume raw walrus but conventionally cook bear meat. Therefore trichinellosis outbreaks among Inuit people have been attributed to walrus meat regardless of the fact that the infection rate for trichinellosis in walrus (2 – 4%) is far lower than in the bear (60%) (Proulx *et al.*, 2002).

Trichinellosis in herbivorous mammals is rare. Natural infection of trichinellosis in cattle and sheep has, however, been reported in China (Wang *et al.*, 2007) and human trichinellosis outbreaks have been attributed to mutton (Wang & Cui, 2001a). In addition there have been reports of human trichinellosis attributed to horse meat in France and

Italy (Ancelle *et al.*, 1988; Pozio *et al.*, 1988). There is no evidence of *Trichinella* infection in domestic herbivores in Thailand. Outbreaks related to the consumption of meat from domestic herbivores such as beef have not been recorded even though it is commonly eaten raw in particular areas, including the areas in this study.

Considering individual knowledge and perception related to trichinellosis of the population under study, the majority of people were able to distinguish raw and cooked food. Most of them knew that lime juice or alcohol does not kill the parasites and microbes in raw meat. Everyone was aware of food-borne diseases and usually associated food-borne diseases with diarrhoea; only a few could specify trichinellosis by name. Only about 21.05% of the cases and 22.56% of the controls did not regularly take de-worming medication (< two times a year). These findings show that people in the study areas have the basic knowledge to prevent trichinellosis. This knowledge and practices may have resulted from the education campaigns instituted in the opisthorchiasis control programme (Jongsuksuntigul & Imsomboon, 2003).

The prevalence of epilepsy was at 0.75% among case households and at 4.51% among control households. Having an epileptic case in a family was about 0.17 times as likely to be associated with trichinellosis cases as than if a family member did not have epilepsy with a *p*-value 0.06. This was in contrast to the belief that epilepsy increased risk of trichinellosis. The borderline significance of having an epileptic case in a family as a protective factor of trichinellosis may occur by chance (OR 0.17; *p* = 0.06). Otherwise one explanation was that a family with an epileptic in the household consumed less pork than a family without an epileptic case. Some people in Thailand believe that epilepsy is caused by eating pork and so those with an epileptic case in a family may refrain from consuming pork. Saengsuwan *et al* (2009) showed that 38.9% (324/831)

of the survey participants in five north-eastern provinces in Thailand believed that epilepsy is transmitted by eating pork.

4.5.3 Risk and protective factors for human trichinellosis in Nan and Chiang Rai Provinces

4.5.3.1. Consumption of raw pork of wild boar

Trichinella transmission to the human population requires two factors, namely the consumption of raw or undercooked meat consumption and the presence of *Trichinella* in domestic or wild animal food sources (Dupouy-Camet & Murrell, 2007). In Nepal, where raw pork consumption occurs in the general population, human trichinellosis does not occur because pigs are free from *Trichinella* (Karn *et al.*, 2008). In Finland where trichinellosis present in domestic pigs and wildlife (Oivanen *et al.*, 2002a), there are no infections in humans due to the custom of restricting consumption to well-cooked meat (Pozio *et al.*, 1998). These examples provide an explanation as to why consumption of raw wild boar meat has been identified as a risk factor for *Trichinella* transmission to the population in this study, despite the fact that other meats, including pork, beef, dog and other wildlife, are also consumed raw.

Historically cases of trichinellosis in the Northern Region of Thailand have been mainly attributed to the consumption of pork reared in highland areas (Dissamarn & Indrakamhang, 1985). At that time, the infection rates of *Trichinella* in pigs in the highland areas was estimated at 11.4 – 32.3% (Dissamarn & Aranyakananda, 1975; Vitoorakool *et al.*, 1989). In recent years a serological survey investigating trichinellosis in 330 pigs in the Northern Region, including some samples from pigs in highland areas, did not detect evidence of infection (Vitoorakool *et al.*,

2007). However, this could possibly be due to the small sample size. In a survey with a sufficient sample size in Nan Province (see Chapter 3) an estimated seroprevalence of 0.97% (95% CI 0.46 – 1.48%) in pigs in the highland areas was obtained. This confirmed that the prevalence of trichinellosis prevalence in pigs in these areas was substantially lower when compared with previous decades. This could be the primary factor giving the low risk of *Trichinella* transmission from pigs to humans, with the risk from consumption of wild boar meat becoming more prominent. The changing epidemiology of trichinellosis in Thailand is similar to that which has been reported in USA where the prevalence of trichinellosis in domestic pigs has significantly decreased; instead wildlife have become an important source of human trichinellosis. During 1991 – 1996, 1997 – 2001 and 2002 - 2007 trichinellosis cases attributed to wild game meat in USA increased from 40% (Moorhead *et al.*, 1999) to 43% (Roy *et al.*, 2003) and 63% (Kennedy *et al.*, 2009), respectively.

This finding supports the observation that the prevalence of trichinellosis in pigs has decreased. However, it did not conclude that the risk of *Trichinella* transmission from pigs to humans is negligible. As in the USA, trichinellosis in humans through eating raw pork still occurs and some of the outbreaks that occurred in Thailand in recent years have implicated pork (A. Thorkee Personal communication). To monitor the potential sources of human trichinellosis in Thailand, information regarding meat implicated in outbreaks should be collected for every individual case. This information should be regularly disseminated to the public.

4.5.3.2. Raw meat consumption at social gatherings

Trichinellosis in Thailand usually occurs following a festival, ceremony or at other social events where people are gathered (Khamboonruang, 1991). It is also observed that trichinellosis cases are more likely to be

detected when many cases have occurred at the same time rather than as isolated cases (CDC, 1996). This may be why cases related to social gatherings predominate in reports. The work described here supports the observation that trichinellosis is associated with social gatherings in the Northern Region of Thailand. Having consumed raw food at a social gathering increases the risk of being a trichinellosis case by 3.89 times (95% CI 1.43 - 10.62).

A consumer behaviour survey in Thailand in 2005 found that while 0.7% of Thai population prefers to consume raw or undercooked meat the majority preferred boiled (57.0%) or pan fried (22.7%) meat (National Statistical Office, 2005). Although the custom of raw meat consumption does prevail in the Northern Region, conventionally, on a day-to-day basis, well-cooked meat is consumed. This is similar to what has been observed in Papua New Guinea (Owen *et al.*, 2005) where, although more than 80% of population in that study reported consuming meat raw, raw meat is not regularly eaten. In the cases of the Northern Region of Thailand, particularly in rural areas, local raw foods are considered to be the special dishes to be served at special occasions, *e.g.* wedding ceremonies and the New Year (Faichid, 2002). Similar traditions are also observed in Laos and are linked to trichinellosis outbreaks (Sayasone *et al.*, 2006).

4.5.3.3. Belief that alcohol kills parasites and microbes in raw meat

Instead of increasing the risk of trichinellosis, the incorrect perception that alcohol kills parasites and microbes in raw meat was observed to be associated with a decrease in the risk of trichinellosis in the study population. Wilcock *et al.* (2004) discussed the disparity between knowledge and behaviour of consumers. Consumer cooking preferences may not be affected by their knowledge and individuals may not associate what they know about the risks with their own practices

(Wilcock *et al.*, 2004). In terms of the population under study in the current work, the participants who perceived that alcohol kills parasites and microbes in raw meat may consume raw meat less than those who know this to be a misconception.

In China there were two trichinellosis outbreaks where people who consumed alcohol and shared a meal with cases did not succumb to infection with the parasites. Thus, it was believed that alcohol consumption can inhibit the establishment of *Trichinella* infection (Takahashi *et al.*, 2000). However, two studies in mice show that ethanol increases the chance of infection, rather than decreasing it (Na *et al.*, 1997; Sukul *et al.*, 2005). A slower rate of expulsion of intestinal adult worms and a higher fecundity rate for the female worms were observed in ethanol-fed mice when compared with paired controls (Na *et al.*, 1997). As 48.12% of the cases were individuals who regularly consumed alcohol, this study supports the proposal that alcohol does not inhibit trichinellosis infection.

4.5.4 Clinical symptoms of trichinellosis

This study collected data through recollection of past exposure and as such could be exposed to error in data collection as some participants may have forgotten relevant information. The findings showed that clinical symptoms compatible with trichinellosis were reported by the cases more frequently than by the controls. During interview some participants also volunteered the information as to how they recalled past exposure. This implied that participants would be able to recollect past exposure to an appropriate level for the study.

More than one year after an outbreak, levels of myalgia and calf pain were still significantly higher in cases compared with the controls. Chronic trichinellosis may occur but its long-term effects may be under-recognized and under-reported. Nontasut *et al* (1999) observed the

clinical manifestations of disease in the three trichinellosis patients in Thailand for six months after infection. They found that the patients still had myalgia and muscle weakness, which was also observed in this study. The observation of muscle weakness in cases of *T. papuae* five months after the infection was postulated to be from hard work rather than related to the past infection (Kusolsuk *et al.*, 2010). Nemet *et al.* (2009) after performing a two year follow-up study in a group of former trichinellosis patients (n = 699), proposed that trichinellosis complications may remain for over two years and they observed trichinellosis-related complications among 9.4% of their cases (Nemet *et al.*, 2009).

4.5.5 Changes in trichinellosis risk-associated behaviour among cases post trichinellosis outbreak

About one year after a trichinellosis outbreak, consumption of raw beef, wild boar and wildlife among the cases decreased to levels in this group that were not significantly different from the controls. The practices of regular alcohol consumption and the consumption of raw pork remained significantly higher in the cases than in the controls. This result shows that the cases that had experienced trichinellosis did not necessarily change their behaviour. This could be why there are repeated reports of trichinellosis in Thailand in the same or adjacent villages (Bureau of Epidemiology, 1981-2008; Kusolsuk *et al.*, 2008). Based on this knowledge, it is essential to continue public education promoting the consumption of cooked-meat and encouraging the practices to prevent the infection in domestic food animals, particular pigs.

4.5.6 Limitations of the study

There are three limitations to this study related to the study design and the selection of the study population. First, age and sex were used as matching factors and therefore could not be assessed as risk factors. Secondly, there may be errors in data sources because of imperfect memory in both the cases and controls. Lastly, the study focused on a high-risk population in the trichinellosis endemic areas of Thailand. Here geographical influences, such as trichinellosis in pigs, the type of pork products available and the food consumption habits in the study areas may be different from other areas in Thailand. Therefore these results cannot be generalized or extrapolated to other settings.

4.6. Summary

Data analysed from an age-matched, sex-matched neighbourhood case-control study in Nan and Chiang Rai Provinces in the Northern Region of Thailand showed that three factors were associated with an individual being a trichinellosis case. Consumption of raw pork from wild boar and the consumption of raw foods at a social gathering increased the risks of trichinellosis. The belief that alcohol kills parasites or microbes in raw meat reduced the risk of individual being a trichinellosis case. Clinical symptoms compatible with trichinellosis, such as myalgia and calf pain, were reported in cases one year after infection suggesting these are long-term complications as sequels of acute trichinellosis. Cases in the study who had experienced trichinellosis did not always necessary change their risk behaviour patterns. They continued consuming raw pork, the higher number of cases consuming raw pork relative to controls about a year following an outbreak.

5. Chapter 5 – A qualitative study: appraisal of knowledge, attitudes and practices relevant to trichinellosis in communities in Nan Province, northern Thailand

5.1. Study aims

This study aims to generate information on the knowledge, attitude and practices relevant to trichinellosis in various ethnic communities in Nan Province, northern Thailand. Its goal is to expand on the information about the factors influencing trichinellosis, cross-referencing from the epidemiological perspective in the previous chapters to a community perspective. Specifically the study was to:

- (i) Identify ability to identify local raw foods and practice of consuming these foods uncooked in the communities,
- (ii) Identify the consumption of the particular types of meat and consumption of those meats uncooked in the communities,
- (iii) Identify whether the communities have had received information of selected food-borne disease and trichinellosis,
- (iv) Identify attitudes within communities towards specific practices relevant to trichinellosis occurrence in pigs and humans including:
 - a. free-range scavenging pigs
 - b. home-slaughtering of pigs
 - c. hunting
 - d. raw meat consumption
- (v) Identify the level of perception within the communities on the practicability of two prevention measures, specifically
 - a. the resources and willingness to cook and consume meat using heat

- b. the resources for building pig pens and willingness to confine the pigs for rearing

5.2. Introduction

There are a number of epidemiological studies that have investigated the prevalence of trichinellosis and associate factors. For example, cross-sectional surveys were conducted to determine prevalence and to identify risk factors of trichinellosis in humans in Greenland (Moller, 2007), Papua New Guinea (Owen *et al.*, 2005) and in semi-rural communities in Mexico (de-la-Rosa *et al.*, 1998). These studies revealed trichinellosis is significantly associated with factors such as age, gender, occupations, residential areas, and type of foods. The findings in various settings can be different from one another. This information assists in drawing control measures corresponding to specific factors associated with infections in a given area. In addition, information from epidemiological studies is used to demonstrate disease status in the country when it is required from an international trading partner. These are also used to compare the disease status in different areas or to monitor the trend of disease in the same region. Because of these reasons, epidemiological studies are always a gold standard to investigate any diseases or health problems (Baum, 1995) including trichinellosis. Nonetheless, these may not provide all the information needed to design an appropriate control strategy. This can be a result of limitations in epidemiological tools or insufficient resources to conduct an additional epidemiological study. Baum (1995) stated that some information related to public health problems would only emerge from qualitative studies using methods, such as observation or focus groups interview. Baseline information including people's beliefs and their practices in a community is needed in order to formulate messages to promote disease control programme that can be adopted by local people.

Trichinellosis has maintained its foci in Thailand in Northern Region. People living in this area belong to various ethnic groups. They have their own culture and belief that influence their behaviour and practices associated with trichinellosis. Although, trichinellosis has been recognized in this region for about five decades, there were limited studies looking at factors influencing trichinellosis among people of different ethnic groups in this region. This information would complement results from the epidemiological studies and help in drawing appropriate and sustainable preventive measures.

5.3. Materials and methods

5.3.1 Study method

The study method used was focus groups (FGs) interview in a community. A “community” in this study refers to a group of people living in the same locality and under the same government; specifically this means people living in the same village. It was decided that this study method would allow greater access to people of different ethnic groups on their thoughts, opinions and practices in the time and resources allowed for the study.

5.3.2 Participant recruitment

Twenty-four FGs were selected purposively and by convenience. The FGs were selected for the purpose of including the communities of Thai, the major ethnic group, and the other four major hill tribe ethnic groups residing in Nan Province including Hmong, Khmu, Lua, and Yao. For convenience the FGs were chosen from 12 of 61 villages in Nan Province where the study to determine the production and management practices in pigs and seroprevalence had taken place (Chapter 3). In addition, participants were recruited voluntarily in the study following proposal by the village head.

Twenty-four FGs in the study included 12 FGs of male and female each. One male and one female FG were recruited from each village. The members in each group were interviewed at the same time but male and female FGs were interviewed separately to encourage the women communication. Research has demonstrated that genders affects the way people communicate, *i.e.* men and women have different behaviour (Brown, 2000). Men's speaking styles are more dominant in mixed-sex conversation, men speak longer, interrupt more, speak more about themselves and take on more of a leadership position than do women (Brown, 2000). Participants in FGs were any adults aged ≥ 15 years, living in the village and who were voluntarily participated in the study following proposed by the village head. Ten participants were invited to participate in each FG. There were six FGs of male and female Hmong, two FGs of male and female Khmu, four FGs of male and female Lua, four FGs of male and female Thai and eight FGs of male and female Yao. More Hmong and Yao were selected than other groups because the study in Chapter 3 revealed that a higher number of trichinellosis seropositive pigs belonged to these groups. One Khmu village present in the study in Chapter 3 was selected and only two FGs in Khmu village were recruited into the study.

5.3.3 Ethical issues

The study ensured that verbal consent was obtained from participants before data collection was performed. Before obtaining verbal consent from participants, the researcher introduced name and institution of the researcher and staff, the reason why participants and the areas were selected, purpose of the study, the benefits that participants will be gained from the study, type of activities that participants will be involved, time spent in the study session, risks that may occur, assure that participant can withdraw at any time or refuse to participate and lastly provide the name, contact address and telephone number of the

researcher and the chief of District Livestock Office to contact if any questions arise (Creswell, 2009).

5.3.4 Data collection

FGs interviews were undertaken by the researcher and two assistants between December 2008 and February 2009. The FGs interviews took place at assembly hall in the village, as arranged by the village heads (see Appendix 15). The date and time of meeting was arranged through the village heads, based on participants' convenience. Meetings were held either at mid-day, in the afternoon or the evening. Data collection for each group took approximately one hour which included conclusion statements, acknowledged the co-operation of participants and educating participants about trichinellosis and preventive measures at the end of the session.

Data were collected by interviewing participants in the FGs individually and in groups using close-ended and open-ended questions. Pictures taken by the researcher in Nan Province and information from newspaper and fact sheets about diseases published by public health organization for public health education (see Appendix 10, Appendix 11, Appendix 12, Appendix 13& Appendix 14) were used to elicit questions. Quantitative and qualitative data were obtained from the FGs. Three interview methods were used: voting interview (5.3.4.1), scoring interview or rating items (Krueger & Casey, 2009) (5.3.4.3) and open-ended interview (5.3.4.2). Quantitative data were obtained when the researcher directly asked participants the question and participants responded to the question individually or in a group. Voting interview with close-ended question was used to collect individual respond and scoring interview was used to collect data of group respond. Qualitative data were obtained when the researcher interviewed participants with open-ended questions. Any participants in the FGs who spoke freely had their views noted in

correspondence to the relevant question. The information that was collected in the study and the interview methods that were used for data collection are shown in Table 5-1.

Table 5-1 Detail of data, data collection methods and acquired information

Type of data	Interviewing methods	Information
Quantitative	Voting interview	Ability to correctly identify raw foods Consumption of raw foods The type of meat consumed The type of meat consumed uncooked The receipt of information about <i>Streptococcus suis</i> infection The receipt of information about trichinellosis
Qualitative	Open-ended interview	Attitudes towards free-range scavenging pigs Attitudes towards home-slaughtering of pigs Attitudes towards hunting Attitudes towards raw meat consumption
Quantitative	Scoring interview	Level of practicability of heat cooking meat Level of resources for heat cooking meat Level of willingness to heat cooking meat Level of consumption only heat-cooked meat Level of practicability to build pig pens Level of resources to build pig pens Level of willingness to build pig pens Level of totally confine pigs in pens

5.3.4.1. Voting interview

The voting interview used in this study was adapted from the pocket chart voting method and the informal confidential voting interview method (Gregson *et al.*, 2004). The pocket chart voting method is a tool that can be used to collect information on the individual practices and behaviours in a rural community, such as treatment seeking behaviour, hand washing, use of water source and others (Ahmadb & Akhtar, 1996; Guzha, 2002; Anon, 2006). This method uses sets of pictures depicting practices and behaviours or other activities under investigation that may be present in the community. Participants indicate their response by placing their votes on the corresponding picture. The informal confidential voting interview method is based on the pocket chart method but this method simply uses questions, allowing respondents to indicate their answer and place it in a secret voting box. It is a tool used to collect information on private topics such as sexual behaviour and is used to reduce embarrassment and to ensure confidentiality (Gregson *et al.*, 2004).

In this study, pictures of raw foods or information about the diseases of interest (*S. suis* infection and trichinellosis), related to the topic under investigation, were shown or read aloud to participants. A demonstration to ensure that participants understood the question was performed before allowing them to indicate their answer by voting. Although the topic of investigation is not as sensitive as sexual behaviour, questions about raw food consumption may cause embarrassment (Owen *et al.*, 2005). Therefore, this study used secret voting to reduce embarrassment and this was outlined to the participants. Two voting boxes, in different colours, were used for storing the voting tokens representing the binary responses (yes/no). A stone was used as a voting token. These were distributed to each participant before each corresponding question. The corresponding

questions (Table 5-2) were read out one at a time after participant had seen the picture or heard the information about the disease of interest. Participants were informed to make a vote once of the corresponding questions. The votes were counted and recorded immediately after each vote.

Three local raw foods, *nhaem*, *larb* and *luh*, were used as models of raw foods to determine the ability to correctly identify a raw food and the consumption of raw foods in the communities. Specific types of meat were explored to identify the variety of meat consumption in the community either cooked or raw. Nine types of meat were chosen to include meat from domestic livestock, fish, wild mammals and reptile and companion animals. These were pork, beef, poultry, fish, wild boar, bear, snake, dog and cat.

Trichinellosis has been recognized in Thailand since the 1960s and public education has been established since that time through public education channels, such as radio, broadcasts, T.V. and the distribution of leaflets (Dissamarn & Indrakamhang, 1985). To determine whether communities' have had received information about food-borne diseases acquired from raw meat consumption besides trichinellosis, *S. suis* infection was used as a representative disease. This is because *S. suis* infection has emerged as a food-borne zoonoses in Asia in the few years prior to study being undertaken. There were outbreaks of *S. suis* infection in China (Yu *et al.*, 2006), Vietnam (Mai *et al.*, 2008) and Thailand (Fongcom *et al.*, 2001). Public awareness regarding *S. suis* infection had been previously addressed rigorously through several communication channels. Information about both *S. suis* infection and trichinellosis were read aloud instead of using the disease name. This was based on the researcher's belief that participants may not remember the disease name. Information about these two diseases was obtained from newspaper and from fact sheets of diseases published by the Ministry of Public Health

Thailand and Mahidol University. The information covers the causative agent, mode of transmission, clinical symptoms and preventive measures.

Table 5-2 Summary of questions and indicators to obtain information using voting interview

Question to individual participants	Voting indicator	
Is this a cooked or raw food? (show picture of food to ascertain if participant knows the food)	Yes	No
Do you eat this food without heating?	Yes	No
Do you eat this type of meat? (specify type of meat for example pork, beef and etc)	Yes	No
Do you eat this meat in raw form? (specify type of meat for example pork, beef and etc)	Yes	No
Have you ever heard about this information from any sources? (read aloud news and information about <i>S. suis</i> infection)	Yes	No
Have you ever heard about this information from any sources? (read aloud news and information about trichinellosis)	Yes	No

Figure 5-1 Pictures of local foods containing raw meat namely, *larb*, *luh*, and *nhaem*



A: *larb*

B: *luh*

C: *nhaem*

5.3.4.2. Open-ended interview

Open-ended questions are used in organizational research to explore, explain, and/or reconfirm existing ideas (Jackson, 2002). The reason for using open-ended questions is to promote explanations and opinions from participants. A picture of a practice known to be related to trichinellosis was shown to participants, one at a time, to elicit response and discussion. Participants expressed their opinions for about five minutes for each corresponding question. The views of each participant were

noted and were read aloud at the end of each discussion to allow them to amend or add more opinions. The author and two assistants, fluent in the local language and hill tribe languages, took notes during interviews in all sessions to ensure that every view was captured and noted.

Table 5-3 Summary of questions and indicators to obtain information using open-ended interview

Questions to groups of participants	Indicator
What do you think about - free-range scavenging pigs - home-slaughtering of pig - hunting - consumption of raw meat	Participants' views

Figure 5-2 Pictures of activities related to trichinellosis (A) hunting activity, (B) home-slaughtering pig, (C) free-range scavenging pigs, (D) raw meat consumption



5.3.4.3. Scoring interview

A scoring technique (Krueger & Casey, 2009) was applied in this study to determine communities' perception on the practicability and availability of resources for cooking meat and building pig pens and their opinion on the consumption of cooked meat and the confinement of pigs. A score 1 – 5 was used as the measuring scale. The higher the score the higher the agreement, thus, for example, a score of five for the practice of totally confining pigs means that the entire community totally confine pigs. Participants were informed how to score and a demonstration was performed by the author and assistant to ascertain that participants clearly understood the method. Stones were used as score tokens. Five stones were given to a group of participants for each corresponding question. Squares were drawn on a piece of paper or on the floor as the containers of the score tokens for each of the questions. One by one the corresponding questions were read aloud. Participants discussed these topics within the group before making their decision to score. This was done until all of the questions were scored. At the end of scoring session, participants interpreted their scores to the researcher, for example they described that their score of five meant the whole community thought that heat-cook meat is very easy. The score results were then recorded.

Table 5-4 Summary of questions and indicators used to obtain information using the scoring method

Question to participants	Score indicator				
	1 very low	2 Low	3 medium	4 high	5 very high
How easy is it - to heat-cook meat - to build a pig pen	1	2	3	4	5
Do you have enough resources - to heat-cook meat - to build a pig pen	1	2	3	4	5
Does the community prefer - the custom of consuming only cooked meat - totally confining pigs	1	2	3	4	5
What is the level of practice in the communities for - consumption only of cooked meat - totally confinement of pigs	1	2	3	4	5

5.3.5 Reliability and validity

Silverman (1993) recommended two ways to achieve reliability and validity of data generating from interview. First, using standardized interviews (*i.e.* use specific question to interview present or past behaviour rather than hypothetical situations), secondly, following a standardized protocol. This means the interviewer should ask each question precisely as it is worded and in the same order that it appears on the schedule. In this study, the researcher was the only interviewer in every FGs interview sessions. The interview followed the protocol and started with voting interview, followed by open-ended interview and at last the scoring interview. Participants' views were validated by their confirmation at the end of each question. The meaning of score was described in words by participants to ascertain that the researcher and participants understood and interpreted the responses in the same ways.

5.3.6 Data management and analysis

All data were recorded in Microsoft Excel spreadsheet (Microsoft Office Excel 2003, Ink). Participant demographic data (age, sex, and ethnicity) and occupation were recorded individually. Data collected from voting interviews, scoring and open-ended questions were recorded at community level.

5.3.6.1. Analysis of quantitative data

Participants' voting was analysed to identify communities' knowledge and practices relevant to trichinellosis (see Table 5-1). Relative frequency, proportions of respondents in each FGs, were calculated from individual voting to roughly determine the level of that knowledge or practices whether it was rare, moderate or commonly. The level of perception within the communities on the practicability of two prevention measures relevant to trichinellosis (see Table 5-1) was determined from very low to very high corresponding to participants' scoring: very low (score = 1), low (score = 2), medium (score = 3), high (score = 4) and very high (score = 5).

5.3.6.2. Analysis of qualitative data

Participants' views were analysed to identify communities' attitudes whether they were against or in favour of the practices relevant to trichinellosis (see Table 5-1). Data were analysed using content analysis. Content analysis is defined by Holsti (1969) as any technique for making inference by objectively and systematically identifying specified characteristics of message. The technique rely on coding and categorising of the data (Holsti, 1969; Stemler, 2001). The analysis followed a *priori* coding approach (Stemler, 2001) in which the themes were established prior to the analysis. The classification of views into themes was developed based on the focus of this study relevant to trichinellosis occurrence and prevention. Two themes were used in this study, they are

for and against. For example participants' view that supports hunting was classified into a theme 'for hunting'. Participants' views were categorised into one of the pre-determined themes. Participants' views from each FGs noted by the researcher and assistants were put together on the same day after the FGs interview. Similar views from each FG were recorded once. The analysis was undertaken after all the FGs were interviewed. Participants' views were read repeatedly to identify themes. Each view was coded with specific colour corresponding to the pre-determined themes. Views in each theme were grouped into sub-theme. For example views against hunting with concerned on law and rule were grouped together.

5.4. Results

In total 238 participants were recruited into this survey. There were 123 males and 115 females from 12 villages in Nan Province. The number of participants in each focus group ranged from 3 to 18 for men and 5 to 18 for women. They included 64 Hmong in three villages, 23 Khmu in one village, 44 Lua in two villages, 45 Thai in two villages and 62 Yao in four villages (see Table 5-5). The overall mean age of the participants was 42.1 years old (range 17 - 75). The specific mean age for each gender was 45.1 years old for men (range 20 - 75) and 38.9 years old for women (range 17 - 75). All of the participants reported that their main occupation was related to agriculture.

Table 5-5 Study sites and composition of participants

District	Tambon	Village	Participant's ethnicity ^a	Number of participants	
				Male	Female
Thung Chang	Thung Chang	No.7	Hmong (H1)	18	5
	Ngob	No.2	Khmu (K1)	13	10
	Ngob	No.6	Lua (L2)	11	7
Chiang Klang	Phra That	No.1	Thai (T1)	14	10
Bo Kluea	Bo Kluea Tai	No.13	Lua (L1)	12	14
	Bo Kluea Tai	No.5	Thai (T2)	9	12
Tha Wang Pha	Pha Tor	No.6	Hmong (H3)	9	7
	Pha Thong	No.6	Yao (Y1)	9	10
Muang	Sa Nian	No.14	Hmong (H2)	12	13
	Sa Nian	No.10	Yao (Y2)	3	6
	Sa Nian	No.11	Yao (Y3)	8	15
	Sa Nian	No.16	Yao (Y4)	5	6
Total				123	115

^a Abbreviation in a bracket refers to that specific FGs

5.4.1 Ability to correctly identify local raw food in the communities

The proportion based on participants voting in each of 24 FGs indicated that the majority of communities in this study could correctly identify the three local foods¹¹ namely, *nhaem*, *larb* and *luh* as uncooked food (Figure 5-3 A-C). Among the three raw foods *nhaem* was the least to be correctly identified as raw food and *luh* was most correctly identified as raw food.

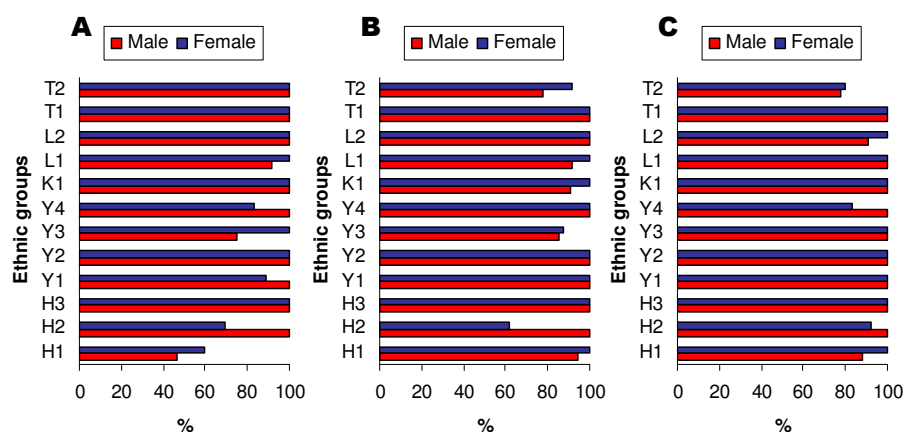
All of the participants in nine male FGs and eight female FGs from five ethnic groups could correctly identify *nhaem* as raw foods (Figure 5-3 A). The smallest proportion of community with ability to identify *nhaem* as uncooked food was 47% (7/15 male) in a Hmong FGs.

All of the participants in seven male FGs from four ethnic groups and eight female FGs from five ethnic groups could correctly identify *larb* as raw foods (Figure 5-3 B). The smallest proportion of community with ability to identify *larb* as uncooked food was 62% (8/13 female) in a Hmong FGs.

All of the participants in seven male FGs from four ethnic groups and eight female FGs from five ethnic groups could correctly identify *luh* as raw foods (Figure 5-3 C). The smallest proportion of community with ability to identify *luh* as uncooked food was 78% (7/9 male) in a Thai FGs.

¹¹ Three local foods contain raw meats referred to *nhaem*, *larb*, and *luh*. *Nhaem* is a preserved food made from raw meat; *larb* and *luh* are fresh prepared foods made from raw meat.

Figure 5-3 Proportion of communities with ability to correctly identify local food containing raw meat namely, (A) *nhaem*, (B) *larb* and (C) *luh*, as uncooked foods



Note: Abbreviations referred to specific FGs as shown in Table 6.5

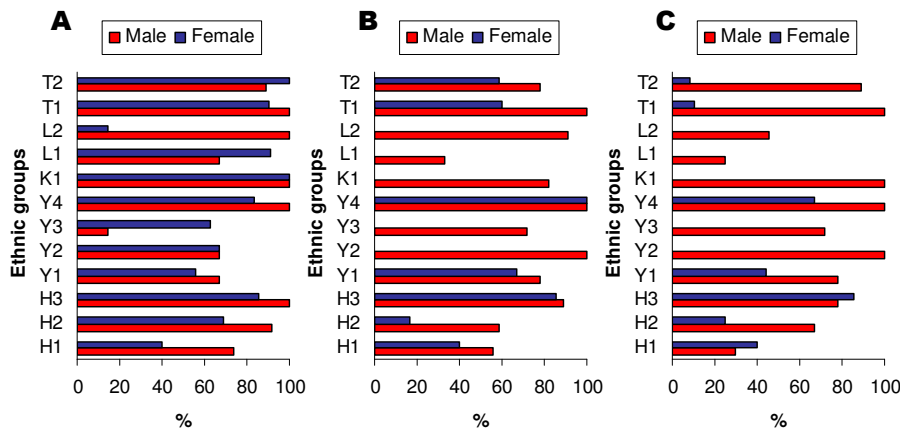
5.4.2 Practice of raw meat consumption in the communities

Among the three local raw meat products *nhaem* was the only product consumed raw by men and women in all of the study communities and it was most commonly eaten raw by both men and women. Men in every community consumed these raw meat products that vary in the proportions among the communities. None of women participants consume raw *larb* and *luh* in some communities of hill tribe ethnic groups (Figure 5-4 A-C).

- All of the participants in five male FGs of Hmong, Khmu, Lua, Thai and Yao and two female FGs of Thai and Khmu consumed raw *nhaem* (Figure 5-4 A). *Nhaem* was consumed raw by men and women participants in every FGs (14 – 100%).
- All of men in one FGs of Thai, two FGs of Yao and all of women in one FGs of Yao consumed raw *larb*. None of women in two FGs of Yao, one FGs of Khmu and two FGs of Lua consumed raw *larb* (Figure 5-4 B).

- All of men in one of FGs of Thai, one FGs of Khmu, and two FGs of Yao consumed raw *luh*. None of women in five FGs consumed raw *luh*: two FGs of Yao and one FGs of Khmu, two FGs of Lua (Figure 5-4 C).

Figure 5-4 Estimated percentages of communities consuming local food containing uncooked meat: (A) *nhaem*, (B) *larb* and (C) *luh*



Note: Abbreviations referred to specific FGs as shown in Table 6.5

5.4.3 Consumption of specific type of meats in the communities

Data on communities consuming specific type of meats were obtained from 24 FGs. The study explored the consumption, either cooked or raw, of nine different meats: pork, beef, fish, poultry, pork from wild boar, bear, snake, dog and cat. Large proportion of participants both men and women across all of the communities consumed pork, beef, fish, poultry and pork from wild boar. These meats and dog and bear were consumed in every community. The proportion of communities' consumption of these meats differed across the communities as well as between genders. Snake and cat were not consumed in every community. Cat was rarely consumed in the communities.

- All of the participants in every FG consumed pork, except in two Lua male FGs where 92% (11/12) and 91% (10/11) of participants consumed pork.

- All of the participants in every FG consumed beef, except in two FGs of Yao, a Khmu FGs and two FGs of Thai where 64 – 92% of participants consumed beef.
- All of the participants in every FG consumed fish.
- All of the participants in every FG consumed poultry except in a FGs of Khmu, two FGs of Lua where 83 – 92% of participants consumed poultry.
- All of the participants in every FG consumed wild boar meat, except two FGs of Thai where 60 – 92% of participants consumed wild boar meat.
- All of the participants in 13 FGs consumed bear: two female FGs of Hmong, four female FGs of Yao, two male FGs of Hmong, three male FGs of Yao, one male FGs of Khmu and one male FGs of Lua communities. None of participants consumed bear in four FGs: one female FGs of Hmong, Lua and Thai and one male FG of Yao. Participants in seven FGs consumed bear 17 – 71%.
- All of the participants consumed snake in only one male Hmong FG. None of participants in seven FGs consumed snake: one female FGs of Hmong, Lua and both female and male Yao FGs in two villages. Participants in 16 FGs consumed snake 7 – 75%.
- All of the participants in four FGs consumed dogs. They included male FGs of Yao, Khmu, Lua and Thai. None of female participants consumed dogs in five FGs, four FGs of Yao and one of Lua. Participants in 15 FGs consumed dogs 14 – 92%.
- Cats were rarely eaten in the study communities. Only two FGs of Yao, male and female in the same village, reported consuming cat 7 – 13% (1/14 female; 1/8 male).

5.4.4 Consumption of specific type of raw meats in the communities

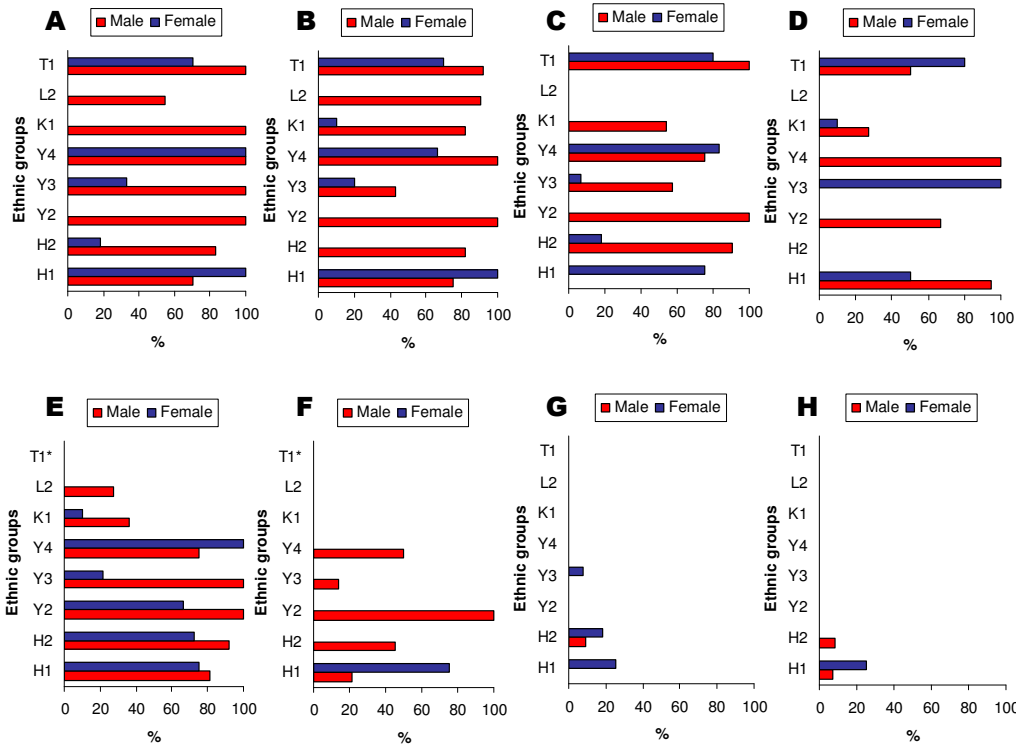
Data on communities consuming specific type of raw meat were collected from 16 FGs: four FGs in two Hmong communities, six FGs in three Yao communities, two FGs in a Khmu community, two FGs in a Lua community and two FGs in a Thai community. The proportions of participants in each FGs consuming specific types of raw meats are shown (Figure 5-5).

Some meats could be consumed raw by large proportion of men across the communities. Pork, beef and pork from wild boar could be consumed raw by men across the communities. Other types of meat were consumed raw only in some communities. The proportions of men consumed raw meat were generally higher than that of women. Snake and dog were rarely consumed raw. None of the FGs indicated that communities consumed raw cat.

Men in every FG consumed raw pork (55 – 100%), beef (43 – 100%), and pork from wild boar (27 – 100%). Men in six of eight male FGs (Hmong, Yao, Khmu and Thai) consumed raw fish (54 – 100%). None of men in one of Hmong and Lua FGs consumed raw fish. Men in five of eight male FGs (Hmong, Yao, Khmu and Thai) consumed raw poultry (27 – 100%). None of men in three FGs (Hmong, Yao and Lua) consumed raw poultry. Men in five of seven FGs (Hmong, Yao and Khmu) consumed raw bear (14 – 50%). None of men in one of Khmu and Lua FGs consumed raw bear. Men in only one Hmong FGs consumed raw snake (9%) and men in two Hmong FGs consumed raw dog (7 – 8%). None of men in any FGs consumed raw cat.

Women in five FGs (Hmong, Thai and Yao) consumed raw pork (18 – 100%). None of women in three FGs (Khmu, Lua and Yao) consumed raw pork. Women in five FGs (Hmong, Yao, Khmu and Thai) consumed raw beef (10 – 100%). None of women in three FGs (Hmong, Yao and Lua) consumed raw beef. Women in six FGs (Hmong, Yao and Khmu) consumed raw pork from wild boar (10 – 100%). None of women in two FGs (Lua and Thai) consumed raw pork from wild boar. Women in five FGs (Hmong, Yao and Thai) consumed raw fish (7 – 83%). None of women in one Yao, Khmu and Lua FGs consumed raw fish. Women in four FGs (Hmong, Yao, Khmu and Thai) consumed raw poultry (10 – 100%). None of women in four FGs (Hmong, Yao and Lua) consumed raw poultry. Only women in one Hmong FGs consumed raw bear (75%) and raw dog (25%). Women in two Hmong and one Yao FGs consumed raw snake (8 – 25%). None of women in any FGs consumed raw cat.

Figure 5-5 The consumption of specific types of raw meat in the communities: (A) pork, (B) beef, (C) fish, (D) poultry, (E) wild boar, (F) bear, (G) snake and (H) dog



* No data from male group* No data from male group

Note: Abbreviations referred to specific FGs as shown in Table 6.5

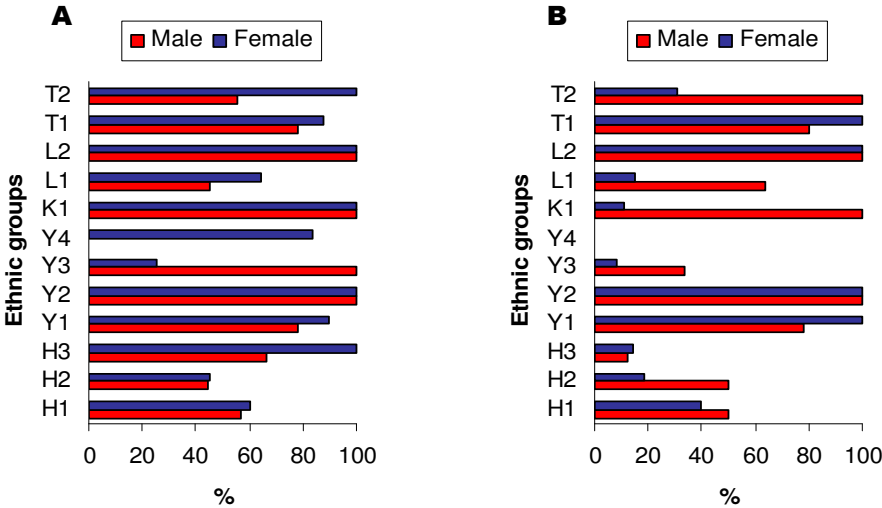
5.4.5 Communities had received information about *S. suis* infection and trichinellosis

Communities that had received information about *S. suis* infection and trichinellosis from any public education channels were explored on 24 FGs. The information had been received in most of the communities. The level of communities that had received information varied widely among the communities from low to high. In general women in the communities have heard about *S. suis* infection more than men, on the other hand, men in the communities have heard about trichinellosis more than women.

All communities have had received information about *S. suis* infection. Men in every FG, except one FG of Yao, have had received information about *S. suis* infection (44 – 100%). Women in every FG have had received information about *S. suis* infection (25% to 100%).

All communities, except one of Yao communities, have had received information about trichinellosis. Men in every FG, except one FG of Yao, have had received information about trichinellosis (13 – 100%). Women in every FG, except one FG of Yao, have had received information about trichinellosis (8 – 100%). In one community participants have had received information about trichinellosis from adjacent village where trichinellosis outbreak occurred in past few years.

Figure 5-6 Community have had received information on (A) *S. suis* infection and (B) trichinellosis



Note: Abbreviations referred to specific FGs as shown in Table 6.5

5.4.6 Communities’ attitudes towards raw meat consumption

Similar views from respondents from each FG was noted as one view, in total there were 64 different views responded from the 24 FGs to the question as to what participants think about raw meat consumption: 37 views were cited by men and 27 views were cited by women. More views against raw meat consumption were cited by women (22) than men (19). On the other hand, more views were cited by men (18) more than women (5) in favour of raw meat consumption. There were more views against raw meat consumption than in favour of raw meat consumption.

Responses against raw meat consumption were concerns that raw meat consumption is related to illness, is un-hygienic and that it is a bad example to children as well as a bad manner to consume raw meat in a public place. Participants in most of the FGs, particularly female FGs, were commonly concerned about parasites and a bad manner to consume raw meat in a public place.

Responses in favour of raw food consumption included the participants' views expressing their personal preference for raw meat consumption, social acceptance of raw meat consumption and optimistic bias (the bias that occurs when people believe that they are less at risk from a hazard than other people). Raw meat is considered delicious, irresistible and tasty with alcohol. Eating of raw meat is acceptable in private places and raw meat is accepted as a type of food. Raw meat is consumed in only small amount and is consumed only on particular occasions. Raw meat consumption was more favourite among men than women.

Table 5-6 Communities' attitudes against and for raw meat consumption from views of male and female participants in 24 FGs comprising of Hmong, Khmu, Lua, Thai and Yao ethnic groups in Nan Province

Themes	Respondents ^a											
	H1	H2	H3	K1	L1	L2	Y1	Y2	Y3	Y4	T1	T2
Against												
Illness and hygiene												
▪ Unhygienic				+#								
▪ Parasites	+#	#	+#	#		#	#	+	+	+#		#
▪ Unsafe food				#	+							
▪ Diarrhoea						+#			+	+#		
▪ Illness						+		#	+#			
▪ Microbes						+			+#			
Social un-acceptable												
▪ Bad example for children		+						+#				
▪ Eating raw meat in public is a poor manner					+#	#	+#	#	#			+#
For												
Personal preferences												
▪ Delicious			+	+						#	+	
▪ Irresistible			#				#	+	+		#	
▪ Tasty with alcohol		+			+				+	+		
Social acceptant												
▪ It is appropriate to eat in private										+	+	+
▪ It is a type of food	+								+			
Optimistic bias												
▪ Eat raw meat only on special occasions			#							+	+	+
▪ Eat a small amount of raw meat										+		

^a + = male respondents; # = female respondents

Note: the abbreviations refer to respondents from specific FGs in table 6.5

5.4.7 Communities' attitudes towards hunting

In total 109 views were received from the question about what the participants think about hunting. Sixty-one views were cited by men and 48 views were cited by women. There were equal number of views against hunting cited by men and women (26). There were more views in favour of hunting cited by men (35) than by women (22). There were slightly more views in favour of hunting than against it.

Views against hunting included participants' concerns that hunting is not necessary, against the law and village' rules, against wildlife conservation and immoral, and that hunting can be dangerous. Participants in FGs from all ethnic groups were concerned about wildlife conservation and hunting being illegal and against the village's rule. FGs of hill-tribe ethnic groups were concerned about gun-related accidents and acquiring infection when hunting.

Views in favour of hunting included the participants' concerns, mostly from FGs of hill-tribe ethnic groups, describing hunting as essential for people's livelihoods. Hunting provides food for household consumption. Extra meat is taken to sell or barter. Hunting is a way to protect crops from wildlife and to protect humans from wildlife.

Table 5-7 Communities' attitudes against and for hunting from views of male and female participants in 24 FGs comprising of Hmong, Khmu, Lua, Thai and Yao ethnic groups in Nan provinces

Themes	Respondents ^a											
	H1	H2	H3	K1	L1	L2	Y1	Y2	Y3	Y4	T1	T2
Against												
Un-important												
▪ It is not necessary for nowadays as meat can be purchased from market											+	
▪ It is waste of time and not necessary	#	#						#	+			
Against law												
▪ Illegal		+#		+#		+#	#		+	+#		+
▪ Being fined								#	+	#		
▪ Violate village's rule											#	
Conservation and moral												
▪ Wildlife should be reserved	+	#	+#	#	#	#			+		+#	+#
▪ It is immoral to kill wildlife			#		+#	+	+				#	+#
Hazard												
▪ It is dangerous from miss-shooting	+	+#	#			+	+#	+	+	#		
▪ Get infection in the forest while go hunting								+	+			
For												
People livelihood												
▪ People livelihood	+	+#		+		#	+#		+	+#		
▪ Share meat										+		
▪ Source of food	+	+#	+#	+	+#	+#			+	+#		
▪ Save money		+	+	+		#	#	+		+#		

▪ Excess meat is sold		#	+	+	#	#			+			
▪ Meat is taken to barter					#							
▪ Protect crop	+	+#	+		+	+	+#	+#	+#	+		
▪ Protect from wildlife attack		+			+	#				#	+	

^a + = male respondents; # = female respondents

Note: the abbreviations refer to respondents from specific FGs in table 6.5

5.4.8 Communities' attitudes towards home-slaughtering of pigs

In total there were 73 views responded to the question of what participants think about home-slaughtering of pigs. Thirty-nine views were cited by men and 34 views were cited by women. More views were cited by women (11) against home-slaughtering of pigs than by men (8). On the other hand, more men (31) were in favour of slaughtering the pigs at home than women (23). There were more views in favour of home-slaughtering of pigs than against it.

- Views against the home-slaughtering of pigs included concerns on the hygiene and safety of meat obtained from home-slaughtered pigs. Women were more concerned than men that it is immoral to kill animals.
- There were consistent views obtained from participants across all communities in favour of the home-slaughtering of pigs. The practice is accepted as part of people's livelihoods. It provides meat for household consumption, saves money and extra meat can be taken for sale. It is part of the tradition of ritual sacrifices. Views were expressed that meat from home-slaughtered pigs has greater quality than the one that has been purchased from market. The slaughter of pigs at home is not a regular practice and meat is primarily bought from the market.
- Participants' views regarding hygiene and safety of home-slaughtering pigs were both accepted and unaccepted it.

Table 5-8 Communities' attitudes against and for home-slaughtering of pigs from views of male and female participants in 24 FGs comprising of Hmong, Khmu, Lua , Thai and Yao ethnic groups in Nan Province

Themes	Respondents ^a											
	H1	H2	H3	K1	L1	L2	Y1	Y2	Y3	Y4	T1	T2
Against												
Unaccepted for hygiene and safety												
▪ Unhygienic process		+		+#		+#	+				+#	#
▪ Causes food poisoning		+				+					#	
▪ Unsafe because of no meat inspection						#						
Moral												
▪ It is immoral to sacrificed a live animal				#	#		+		#	#		#
For												
People's livelihood												
▪ People's livelihood	+	+#	+		+#		+	+#	+#	+#		+#
▪ A process to get meat	+#		+		+#		#	#	+#	#		
▪ Save money		#	+				+	+			+	
▪ Extra meat is sold					+				+#			
▪ Tradition for ritual sacrificed					+#		+		+#	+	#	+#
Accept for quality of meat												
▪ Meat from home-slaughtered pig is of higher quality than meat purchased from market								+				
Accept for hygienic process												
▪ Hygienic process			#		+			+#	+			+
Accept that it is not a regular practice												
▪ It is not a regular practice									#			+
▪ Primarily buy meat from market						#				+	#	

^a + = male respondents; # = female respondents

Note: the abbreviations refer to respondents from specific FGs in table 6.5

5.4.9 Communities' attitudes towards free-range scavenging pigs

There were 110 views in total to the question what participants think about scavenging pigs. Sixty-two views were cited by men and 48 views by women. Views against free-range scavenging pigs were cited more by men (49) than by women (42). Views in favour of free-range scavenging pigs were also cited more by men (13) than by women (6). There were more views against free-range scavenging pigs than views in favour of it. Views against free-range scavenging pigs included participants' concerns that it is an un-acceptable practice. Meat from free-range scavenging pigs is un-acceptable in terms of quality and safety. The practice is against the village's rules that prohibit free-roaming pigs rearing. Some views described the practice as an out-of-date. Free-range scavenging pigs may be bitten by dogs. They may obstruct the road and cause road accidents. Participants' views against free-range scavenging pigs that persisted across most of the FGs included that pigs destroy crops, disturb neighbours, and have parasites and that it is a practice that against the village's rules.

Views in favour of free-range scavenging pigs included participants' acknowledgement that the practice is acceptable and that the quality and safety of meat from free-range scavenging pigs is acceptable. These views were obtained exclusively from FGs of the hill tribe ethnic groups (Hmong, Lua and Yao). Free-range scavenging pigs are considered a good practice for the management of the sow and piglets. Participants described that the survival rate of piglets is greater in this sort of rearing than when they are in a pen. Free-range pigs rearing in farmland is considered good practice because pigs fed themselves on agricultural waste/ products and they grow well. Views from some communities

pointed that free-range scavenging pigs grow naturally, thus, their meat is safe. Additionally, they believed that meat from home-raised pigs is higher in quality than that from commercial pigs.

Table 5-9 Communities' attitudes against and for free-range scavenging pigs from views of male and female participants in 24 FGs comprising of Hmong, Khmu, Lua, Thai and Yao ethnic groups in Nan Province

Themes	Respondents ^a											
	H1	H2	H3	K1	L1	L2	Y1	Y2	Y3	Y4	T1	T2
Against												
Unaccepted in the practice												
▪ Pigs receive insufficient food and are not growing well			#		+		#		#			
▪ Untidy environment because of pig foul	+	#	+#		#	+		#	+#	+#	+#	#
▪ Pigs destroy crop		+	+#		+#	+		+#	+#	+#	+#	#
▪ Pigs disturb neighbours		#	+	#	+#	+	#	+	+#	#	#	
▪ Unhygienic practice											#	
Unaccepted in the quality and safety of meat												
▪ Pigs are raised in unhygienic condition therefore meat is poor in quality	+						#					
▪ Pigs have parasite	+		#	#	#	+#	+#	+#	+	#	+	
▪ Meat is a source of disease			+		+	#						+
▪ Pigs eat sewage	+	+#			#		+	#	+	+		#
Against village's rule												
▪ Against village's rule that prohibit free-roaming pig rearing	+	+			+	#	+	+	+#	+	+	
Other												
▪ Out-of-date practice				+							+	
▪ Pig is bitten by dog						+#						
▪ Pig obstruct road and cause road accident								+		+	+	
For												
Accept in practice												
▪ Safe practice for sows and piglets							+		#	+		
▪ Free-range pig rearing in farmland is a good	+	+#			+					+		

practice, pig is fed on agricultural by products												
▪ Free-range pigs receive extra food and grow well		+	#		+				#			
Accept in quality and safety of meat												
▪ Pigs grow naturally so that their meat is safe		+#			+		+					
▪ Meat from home-raised pig is higher in quality than meat from commercial pigs	+#	+										

^a + = male respondents; # = female respondents

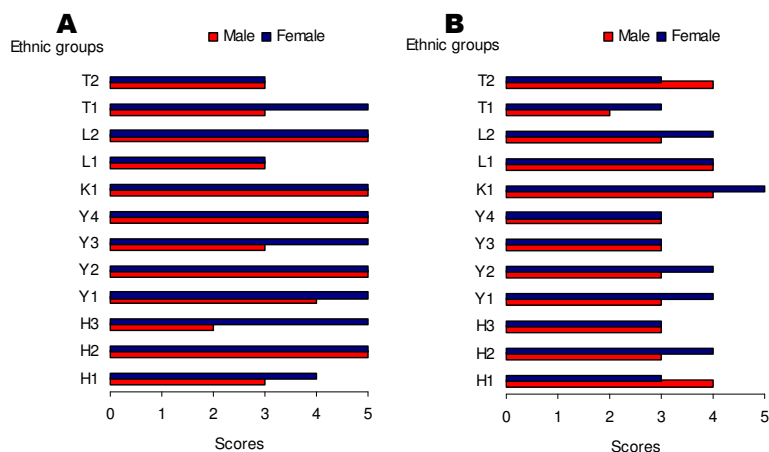
Note: the abbreviations refer to respondents from specific FGs in table 6.5

5.4.10 Communities' perception related to consumption of cooked-meat

Participants across the communities perceived that the resources for heat-cooking and the practicability of heat-cooking meat were at the very high level. Only one FG of Yao reported that resources for heat-cooking were high (score = 4). The rest of the FGs reported that resources for heat-cooking were very high (score = 5). Every FG unanimously agreed that heat-cooking meat was very practicable/practical (score = 5). Participants added an additional explanation to this question in that it is easier to prepare heat-cooked food than to prepare the raw food such as *nhaem*, *larb* and *luh*, as not everyone can make these.

The preference or willingness to consume only cooked meat was not generally very high across the communities and none of the communities perceived that the entire communities consumed only cooked-meat. There were 10 FGs in which both men and women in the same villages agreed that the communities' preference on the consumption of only heat-cooked meat was very high (score = 5) (Figure 5-7 A). These FGs were from one of Hmong communities, two of Yao communities, one Khmu community, one of Lua communities. Only one female FG of Thai communities reported that the communities' preference for the consumption of heat-cooked meat only was very high. There was no community where both men and women FGs reported that the entire community consumed only heat-cooked meat (Figure 5-7 B).

Figure 5-7 Scores illustrated communities' perception on (A) preference to consume only cooked-meat and (B) practice of consumption only cooked-meat



Note: Abbreviations referred to specific FGs as shown in Table 6.5

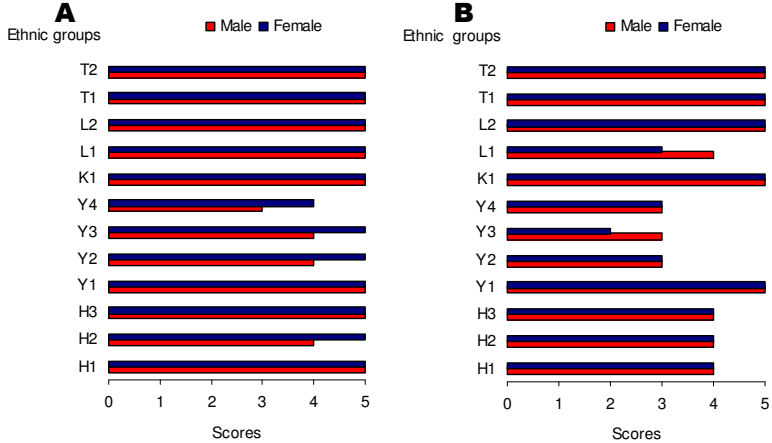
5.4.11 Communities' perception related to confining pigs

In terms of the availability of resources for building pig pens in the community, there were only two female FGs in two Yao communities where participants reported that there were low or average resources (score = 2 – 3). Two female FGs, one of the Hmong and one of the Yao communities, reported that practicable/feasibility to build a pig pen was low (score = 2). All of men FGs reported that both resources for building pig pens and the practicable to build pig pens was very high (score = 5).

Only four male FGs reported that willingness or preference to totally confine pigs was medium or high (score = 3 – 4) (Figure 5-8 A). These included one of the Hmong and three of the Yao communities. The rest (20 FGs) reported that willingness or preference to totally confine pigs was very high (score = 5). In terms of the practice of total confinement of pigs in the communities, 14 FGs, both male and female from seven villages, reported that not all of the pigs in the communities were totally confined (score 2 – 4). These included all of Hmong communities, three

of four Yao communities and one of two Lua communities. There were 10 FGs of male and female in five villages who reported that the practice of total confinement of pigs was very high in the communities (score = 5) (Figure 5-8 B). These included one of the four Yao communities, one Khmu community and both of the Thai communities. They described that all of pigs in the communities were confined.

Figure 5-8 Scores illustrated communities' perception on (A) the willingness of totally confine pigs (B) and practice of totally confine pigs in the community



Note: Abbreviations referred to specific FGs as shown in Table 6.5

5.5. Discussion

This survey collected data from FGs chosen from the Hmong, Khmu, Lua and Yao hill-tribe ethnic groups and the Thai ethnic group in Nan Province. The study aimed to generate information about knowledge, attitude and practices in the communities that are relevant to trichinellosis. All of these communities had no reported cases of trichinellosis in humans. The findings for four topics relevant to trichinellosis are discussed: raw meat consumption, hunting, scavenging pigs and the home-slaughtering of pig.

5.5.1 Raw meat consumption

The case-control study (Chapter 4) in the Thai ethnic groups revealed high prevalence of raw meat consumption practice, particularly pork (cases = 93.2%; controls = 82.7%), beef (cases = 88.0%; controls = 73.7%) and wild boar (cases = 44.4%; controls = 27.1%). Based on cross-reference of responses in this current study on the consumption of local raw foods, the consumption of specific types of raw meat and the level of consumption only cooked food, in the hill-tribe ethnic groups and the Thai ethnic groups, it showed that raw meat consumption were practised across the communities irrespective of the ethnic background of people. About a half of people in all communities were believed to practise raw meat consumption. These findings highlighted and supported the understanding that the consumption of raw meat has been adopted by people of all ethnic groups living in the study area.

Nhaem was most commonly consumed raw by men and women in all ethnic groups, while *larb* and *luh* were mostly consumed by men of all ethnic groups but not by women. The viability of *Trichinella* larvae in these foods has been documented (Dissamarn *et al.*, 1966; Keittivuti, 1983). *Nhaem* is a fermented raw food product. The preparation process of *nhaem* with optimum salt, temperature and time can inactivate *Trichinella* larvae. Fermented *nhaem* according to commercial standards are able to inactivate *Trichinella* larvae (Keittivuti, 1983). Whereas, *larb* and *luh* are freshly prepared and consumed, *Trichinella* larvae remain viable and thus infective in these foods (Dissamarn *et al.*, 1966). Dissamarn and Aranyakananda (1975) examined *nhaem* sold in northern provinces during 1964 – 1973. They identified *Trichinella* larvae in 0.12% of samples (23/18,765), however, those larvae were no longer infective. Gamble *et al.* (2000) mentioned that the processing methods such as curing, and smoking cannot be controlled reliably. It was true for homemade *nhaem*, trichinellosis outbreaks involving consumption of

homemade *nhaem* does occur occasionally in Thailand (Khamboonruang & Nateewatana, 1975; Khumjui *et al.*, 2008).

Raw meat consumption patterns were generally similar among all ethnic groups. These patterns correlated with the occurrence of trichinellosis in Thailand from 1962 to 1983 (Dissamarn & Indrakamhang, 1985) and 1981 to 2008 (Chapter 2) which the major sources of infections were pork from domestic pigs and wild boar. It is found in this study that various types of meat were consumed across the communities including the meat of reptiles like snakes and companion animals such as dogs. However, meat from the other species besides pork, beef and wild boar was consumed to a lesser extent and it was rare to consume these meats in uncooked form. Cat was rarely consumed due to a belief that killing a cat is a sin. Although, dogs and snakes were consumed by people of all ethnic groups, they were not commonly consumed as raw food. Meat from wild boar and bear were likely to be consumed raw by men of hill-tribe ethnic groups than men of Thai ethnic group probably due to its easier accessibility in the area where the hill-tribe ethnic groups are living.

This study showed that majority of participants in all ethnic groups can distinguish between raw and cooked food. They were capable of correct identification the local raw foods. This is consistent with data obtained in Chapter 4 where only a low proportion of cases and controls (2.25% and 4.51%, respectively), all from the Thai ethnic group, could not correctly identify the three common local raw foods. Regardless of knowing that these foods are raw about a half of communities consumed those foods uncooked. Faichid (2002) and Kaewpitoon *et al.* (2007) pointed out that there are also some people who believed that acid in lime juice and ants can kill parasites. Thus, they consumed the food uncooked by adding lime juice and ants. This reason may be partly explain why people continue consuming the raw foods.

There are more reasons to explain the continuing practise of raw food consumption in the communities. Through the views of the communities in terms of people attitudes towards raw meat consumption, people of all ethnic groups were prominently against the consumption of raw meat. Their views reflected awareness of health risk from raw meat consumption, particularly concerned about parasites in raw meat. In addition, information about diseases acquired from raw meat have been distributed to every community although it might not have been received throughout the communities. Previous studies found that communities' knowledge was not consistent with their actual food hygiene practices (Pinfold, 1999). This was also the case from the findings in these communities. Although the communities knew about the diseases and they were aware of parasites in raw meat, the practice of raw meat consumption was common in the communities. Participants considered the local foods, when eaten uncooked are 'delicious' and 'irresistible'. This is in line with what has been reported from a Yao community in Phayao Province in the Northern Region where 33.70% of the community stated that raw fish *larb* is tastier than cooked *larb* (Faichid, 2002). This perception may be the main reason why people continue to consume raw food. Three additional reasons proposed by participants in this study could explain continued raw food consumption. First, though known as raw foods by the majority of people, these sit in a unique cultural niche. According to Faichid (2002) some local raw foods of the Northern Region are not just food but they are key factors for social functions. For example *larb* is served on particular occasions to bestow high honours on guests at a wedding ceremony, celebrating the move to a new house, at a New Year festival, or other auspicious events. Secondly, raw foods are considered to be 'tasty when eaten together with alcohol'. A personal preference on consuming alcohol together with raw meat is consistent with the result detailed in Chapter 4. Among trichinellosis cases, they have continued their practice of alcohol and raw pork

consumption despite having experienced the infection. Kusolsuk *et al.* (2010) has also mentioned that raw foods are usually served with alcohol. Lastly, the communities believed that it is acceptable to consume raw foods in a small amount and only on special occasions. This view could reflect the communities' actual practice of raw meat consumption as supported by the previous study. Faichid (2002) reported that the majority (88.2%) of the Yao community consume raw fish \leq once a month (Faichid, 2002). The belief that it is acceptable to consume raw foods in a small amount and not regularly may imply an optimistic bias; the bias that occurs when people believe that they are less at risk from a hazard than other people (Miles *et al.*, 1999). Parry *et al.* (2004) studied the differences in perception of risk between people who have and who have not experienced *Salmonella* food poisoning. They demonstrated that there was a significant optimistic bias for people in perceiving the risk associated with food poisoning, particularly among people who have not had an adverse experience linked to the hazard (Parry *et al.*, 2004). The communities in the current study as mentioned earlier have not experienced trichinellosis outbreak and therefore some people have maintained such misperception.

From the participants' attitudes in favour of raw meat consumption, changing food consumption habits particularly among adult population is not an easy task. However, the adults have been made aware of the risks from raw meat consumption and they did not want children to consume raw foods. There were views stating that 'raw food consumption is a bad example to children'. Sithithaworn *et al.* (2007) has mentioned to the control of opisthorchiasis (liver fluke infestation) in Thailand that the sustainable control programme should be focused on schoolchildren who will then be a liver fluke-free generation. This measure can also be applied for the control of trichinellosis. In addition, parents and adults should be educated in the same way so that they will be aware not to encourage their children to consume raw foods.

This study, through the response of participants on the consumption of local raw foods and the consumption of specific types of raw meat by men and women, showed the gender differences on the prevalence of raw meat consumption. Women from various ethnic groups mostly reported consuming all types of raw meat less than men. A survey of consumer food-handling and food-consumption practices in USA and a survey on consumption pattern in Canada also showed that men were more likely to report risky food-consumption behaviour practices than women (Altekruse *et al.*, 1999; Nesbitt *et al.*, 2008). The less prevalence of raw meat consumption in women than in men correlates with the finding of fewer female trichinellosis cases (Chapter 2). This pattern is the same in most countries where trichinellosis is present such as in USA (Moorhead *et al.*, 1999; Roy *et al.*, 2003; Kennedy *et al.*, 2009), Canada (Serhir *et al.*, 2001), Greenland (Moller, 2007), Papua New Guinea (Owen *et al.*, 2005) and China (Wang *et al.*, 2006b). Exception are sub-rural area in Mexico where a study showed that there were more female cases than male (de-la-Rosa *et al.*, 1998) and in Romania that the surveillance data showed almost equal number of male and female cases. Type of raw food products that are available in each country may influence the prevalence of consumption of the raw (Neghina *et al.*, 2009) foods by men and women and thus they have different chance of becoming infected. In the case of Thailand, not only the proportion of women consuming raw meat was smaller than men but also the type of raw meat that they commonly consumed does not associate with a high risk of trichinellosis.

5.5.2 Hunting

Hunting is one of the activities that have been related to trichinellosis in humans. It provides source of *Trichinella* to humans directly through wildlife meat or indirectly through contamination of wildlife meat into food chain of domestic animals and that animals are consumed by humans. During 1990 – 2006 there were 57 outbreaks of trichinellosis in Bulgaria implicating wild boar meat. These outbreaks related to hunting season (Kurdova-Mintcheva *et al.*, 2009). The transmission of *Trichinella* from wildlife to pigs and then from pigs to humans has occurred when wildlife carcasses have been used to feed pigs (Jarvis *et al.*, 2002). Game hunting and the use of wildlife carcasses to feed animals are not the practices present in the study communities. Hunting occurs mainly for subsistence purposes and wildlife meat is taken mainly for human consumption. Tungittiplakorn and Dearden (2002) reported from a study in Thai Hmong communities that hunting has declined since the adoption of production of cash crops. The time commitment to this type of agriculture activity, since it started in the 1990s has changed working and social practices. Hunting is now practiced mainly for social rather than economic reasons. In this study, women in some Hmong and Yao communities viewed hunting as not essential and a waste of the time. This is in accordance with what had been reported by Tungittiplakorn and Dearden (2002). However, communities of all ethnic groups, particularly among hill-tribe ethnic groups still widely accepted hunting as part of their livelihood. Hunting provides a food source for the households and a source of income or for trading through barter.

Although laws as well as village's rules prohibit hunting and people there are well aware of them, hunting activity has continued. The activity will facilitate *Trichinella* transmission from wildlife to people and free-roaming pigs. In these communities where hunting is widely accepted and where wildlife is a source of food there should be education on the

risks of disease from wild food and the prevention of *Trichinella* to domestic pigs.

5.5.3 Free-range scavenging pigs

Free-range scavenging pigs is the significant risk factor of trichinellosis in Thailand (Chapter 3), Vietnam (Vu Thi *et al.*, 2009) and China (Cui *et al.*, 2006a). They are predominantly found in mountainous areas, the areas occupied by the ethnic minorities. Based on two reports mentioned about pig production in mountainous areas of North-western Vietnam, free-range scavenging pigs were found in Hmong villages (Vu Thi *et al.*, 2009) but not in the Black Thai villages where the practice of free-range scavenging pig rearing used to occur in the past decades (Lemke & Zarate, 2008). This current study identified a practice of free-range scavenging pigs rearing in Hmong villages as that found in Vietnam and in some Lua and Yao villages. The willingness and actual practice to totally confining pigs both in Hmong and some Yao communities were not as high as in other ethnic communities. It is consistent with the evidence of trichinellosis sero-positive pigs. The study in Chapter 3 found greater numbers of trichinellosis sero-positive pigs in these ethnic communities when compared with others (Chapter 3).

Participants' views reflected problems perceived in raising sows and piglets in a pen and the shortage of food for pigs as the reasons for allowing pigs to scavenge. According to many participants' views piglets survive better outside a pen. Death of piglets in an inappropriately-designed pen (Svendsen *et al.*, 1986) from being crushed or falling from the pen, could be one of a reasons responsible for the lower survival rate of piglets reared in a pen when compared with being reared outside a pen. Problem in pig husbandry management due to inappropriately-designed pens and a shortage of food for the lactating sow has been observed in smallholder pig production in the mountainous areas of North-west Vietnam (Lemke & Zarate, 2008). In terms of pig feed

insufficiency, Nakai (2008) observed pig production in a Hmong community in Nan Province and found that the feed for the pigs was insufficient in the rainy season.

Besides the constraints, free-range pig rearing may be a traditional practice similar to the rearing of free-range duck in rice field in Thailand and Vietnam. Free-range pig rearing, where the pigs are allowed to roam free in farmland (pastured pigs), is practised by some Hmong, Yao and Lua ethnic communities. Their pigs have to be self-sufficient in finding food. Because they are free-roaming in farmland they are exposed to wildlife animals and cross-breeding with wild boar occurs. Some pigs are brought back from the farmland areas to be reared in pens in residential areas. Cross-bred offspring of domestic pig and wild boar were observed in some communities supporting the participants' views that they actually reared free-range pigs in farmland and that in the farmlands there is high degree of interactions between domestic pigs and wild animals. This practice adds another portal for *Trichinella* transmission between domestic pigs and wild animals. In this situation *Trichinella* may be transmitted from animal-to-animal through ingestion of carcasses or faeces contaminated with the parasites.

5.5.4 Home-slaughtering pigs

Similar to hunting, the home-slaughtering of pigs was widely accepted across the communities. Home-slaughtered pigs were utilised for food, to be sold as meat and most importantly, to be used in ritual sacrifice ceremonies. Nakai (2009) observed the pig consumption pattern in one Hmong community in Nan Province for about two years and found that socioeconomic changes, the introduction of the national educational system and Christianity did not change the performance of ritual events and the slaughtering of pigs in the communities (Nakai, 2009). Participant mentioned that the home-slaughtered of pigs tended to be less common. With money they earn from cash crops the communities are

able to buy meat from the market (Tungittiaplakorn & Dearden, 2002). In addition, mobile food sellers were observed throughout the areas that can be access by vehicles (author's personal observation).

Based on the findings in Chapter 3, a significant cluster of trichinellosis sero-positive pigs was identified in the communities practising the home-slaughter of pigs and practising free-range scavenging pig rearing. Most communities were already aware that free-range pigs scavenged on sewage. None of them were aware of the risk of *Trichinella* transmission from pig-to-pig through meat that is left on the ground during home-slaughtered and butchering of pigs (or wildlife). This is one aspect of knowledge that must be conveyed to the communities in order to prevent *Trichinella* transmission to their pigs.

5.6. Limitations and constraints

Based on the design of this study, the results represented the ideas of participants but not the entire population. In addition, this study explored only about a half of hill- tribe ethnic groups in Thailand. Furthermore, information obtained may be limited when few numbers of groups representing in the category, *i.e.* one Khmu group. The accepted numbers of FGs for each category of individual are three to four groups (Krueger & Casey, 2009). It has been believed that information from three to four groups is saturated (no more new information) (Krueger & Casey, 2009). Some constraints of conducting this FGs interview appeared – one was to get a representative sample. Because FGs were recruited on a voluntary basis, the problem occurred where some FGs contained fewer participants than planned. Kruger and Casey (2009) mentioned that too small group limit range of experience, an ideal size of five to eight participant for each FG was recommended.

5.7. Summary

This study contributes to the understanding of knowledge, attitudes and practices relevant to trichinellosis in the communities of different ethnic groups in Nan Province. The results indicate that most communities irrespective of ethnic groups have had received educational information about trichinellosis. Communities are concerned about illness and diseases, particularly parasite infestations, from the consumption of raw meat. However, they continued to consume raw meat because of their preference for its taste and in the belief that they are less at risk by consumption of raw meat in a small amounts and irregularly. Many types of meat were consumed in the communities. Among these meats, pork, beef and pork of wild boar were most commonly eaten raw. Raw meat consumption occurred in all of the communities regardless the people ethnicity. Hunting and the home-slaughter of pigs were widely accepted as part of people's livelihoods. Hunting was conducted for subsistence purposes in order to provide food for human consumption. The home-slaughtered of pigs was performed mainly to provide meat for ritual sacrificial ceremonies. Free-range scavenging pigs were presented in low numbers in some hill tribe ethnic communities. Reasons given as to why pigs were not confined included, primarily problems with piglets survival in an inappropriately designed pens and the shortage of foods for the confined pigs.

6. Chapter 6 - General discussion

The first trichinellosis outbreak in Thailand was reported in 1962 in Northern Region (Menakanit *et al.*, 1962; Boonthanom & Nawarat, 1963) and since then cases have been continually documented. Trichinellosis has been listed as a notifiable disease in Thailand since 1969. The continual reporting of cases through the national surveillance system led to Thailand being named as one of the trichinellosis endemic countries in Asia and the Pacific Rim (Takahashi *et al.*, 2000).

Trichinella infections are commonly acquired when meat from infected pigs is consumed raw. Although the custom of raw meat consumption is prevalent in the Northern and Northeastern Regions in Thailand (Office of the National Culture Commission, 1996), the majority of cases were found in the Northern Region. This implies that *Trichinella* infected pigs, the major source of human trichinellosis, were found only in the Northern Region. To prevent the public from the infections the *Trichinella* examination programme at municipal abattoirs was initiated in northern Thailand in 1967 (Dissamarn & Indrakamhang, 1985). All of pigs slaughtered at municipal abattoirs were screened for *Trichinella*. However, there were no confirmed cases of *Trichinella* infection at municipal abattoirs during 1971 – 1983 (Dissamarn & Indrakamhang, 1985), thus, the inspection programme ended. The only control measure currently in place is public education campaigns on consumption of thoroughly cooked meat.

Because the national surveillance reports showed that trichinellosis was still being reported in Thailand (Bureau of Epidemiology, 1981-2008), the current work firstly looked into the epidemiological characteristics of trichinellosis cases in Thailand over a 28 year period from 1981 to 2008 (Chapter 2). Results showed that the majority of cases were maintained in rural areas in the Northern Region. The outbreaks were associated with

the consumption of raw meat, mostly from domestic pigs or wild boar. As both pigs and wild boar were implicated as sources of infection, Chapter 3 investigated trichinellosis prevalence in domestic pigs and in wild animals (wild boar, barking deer and monitor lizard). The management practises associated with trichinellosis in pigs were also investigated in Nan Province, one of the five northernmost provinces with a high incidence of human trichinellosis. Infection was found only in domestic pigs and was associated with pigs that were free ranging. The next stage of the investigation (Chapter 4) focussed on the characteristics of people who had been diagnosed with trichinellosis (case positive) and controls (case negatives). It was found that being a case was associated with consuming raw wild boar and consuming any raw meat at social gatherings. Finally in Chapter 5, a qualitative approach was utilised, through focus groups interviews, to further investigate what factors may place humans at risk of *Trichinella* infection. It was found that the consumption of raw meat was still present at a high level across the communities, regardless of the ethnic background, even though they knew that raw meat consumption could be harmful. In addition, scavenging pig production which is the only risk associated with trichinellosis in pigs, is still being practising in some rural areas. The continued presence of raw meat consumption suggests that attempts to inform the population of its danger have not yet met with success. Therefore, prevention of human trichinellosis can not rely only on the public education currently in place.

6.1. Factors influencing trichinellosis

“There is hardly a common disease which is not uncommon some where else” (Rose, 1987).

Rose (1987) stated that environments and lifestyle influenced the incidence rates of most diseases and that the adverse factors or risk factors and diseases cluster in certain areas. This is true for trichinellosis where the distribution is varied depending on geographical characteristic

and cultural eating habits of people in the areas (Pozio, 2007). In many countries or regions trichinellosis is mostly found in mountainous areas for example trichinellosis in wildlife in Western Europe (Italy and France) (Pozio, 2008), trichinellosis in pigs in Vietnam, China and Thailand.

In the current work trichinellosis foci were found and maintained almost exclusively in northern Thailand where there are vast areas of mountains and forest (Chapter 2). Mountainous areas (highland) in Thailand are similar to those in Western Vietnam (Lemke & Zarate, 2008; Vu Thi *et al.*, 2009) and are occupied by various ethnic minorities.

In mountainous areas, the production of pigs by ethnic minorities was predominant and mainly occurred for the farmers own use (Chapter 3&5). Traditional pig rearing (including scavenging pig production in residential areas and scavenging pigs in farmland or pastured pig production) has been practised for many years and is still accepted as a good practice by some ethnic groups *i.e.* Hmong, Yao and Lua (Chapters 3&5). Wildlife and domestic pig interaction has been previously suggested as a means of *Trichinella* transmission from wildlife to pigs in these mountainous areas (Khamboonruang, 1991). However, although subsistence hunting is common in rural areas, hunting wildlife is prohibited by law both in most Asian countries including Thailand (Department of National Parks Wildlife and Plant Conservation, 2009; Drury, 2009), thus the investigation on trichinellosis in wildlife to confirm its potential as a source of *Trichinella* to domestic pigs is limited. Reports from other regions suggested that environment pressure from high prevalence of *Trichinella* infection in wildlife influences the infection in domestic pigs. For example, an increased prevalence of trichinellosis in wild boar in Poland and the migration of raccoon dogs from Poland to Germany were the potential sources of *Trichinella* to domestic pigs in a small farm in Germany (Pannwitz *et al.*, 2010). On the

other hand, there was also a study which provided evidence to support that *Trichinella* transmission from domestic pigs to wildlife (Murrell *et al.*, 1987). The preliminary investigation into *Trichinella* infection rate in wildlife caught for human consumption in Nan Province did not find any infected animals (n = 97) and would imply a low infection rate of trichinellosis in wildlife (Chapter 3). Nonetheless, it is not possible to exclude the risk of *Trichinella* transmission from wildlife to pigs, because human trichinellosis implicated wildlife meat, particularly wild boar, as a significant risk factor of being a trichinellosis case (Chapters 2&4). Scavenging pig production (Chapters 3&5) is another factor that explains for the perpetuation of trichinellosis in domestic pigs. Pig-to-pig *Trichinella* transmission occurs when meat of infected pigs that is left on the ground during home-slaughtering of pigs is then fed on by pigs that scavenge in that area (Chapter 3). Trichinellosis in domestic pigs in neighbouring countries were introduced into Nan Province through local trading at border market and illegal movement (Chapters 2&3). Therefore, in Thailand the environmental factors that influenced *Trichinella* transmission to pigs comprised (i) mountainous environments where wildlife interacts with domestic pigs, (ii) the presence of scavenging pig production system practised by the ethnic minorities living in mountainous areas, and (iii) the sharing of an international boundary with other trichinellosis endemic countries where trade and illegal movement of animal and animal products takes place.

Several changes that took place during the 1990s, including political, economic transformations and the impact of war have impacted on the quality of pig production and management, veterinary services and the lifestyle of people. This eventually results in an increase in incidence of trichinellosis in pigs and humans as seen in Russia, Romania, Croatia, Bosnia and Herzegovina, Bulgaria, Serbia and Montenegro, Yugoslavia, Argentina and China (Djordjevic *et al.*, 2003; Cuperlovic *et al.*, 2005; Blaga *et al.*, 2007). This was not the case for Thailand where in fact the

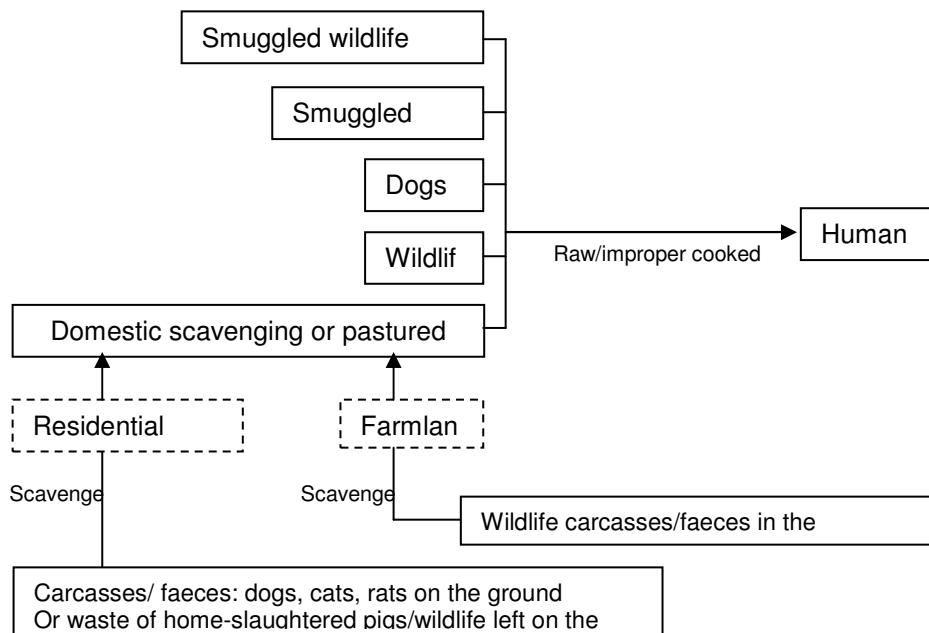
incidence of trichinellosis has significantly decreased over time and the foci of disease have been limited to historical endemic areas (Chapter 2). Trichinellosis outbreaks outside endemic foci occur only sporadically and rarely are repeated in the same area; this implies that sources of *Trichinella* or raw meat consumption have not been established outside the endemic foci. A report indicated that an outbreak of trichinellosis in southern Thailand, which implicated wild boar meat, occurred among people who had moved from the Northeastern Region (Jongwutiwes *et al.*, 1998). Another report revealed that trichinellosis cases in the Northeastern Region, which implicated domestic pork, were imported cases from the Northern Region a trichinellosis endemic area.

Various factors might have accounted for a decreasing trend of human trichinellosis in Thailand. Expansion of medium-scale and large scale pig production, where risks of trichinellosis is negligible, has occurred in Thailand since last two decades and has dominated the overall pig production in Thailand (about 80%) (Cameron, 2000). A decrease in prevalence of trichinellosis in pigs in hill tribe pigs compared with that in the past due to an improvement in pig management practices (Chapters 3&5). Public education on safe food has access to rural communities and raised awareness of food-borne diseases among those in high risk areas (Chapters 4&5). A decline in prevalence of trichinellosis in pigs, previously the major source of trichinellosis in Thailand (Chapter 2), resulted in wildlife sources, particularly wild boar, becoming a significant source of human trichinellosis (Chapter 4). These changes indicated that food safety education, particularly in subsistence communalities has to have emphasis on wild foods, particularly those from wildlife. In addition, to prevent the introduction of *Trichinella* into the food chain, a surveillance of trichinellosis in smuggled wildlife meat and law prohibiting commercial hunting should be strengthened.

6.2. Trichinellosis control measures

To control any disease it is important that risk factors of the disease are identified so that they can be altered to control the infection (Jekel *et al.*, 2007). Based on the epidemiological investigation of human trichinellosis cases and prevalence studies of trichinellosis in some animal reservoir hosts, the potential sources of *Trichinella* in humans in Nan Province included smuggled wildlife, smuggled pigs, dogs, wildlife and domestic pigs (Chapter 2) (see Figure 6-1). While, the potential sources of *Trichinella* in domestic pigs, particularly scavenging pigs and pastured pigs, could be carcasses and faeces of domestic and wild animals and carcasses and faeces of wild animals, respectively. This information helps to guide for risk management of trichinellosis in pigs and humans in Nan Province *i.e.* public education on potential sources of *Trichinella*, pig owner education on pig management practices, surveillance of trichinellosis in high risk groups of animals or products.

Figure 6-1 Schemology of the potential sources of *Trichinella* transmission to pigs and humans in endemic foci in Nan Province



6.2.1 Trichinellosis control in pigs

Risk for *Trichinella* spp. infection is low in the pig production units that practise high levels of bio-security (Gamble *et al.*, 2001). Among different pig production systems potential for uptake of bio-security measures is the poorest in scavenging pig production systems compared with small-scale confined, large-scale confined and large-scale outdoor production systems (FAO/OIE/World Bank, 2010). None of the baseline bio-security measures to prevent trichinellosis in a pig farms, such as avoiding introduction of pigs from outside farms, exclusion of wild pigs and rodents, permanent housing of pigs and avoid non-boiled swill feeding, are utilised in the scavenging pig production system (Table 6-6-1) (FAO/OIE/World Bank, 2010). Whereas in small-scale confined pig production only avoiding introduction of pigs from outside farms is impractical. Meanwhile, in large outdoor pig production systems exclusion of rodents and permanent housing of pigs are impractical (FAO/OIE/World Bank, 2010).

Table 6-6-1 Potential for uptake of selected bio-security measures in different type of pig production systems

Bio-security measure	Type of production systems			
	Scavenging pigs	Small-scale confined	Large-scale confined	Large-scale outdoor
Avoid introduction of pigs from outside farms, markets, or villages	No	No	Yes	Yes
Exclusion of rodents	No	Yes/No	Yes	No
Permanent housing of pigs	No	Yes	Yes	No
Avoid non-boiled swill feeding	No	Yes	Yes	Yes

Source: Good practices for bio-security in the pig sector: issues and options in developing and transition countries (FAO/OIE/World Bank, 2010)

A key strategy in achieving bio-security goals is to engage pig keepers in a participatory approach (FAO/OIE/World Bank, 2010). If risks and protection measures are communicated and are clear to the communities and they accept that actions are feasible and beneficial, they are likely to adopt the measures. In addition, Sithithaworn *et al.* (2007) mentioned that control programmes that demand too many changes may not be successful. In Nan Province scavenging pig production, small-scale pig production and pastured pig production, where pigs are let loose in farmland, were practised (Chapters 3&5). *Trichinella* infected pigs were found both in a small-scale confined pig farm where the pig was purchased from a border market and in the scavenging pig production

systems (Chapter 3). Therefore the measures to prevent trichinellosis in pigs should be focused on these factors.

Generally, in many small-scale pig farms rodent control is not widely adopted while raw swill feed is commonly used. There are reports that suggest that rodents and raw swill feeding are not always the sources of *Trichinella*, hence, these measures could be more relaxed compared with the other two measures. An absence of rodent control measure and feeding leftovers in pig farms were not associated with trichinellosis in pigs in Nan Province (Chapter 3). Even in commercial pig farms Theodoropoulous *et al.* (2009) found this measure is most insufficient in pig farms in Greece. Additionally, several studies indicated that rats are not a reservoir of *Trichinella* but a victim of improper pig slaughtering and rats are instead an indicator of *Trichinella*-infected pig farm. Stojcevic *et al.* (2004) screened for *Trichinella* infection in 2,287 rats collected from 60 pig farms in a period of two years. The study showed that the prevalence of infection ranged from 0.2 – 10.7% and the infected rats were found only on farms with *Trichinella* infected pigs. In terms of feeding, feeding un-boiled garbage or leftovers was not a significant factor influencing trichinellosis in pigs in Nan Province (Chapter 3). However, in the 1950s there were higher prevalence of trichinellosis in pig in the USA that were fed with raw garbage (5.7 – 11.2%) compared with those that did not feed on raw garbage (<1%) (Schwartz, 1960). In pig farms in Hawaii trichinellosis sero-positive pigs were only found in the farms that fed raw garbage to pigs (Dubey *et al.*, 1992). In contrast, a study in the northeastern USA found that the type of feed was not associated with trichinellosis in pigs (Gamble *et al.*, 1999). In pig farms in suburbs of China, trichinellosis was found to be associated with restaurant swill feeding, while in mountainous areas trichinellosis was associated with pigs that were raised outdoors (Cui *et al.*, 2006a).

The benefits of controlling trichinellosis in pigs is two fold, first to prevent infection in pigs and secondly to prevent the infection in other domestic animals and humans. Hill *et al.* (2009) found trichinellosis seropositives in scavenging mammals in the *Trichinella* infected pig farm environment only up to about six months after infected pigs were depopulated. These results suggested that *T. spiralis* was not maintained in scavenging mammals in the absence of infected pigs. In Nan Province dog meat is consumed and mostly consumed cooked (Chapters 4&5), however, there is still a chance of consuming raw dog meat (Chapter 5). Thus prevention of *Trichinella* transmission from pigs to dogs can also lessen the risk of trichinellosis in humans.

Control of trichinellosis in scavenging pig production in Nan Province is challenging but it has a potential to achieve its goal. There is a rule prohibiting free roaming pigs in the villages in Nan Province and the majority of communities have accepted the need to confine pigs (Chapter 5). Although scavenging pigs were still found in some Hmong, Yao and Lua communities, all holdings in some of the communities (Yao and Lua) have practised permanent confinement of pigs (Chapter 3&5). It was only in the Hmong communities where allowing pigs to scavenge was practised across all the study communities (Chapter 5). This is similar to what is found with the Hmong communities in Lao and Vietnam (Gibson & Wilkie, 1998; Vu Thi *et al.*, 2009). Gibson and Wilkie (1998) stated that although they are being encouraged by government officer to confine pigs, the Hmong communities in Lao and Vietnam still practise scavenging pig production. Hence, in the case of Thailand, more attempts may have to be made for pig owners in this ethnic group to alter their risky pig management practices. The constraints of permanent confinement of pigs are insufficient feed availability and poor survival rate of piglets when compared with those kept outside a pen (Chapter 5). These problems have also found in a

smallholder pig farming in North Vietnam (Lemke & Zarate, 2008). Purchased pig food is rarely used in any highland areas in Thailand (Chapter 3), Laos and Vietnam (Gibson & Wilkie, 1998; Van Gansberghe, 2005). The collection and preparing of supplementary food for scavenging pigs in these areas is very labour-intensive and can take up to three hours per day (Van Gansberghe, 2005). To encourage the holders to move towards permanent confinement of pigs requires a solution to the problems of insufficient feed and poor survival rate of piglets in appropriately designed pens. Possible models may be adapted from hill tribe communities that have already practise permanent confining pigs. Lastly, because it is not feasible to avoid introduction of pigs from other farms in a small-scale confined pig production systems, pig holders should be informed to avoid purchasing pigs from high risk sources, this includes pigs from border markets and scavenging pigs from mountainous areas (Chapter 3). Pigs, Pork or wildlife meat sold at border market should be checked and certified *Trichinella*-free. For pigs, pork or wildlife meat sold at border markets, the samples (meat, serum) should be collected and tested for *Trichinella* by regional laboratory centre.

6.2.2 Trichinellosis control in humans

For control of trichinellosis in humans, the rationale is that health education is required to stop people consuming raw meat and/or meat or food that contains meat are free from viable *Trichinella* larvae. Knowledge is quoted an important tool in safe food consumption (WHO, 2010) and one of the five keys recommended for safe food is proper cooking meat. In practical, people intended to consume raw food rather than they did not aware of its harmful. In rural areas of Nan Province most people still consumed raw foods even though they knew the foods are raw and they carry risks of food poisoning (Chapters 4&5).

Previous studies have shown that there are people who lack correct knowledge or have misconceptions on what foods are safe to eat; for example they believed that lime juice or acid in ants can kill microbes in meat (Faichid, 2002; Kaewpitoon *et al.*, 2007). In Nan Province there were people, particularly adults, who continued consuming raw foods because of the preference in the taste of raw foods and the belief that their risk is lessened from consuming raw food only in a small amount and only on special occasions (Chapter 5). These facts indicated that public education alone cannot effectively prevent trichinellosis because there are people who prefer to consume raw foods and accept the risk that this brings. It also indicated that public education is still required and it is important to prevent infection among those who lack correct knowledge and also among children, who are generally fed on what is put in front of them. Risk behaviour could not be easily changed in adults (Chapter 4&5) although it may be possible to reduce the likelihood of the young eating raw meat products. In the case of liver fluke infestation, it is believed that successful control may have to wait until the next generation of adults. They will have received formal education on the risks of consumption of raw meat and should hopefully no longer participate in this activity (Sithithaworn *et al.*, 2007). Results in Chapter 5 indicated that the awareness adults had gleaned from public education they did not want their children to expose to raw food consumption (Chapter 5), hence public education also plays an important role in the success of trichinellosis control.

It is not easy to change consumption behaviour, for instance in Argentina, Ribicich *et al.* (2005) suggested disseminating information to populations to prevent human trichinellosis without modifying their cultural eating habits (Ribicich *et al.*, 2005). The means to test and detect for *Trichinella* in locally raised animals was made available and the population were advised to obtain diaphragm tissue from pigs and send it to the veterinary centre for testing before consuming the pork (Ribicich *et*

al., 2005). In Inuit communities in northern Canada testing harvested walrus meat before consumption is a successful programme to prevent human trichinellosis (Proulx *et al.*, 2002). The test before consumption programme requires that meat can be distributed for consumption only after laboratory examination confirms that *Trichinella* is not found in that meat. This measure has not been undertaken in Thailand, the reasons involve a preference on using fresh meat rather than frozen meat and the inconvenience in sending samples for testing. Raw food in Thailand, *larb* and *luh*, are made of fresh meat and are consumed immediately. *Nhaem* which is a fermented food is also made from fresh meat but is consumed a few days after fermentation takes place. In Thailand meat of *Trichinella* high-risk animals are consumed in rural communities as such it is not feasible to send samples for laboratory testing. Considering that there is not only trichinellosis but also other food-borne diseases (such as *Streptococcus suis* infection and opisthorchiasis) common in Thailand (Bureau of Epidemiology, 2007-2009), public education to consume only cooked meat is more appropriate than *Trichinella* test before consumption. Public education should therefore be continued as one of the important measures to prevent trichinellosis as well as other food-borne diseases in Thailand.

Trichinellosis cases occurred when infected meat distributed through market or through sharing of food in rural communities or both. Infected meat distributed through market can cause large scale outbreak in many areas such as an outbreak from contaminated meat in Turkey that were distributed to restaurant and food vendors and caused hundreds of cases (Akkoc *et al.*, 2009), outbreak caused by contaminated marketed horse meat in France occurred in several foci (Ancelle *et al.*, 1998). Fortunately only the latter accounted for the majority of trichinellosis cases in Thailand. Implicated foods made from home-slaughtered animals are served during festival or ceremonies or meat from home-slaughtered animals are shared in the communities (Chapter 2) and being trichinellosis cases associated with consuming raw meat at social

gatherings (Chapter 4). Commercial pork and its products sold in markets have never been associated with trichinellosis in Thailand (Bureau of Epidemiology, 1981-2008). The only widespread trichinellosis outbreak in Thailand was attributed to smuggled wildlife meat sold at a border market. The infected meat was distributed to several provinces by tourists who visit the market and bought the meat to consume at home (Bureau of Epidemiology, 2004). Because of this, public education campaign in Thailand can be focused on the promotion of consumption and serving of well cooked food at festivals or ceremonies as well as at home. Public education can be carried on through communication channels that are already in place as they have proven to reach the majority of communities even in rural areas (Chapter 5).

6.3. Prospects for future control of trichinellosis in Thailand from an international perspectives

In the context of international trichinellosis control, two measures have been adopted besides public education. These comprise routine test at slaughterhouses (van Knapen, 2000) and certified *Trichinella*-free farm (Pyburn *et al.*, 2005). In the USA, trichinellosis control relies on certified *Trichinella*-free farm. It is considered that good management practices in the farm can effectively prevent *Trichinella* infection in animals (Gamble *et al.*, 2001), hence, the animals have low or negligible risk and unnecessary to test for the infection on routine basis. Several EU Member States has carried out routine test and condemn *Trichinella* infected carcasses. However, it has recognised that hundred of millions of fattening pigs from so called controlled and non-controlled housing¹² has been tested negative for *Trichinella*. Meanwhile, most backyard and free-ranging pigs are not tested but such animals are often included those infected with *Trichinella* and are sources of human infection (EFSA,

¹² Control housing is identified by the application of a specific list of management and husbandry practices

2010). These facts have led the EU to propose cost-effective *Trichinella* monitoring scheme which direct more resources and attention to be spent on the high-risk population and less resources would be spent on populations represent a negligible risk to human health (EFSA, 2010; Alban *et al.*, 2011). The proposed scheme relies on compartmentalisation to identify regions and categories of animals represent distinct risk of *Trichinella*. Certain animal populations are monitored with different intensity within the specified regions. Cost-effective measure that does not compromise public health and the measures enable standardised *Trichinella* monitoring within the member states were keys of trichinellosis control in the EU. For Thailand, the country has already adopted trichinellosis control following international contexts through certified standard farm (FAO, 2003). Compartmentalisation to identify areas and categories of animals with distinct risk of *Trichinella* has not been carried out in this country but it can be undertaken based on existing surveillance data as it has already been in placed in cases of poultry (for HPAI surveillance purpose). However, certain limitations can be foreseen if to test for *Trichinella* in high-risk groups of animals. The high risk group of pigs in Thailand are reared in remote areas far from laboratory centre and hunting wildlife is illegal activity. These animals are slaughtered at home. Compulsory test for *Trichinella* in these animals are impractical. The most important measure to alleviate risk of infection to people in the high-risk areas is to educate public on consumption safe food and educate farmer on good management practices in the farms. Public education also has additional value in it since there are several infectious pathogens that can be transmitted to people via ingestion raw of foods not only *Trichinella*.

6.4. Future research

Permanent housing of pigs is one of the most important bio-security measures to prevent trichinellosis in pigs. Some ethnic minority

communities that traditionally practised scavenging pig rearing have now changed to the practice of permanent housing of pigs. Some communities have maintained the traditional practice and their pigs as well as people who eat raw pork are at risk of trichinellosis. A further in depth investigation is required to understand why communities have changed or maintained pig management practices and what can be done for the communities to practically permanent confine their pigs.

The lack of records as well as signs and/or symptoms of trichinellosis might be due to subclinical infections from the ingestion of pork or pork products with low parasite burdens. Chomel *et al.* (1993) and Owen *et al.* (2005) demonstrated the presence of antibodies against *Trichinella* infection in asymptomatic trichinellosis cases. A study by Tinoco-Velazquez *et al.* (2002) found some cases (4%, n=250) diagnosed as fever of unknown origin related to trichinellosis. An investigation in rural remote areas of northern Thailand where people live on subsistence foods and have maintained the culture of raw food consumption should be undertaken to determine subclinical or undiagnosed clusters of human trichinellosis in order to evaluate the actual situation of this disease and to alert people to prevent this infection.

Surveillance of *Trichinella* infection in animal particularly pigs is an important measure in the disease control programme. The ELISA is a method recommended by the OIE for trichinellosis surveillance purpose (OIE, 2008). Currently, a commercial ELISA test is used for trichinellosis surveillance in animal in Thailand. It costs approximately US\$ 4-5 per sample which is not possible to afford in the long term or to test larger samples. An in-house ELISA test should be developed and standardized to replace the use of commercial ELISA test kit.

7. References

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Appendix 1 Calculation 12-month moving average, centred 12-month moving average and the ratio-to-moving average

Month-year	Monthly cases	12-month moving average	Centred 12-month moving average	Ratio-to-moving average
January 1981	87			
February 1981	0			
March 1981	0			
April 1981	178			
May 1981	0			
June 1981	0			
July 1981	0		20.04	0
August 1981	0		21.29	0
September 1981	0		26.50	0
October 1981	0		19.42	0
November 1981	0		12.00	0
December 1981	19	23.67	14.50	1.31
January 1982	117	16.42	18.42	6.35
March 1982	8	26.16	19.83	0.40

Appendix 2 Average monthly ratio-to-moving-average (seasonal index) and adjusted average monthly ratio-to-moving-average

Month	Average monthly ratio -to-moving-average	Adjusted average monthly ratio-to-moving-average
January	1.0737	122.8741
February	0.7507	85.91001
March	0.81773	93.58092
April	1.039746	118.9884
May	1.527392	174.7945
June	1.126479	128.9141
July	0.355629	40.69814
August	1.006965	115.2369
September	0.545948	62.47822
October	0.855128	97.86074
November	0.69071	79.04476
December	0.695729	79.61914
Total annual seasonal indices	10.48586	1200

Appendix 3 Incidence rates of human trichinellosis in Thailand between 1981 – 2008

Province	Region	Person-year	Cases	Rates ^a
1. Mae Hong Son	North	193826	437	2254.61
2. Nan	North	431168	641	1486.66
3. Chiang Rai	North	1084035	1109	1023.03
4. Chiang Mai	North	1429541	1362	952.75
5. Phayao	North	478981	452	943.67
6. Lampang	North	719921	276	383.38
7. Nong Bua Lam Phu	Northeast	476808	141	295.72
8. Phetchabun	North	898352	248	276.06
9. Kamphaeng Phet	North	648143	169	260.75
10. Chumphon	South	410794	96	233.69
11. Phitsanulok	North	783412	148	188.92
12. Uttaradit	North	452800	71	156.80
13. Nakhon Sawan	North	1035082	149	143.95
14. Uthai Thani	North	296042	26	87.83
15. Kanchanaburi	Central	684950	29	42.34
16. Nong Khai	Northeast	796917	19	23.84
17. Trad	Central	181346	4	22.06
18. Lamphun	North	382796	8	20.90
19. Phrae	North	459285	8	17.42
20. Nakhon Phanom	Northeast	622010	10	16.08
21. Khon Kaen	Northeast	1568620	18	11.48
22. Nakhon Ratchasima	Northeast	2259491	20	8.85
23. Phuket	South	231634	1	4.32
24. Prachin Buri	Central	551451	2	3.63
25. Amnat Charoen	Northeast	357176	1	2.80
26. Tak	North	410349	1	2.44
27. Udon Thani	Northeast	1505894	3	1.99
28. Sukhothai	North	572877	1	1.75
29. Kalasin	Northeast	874255	1	1.14
30. Sakon Nakhon	Northeast	953804	1	1.05
31. Ubon Ratcha Thani	Northeast	1692997	1	0.59

^a Rate per 1,000,000 person-years

Appendix 4 Sex ratios of male to female trichinellosis in Thailand between 1981 – 2008^a

Year	Number of cases		Male : female ratio	$\chi^2_{df=1}$
	Male	Female		
1981	184	100	1.8 : 1	24.84*
1985	234	180	1.3 : 1	7.04*
1986	172	75	2.3 : 1	38.09*
1987	230	126	1.8 : 1	30.38*
1989	163	79	2.1 : 1	29.16*
1990	52	15	3.5 : 1	20.40*
1991	91	18	5.1 : 1	48.89*
1992	232	165	1.4 : 1	11.31*
1994	110	73	1.5 : 1	7.48*
1995	116	126	0.9 : 1	0.41
1996	91	62	1.5 : 1	5.50*
1997	14	9	1.6 : 1	1.09
1998	234	117	2.0 : 1	39.00*
1999	12	4	3.0 : 1	4.00*
2000	79	49	1.6 : 1	7.03*
2002	167	122	1.4 : 1	7.01*
2003	80	46	1.7 : 1	9.17*
2004	76	78	1.0 : 1	0.03
2005	38	35	1.1 : 1	0.12
2006	9	9	1.0 : 1	0
2007	36	24	1.5 : 1	2.40
2008	33	11	3.0 : 1	11.00*

* Significant $p < 0.05$

^a Data of number of trichinellosis cases by gender between 1981 – 2008 was available in 22 years

Appendix 5 Questionnaire for survey of production and management profiles in pig holdings, Nan province (2008)

5.1 English

Questionnaire id.....

Owner name.....

Address.....

Coordinates.....

Altitude.....metres

1) Holder ethnic background and main occupation

1.1 Ethnicity: Thai Hmong Yao Lua
 Khamu

1.2 Religious: Buddhism Christianity Animist

1.3 Occupation: Agriculturist Merchant Labour
 Officer

2) Pig production characteristics

2.1 Number of pigs by category in a holding

Boar: [__]

Sow: [__]

Nursery: [__]

Fattening: [__]

Replacement: [__]

Total: [__]

2.2 Breed: improved-breed native wild boar
 mixed

2.3 Production scheme: own use sell

3) Husbandry management characteristics

3.1 Mode of introduction new pigs:

- born in holding
- buy from market
- buy from mobile seller
- buy from other holding
- gift

3.2 Source of new pigs: born in holding from other holdings
 both

3.3 Feed:

agriculture products

grain

boiled leftover

un-boiled leftover

3.4 De-worming: yes no

3.5 Swine fever vaccination: yes no

3.6 Rodent control: yes no

3.7 Dead pig carcass management:

buried

eat

leave on ground

no dead pig in holding

Thank you very much for your kind co-operation

5.2 Thai

หมายเลข
ตัวอย่าง.....

การสำรวจสุกรรายครัวเรือนในพื้นที่จังหวัดน่าน พ.ศ.๒๕๕๑

ชื่อเจ้าของ.....
ที่อยู่ บ้านเลขที่..... หมู่ที่..... ตำบล..... อำเภอ.....
พิกัด.....
ระดับความสูง..... เมตร

๑) ข้อมูลเจ้าของและฟาร์ม

- ๑.๑ เชื้อสาย ไทยพื้นราบ มัง เหย้า ลัวะ ขมุ
๑.๒ ศาสนา พุทธ คริสต์ นับถือผี
๑.๓ อาชีพ เกษตรกรรม ค้าขาย รับจ้าง พนักงาน/ราชการ

๒) จำนวนสุกร พันธุ์ และจุดประสงค์ของการเลี้ยง

- ๒.๑ จำนวนสุกรในฟาร์ม พ่อพันธุ์ แม่พันธุ์ ดูนม/หย่านม ขุน
 ทดแทน รวม
๒.๒ พันธุ์ พันธุ์ พื้นเมือง หมูป่า ผสม
๒.๓ จุดประสงค์หลักที่เลี้ยง บริโภคเอง จำหน่าย

๓) ลักษณะการจัดการ

- ๓.๑ แหล่งที่มาของสุกร เกิดในฟาร์ม ตลาด รกรเร่
 ฟาร์มอื่น ของขวัญ
๓.๒ แหล่งการนำเข้าสุกร เกิดในฟาร์ม จากที่อื่น ทั้งสองแบบ
๓.๓ อาหารสุกร ผลิตผลเกษตร อาหารสำเร็จ
 เศษอาหารต้ม เศษอาหารไม่ต้ม
๓.๔ การถ่ายพยาธิ ใช่ ไม่ใช่
๓.๕ การทำวัคซีน ใช่ ไม่ใช่
๓.๖ การกำจัดหนู ใช่ ไม่ใช่
๓.๗ การจัดการซาก ฝัง กิน ทิ้งบนพื้น ไม่มีสุกรตายในฟาร์ม

ขอขอบคุณ ที่ให้ความร่วมมือในการสำรวจข้อมูลค่ะ

Appendix 6 Questionnaire for survey of factors associated with trichinellosis in pigs in Nan province, 2008

6.1 English

Questionnaire id.....

1. Pig demographical profiles

1.1 Age (months)

1.2 Sex male female

1.3 Breed native improved-breed
 wild boar mixed

1.4 category boar sow nursery
 fattening replacement

2. Pig husbandry management characteristics

1.1 Source of introduction born in holding from other holding

1.2 Strictly confinement yes no

1.3 Feed contain leftover yes no

1.4 De-worming yes no

1.5 Rodent controls yes no

1.6 Dead carcass management yes no

1.7 Expose to wildlife yes no

1.8 Expose to other domestic animals yes no

1.9 Age introduction to the holdingmonths

2. Pig production characteristics

2.1 registered holding yes no

2.2 Production scheme own use sell

2.3 Holding environment farmland residential area

2.4 Home slaughtered pig yes no

2.5 Total number of pig boar sow nursery
 fattening replacement

3. Holding address and coordinates

3.1 Holding address.....

3.2 Coordinates

3.3 Altitudemetres

4 Holder ethnic backgrounds

4.1 Ethnicity Thai Hmong Yao
Lua Khamu

Thank you very much for your kind co-operation

6.2 Thai

หมายเลขตัวอย่าง.....

การสำรวจปัจจัยด้านการจัดการที่สัมพันธ์กับโรคทริคิเนลโลซิสในสุกรในจังหวัด น่าน พ.ศ.๒๕๕๑

ชื่อเจ้าของ.....
ที่อยู่ บ้านเลขที่..... หมู่ที่..... ตำบล..... อำเภอ.....
พิกัด.....
ระดับความสูง..... เมตร

๑) ข้อมูลสุกร

- ๑.๑ อายุเดือน
- ๑.๒ เพศ ผู้ เมีย
- ๑.๓ พันธุ์ พันธุ์ พื้นเมือง หมูป่า ผสม
- ๑.๔ ชนิดของสุกร พ่อพันธุ์ แม่พันธุ์ ดูนม/หย่านม ขุน ทดแทน

๒) ลักษณะการจัดการ

- ๒.๑ แหล่งที่มาของสุกร เกิดในฟาร์ม จากที่อื่น
- ๒.๒ ชังคอกตลอดเวลา ใช่ ไม่ใช่
- ๒.๓ เลี้ยงด้วยเศษอาหาร ใช่ ไม่ใช่
- ๒.๔ การถ่ายพยาธิ ใช่ ไม่ใช่
- ๒.๕ การกำจัดหนู ใช่ ไม่ใช่
- ๒.๖ การจัดการซาก ใช่ ไม่ใช่
- ๒.๗ สัมผัสสัตว์เลี้ยงอื่น ใช่ ไม่ใช่
- ๒.๘ สัมผัสสัตว์ป่า ใช่ ไม่ใช่
- ๒.๙ อายุที่นำเข้าฟาร์ม.....เดือน

๓) ลักษณะการผลิต

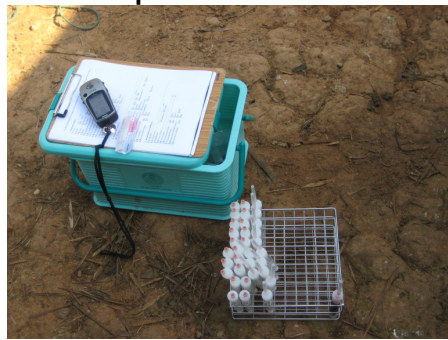
- ๓.๑ ฟาร์มมาตรฐาน ใช่ ไม่ใช่
- ๓.๒ จุดประสงค์หลักที่เลี้ยง บริโภคเอง จำหน่าย
- ๓.๓ สภาพแวดล้อม ในเขตบ้าน พื้นที่การเกษตร
- ๓.๔ การฆ่าหมูที่บ้าน ใช่ ไม่ใช่
- ๓.๕ จำนวนสุกรทั้งหมด พ่อพันธุ์ แม่พันธุ์ ดูนม/หย่านม
 ขุน ทดแทน รวม

๔) เจ้าของ

- ๔.๑ เชื้อสาย ไทยพื้นราบ ม้ง เข่า ลี๊วะ ขมุ

ขอขอบคุณ ที่ให้ความร่วมมือในการสำรวจข้อมูลค่ะ

Appendix 7 Field work for data and sample collection on a survey to determine prevalence and risk factors of trichinellosis in pigs



Appendix 8 Village seroprevalence of trichinellosis in pig: a survey of 61 villages selected randomly from 311 villages; all pigs aged ≥ 2 months old in the selected villages investigated

Villages	Total number of pigs	Number of sero-positive pigs	Village prevalence
1	7	0	0
2	106	0	0
3	30	0	0
4	88	0	0
5	15	0	0
6	23	0	0
7	91	1	0.011
8	111	0	0
9	23	0	0
10	6	0	0
11	45	1	0.022
12	72	2	0.028
13	9	0	0
14	30	0	0
15	167	6	0.036
16	52	0	0
17	8	0	0
18	34	0	0
19	173	0	0
20	25	1	0.040
21	14	0	0
22	141	4	0.028
23	10	0	0
24	19	0	0
25	29	0	0
26	65	1	0.015
27	29	0	0
28	42	0	0
29	20	0	0
30	83	0	0
31	10	0	0
32	1	0	0
33	26	0	0
34	14	0	0
35	15	0	0
36	59	1	0.017
37	10	0	0
38	8	0	0
39	15	0	0
40	38	0	0
41	26	0	0
42	15	0	0
43	18	0	0
44	25	0	0
45	39	0	0
46	108	0	0
47	40	0	0
48	38	0	0
49	28	0	0
50	9	0	0
51	7	0	0
52	18	0	0
53	15	0	0
54	11	0	0
55	7	0	0
56	36	0	0
57	54	0	0
58	12	0	0
59	140	0	0
60	11	0	0
61	43	0	0
	2463	17	

Appendix 9 Questionnaire for a case-control study

9.1 English

Questionnaire no..... case control

Interviewer's name Date of interview
Participant's name..... Home address: House number.....Village number..... Tambon.....District..... Age.....years Gender male/female Ethnicity.....

Part I Risk factors

	yes	no
1) Individual and household characteristics		
1.1 Agricultural occupation		
1.2 Illiteracy		
1.3 Does not have a refrigerator		
1.4 Family member go hunting		
1.5 Family member having epilepsy		
1.6 Family member having acquired hearing-impaired		
2) Individual behaviour		
2.1 Regular alcohol consumption (\geq once a week)		
2.2 Not de-wormed regularly (<two times a year)		
2.3 Consumption of home-slaughtered pig		
2.4 Consumption of native-breed pig		
2.5 Consumption of wildlife		
2.6 Consumption of dog		
2.7 Consumption of raw pork		
2.8 Consumption of raw beef		
2.9 Consumption of raw pork of wild boar		
2.10 Consumption of raw meat of wildlife		
2.11 Consumption of raw meat of dog		
2.12 Consumption of homemade raw food		
2.13 Consumption of purchased raw food		
2.14 Consumption of raw food at a social gathering		
3) Knowledge and perception		
3.1 Inability to identify uncooked food		
3.2 Belief that lime juice kill parasite and microbes in raw meat		
3.3 Belief that alcohol kill parasite and microbes in raw meat		
3.4 Ignorance of food-borne diseases		

Part II Clinical symptoms

	yes	no
During trichinellosis outbreak		
1 Myalgia		
2 Calf pain		
3 Periorbital oedema		
4 Facial oedema		
5 Diarrhoea		
6 Vomiting		
About one year after trichinellosis outbreak		
1 Myalgia		
2 Calf pain		
3 Periorbital oedema		
4 Facial oedema		
5 Diarrhoea		
6 Vomiting		

Part III Behaviour after about one year of trichinellosis outbreak

	yes	no
1 Regular alcohol consumption (\geq once a week)		
3 Not de-wormed regularly (< two times a year)		
4 Consumption of home-slaughtered pig		
5 Consumption of native-breed pig		
6 Consumption of raw pork		
7 Consumption of raw beef		
8 Consumption of raw pork of wild boar		
9 Consumption of raw meat of wildlife		
10 Consumption of raw meat of dog		

Thank you very much for your kind co-operation

9.2 Thai

เลขที่แบบสอบถาม เดส คอนโทรล

การศึกษาปัจจัยเสี่ยงของการติดโรคริดิโนซิสในพื้นที่จังหวัดน่าน

ชื่อผู้สัมภาษณ์ วันที่สัมภาษณ์.....

ข้อมูลบุคคล

ชื่อ.....

อายุ.....ปี เพศ ชาย หญิง

ที่อยู่ บ้านเลขที่.....หมู่ที่.....ตำบล.....อำเภอ.....

เชื้อชาติ.....

ส่วนที่ ๑ ข้อมูลปัจจัยเสี่ยง

๑) ข้อมูลบุคคลและครัวเรือน	ใช่	ไม่ใช่
๑.๑ อาชีพเกษตรกร		
๑.๒ การอ่านเขียนไทยได้		
๑.๓ มีผู้เฒ่า		
๑.๔ มีสมาชิกในบ้านล่าสัตว์		
๑.๕ มีคนเป็นโรคลมชักในครอบครัว		
๑.๖ มีคนทุบในครอบครัว		

๒) พฤติกรรมบุคคล	ใช่	ไม่ใช่
๒.๑ ดื่มสุราเป็นประจำอย่างน้อยสัปดาห์ละครั้ง		
๒.๒ ก่ายพยาธิเป็นประจำอย่างน้อยปีละสองครั้ง		
๒.๓ กินหมูฆ่าตามบ้าน หมูปุ้น		
๒.๔ กินหมูตำหมูเมืองฆ่าตามบ้าน		
๒.๕ กินเนื้อสัตว์ป่า รวมจู ตุ่น เก้ง กวาง หมี (ระบุชนิด.....)		
๒.๖ กินเนื้อ สุนัข หรือแมว		

การบริโภคเนื้อดิบ	สด	ลาบ	หู้	ส้า	แหมม
๒.๗ หมู					
๒.๘ วัว/ควาย					
๒.๙ หมูป่า					
๒.๑๐ สัตว์ป่า					
๒.๑๑ สุนัข แมว					

แหล่งที่มาของอาหาร	ลาบ	หู้	ส้า	แหมม
๒.๑๒ ทำเองที่บ้าน				
๒.๑๓ ซื้อมาจากร้านค้า				
๒.๑๔ กินนอกบ้านตามงานเลี้ยงทุกประเภท งานเลี้ยง ลงแขก สัก สันต์				

๓) ความรู้ความเข้าใจเรื่องโรคทริคิโนซิส		
๓.๑ ลาบ หลู้ ส้า แหนม ที่ไม่คั่ว จัดว่าสุกหรือดิบ	สุก	ดิบ
๓.๒ น้ำส้ม หรือ น้ำมะนาว ทำให้เนื้อสุก	ใช่	ไม่ใช่
๓.๓ การต้มเหล้าฆ่าเชื้อโรค หรือ พยาธิ ได้	ใช่	ไม่ใช่
๓.๔ การกินเนื้อสัตว์ที่ไม่สุกอาจทำให้เจ็บป่วย	ใช่	ไม่ใช่
คำตอบใช่ ทราบได้อย่างไร)ด้วยตัวเอง ตำบลเก่าต่อกันมา ทวี วิทยุ นสพ .เอกสาร เจ้าหน้าที่ ผู้นำชุมชน (
รู้จักโรคที่เกิดจากการการกินเนื้อสัตว์ที่ไม่สุก	ใช่	ไม่ใช่
คำตอบใช่ บอกชื่อโรค หรืออาการ		

ส่วนที่ ๒ อาการป่วย

อาการเจ็บป่วยในช่วงที่มีการเกิดโรคทริคิโนซิส	ใช่	ไม่ใช่
การปวดเมื่อยกล้ามเนื้อทั่วตัว		
การปวดเมื่อยกล้ามเนื้อท้อง		
ตาบวม		
หน้าบวม		
ท้องร่วง		
อาเจียน		
อาการเจ็บป่วยหนึ่งปี ภายหลังเกิดโรคทริคิโนซิส		
การปวดเมื่อยกล้ามเนื้อทั่วตัว		
การปวดเมื่อยกล้ามเนื้อท้อง		
ตาบวม		
หน้าบวม		
ท้องร่วง		
อาเจียน		

ส่วนที่ ๓ พฤติกรรมหนึ่งปีภายหลังการเกิดโรคทริคิโนซิส

	ใช่	ไม่ใช่
๑ ดื่มสุราเป็นประจำอย่างน้อยสัปดาห์ละครั้ง(
๒ ก่ายพยาธิเป็นประจำอย่างน้อยปีละสองครั้ง(
๓ กินหมูฆ่าตามบ้าน)หมูปุ้น(
๔ กินหมูดำ)หมูเมือง (ฆ่าตามบ้าน		

การบริโภคเนื้อดิบ	สด	ลาบ	หลู้	ส้า	แหนม
๕ หมู					
๖ วัว/ควาย					
๗ หมูป่า					
๘ สัตว์ป่า					
๙ สุนัข แมว					

ขอขอบคุณเป็นอย่างสูงสำหรับความร่วมมือและการเสียสละเวลาเพื่อให้ข้อมูลในการศึกษาครั้งนี้

Appendix 10 Fact sheet on trichinellosis published by Department of Preventive Medicine, Siriraj Hospital, Mahidol University, Thailand

บทความทางวิชาการ ภาควิชาเวชศาสตร์ป้องกันและสังคม คณะแพทยศาสตร์ศิริราชพยาบาล

ทริคิโนซิส (Trichinosis)

โดย: ศาสตราจารย์เกียรติคุณนายแพทย์มุกดา ดุทยานานนท์

ภาควิชาเวชศาสตร์ป้องกันและสังคม

คณะแพทยศาสตร์ศิริราชพยาบาล

เมื่อไม่นานมานี้มีข่าวจากหนังสือพิมพ์ เรื่องคนกินเนื้อแก่งและมีอาการไข้ ปวดเมื่อยตามตัว ท้องเสีย และมีอาการบวมที่หนังตา สรุปว่าจะเป็น Trichinosis ทั้งนี้เนื่องจากกินอาหารดิบๆ สุกๆ จากเนื้อแก่งซึ่งมีเชื้อของพยาธิที่อยู่ในกล้ามเนื้อ จึงเกิดเรื่องดังกล่าวขึ้น ถ้ากินอาหารที่สุกก็ไม่มีปัญหา แต่อย่างไร

พยาธิชนิดนี้เรียกว่า *Trichinella spiralis* วงจรชีวิตของโรคนี้คือ หนู และหมู เมื่อหมูที่มีเชื้อตายลงหรือถูกฆ่าและเนื้อมีพยาธินี้อยู่ หนูกินเนื้อหมูเข้าไป หนูก็มีเชื้อนี้อยู่ในกล้ามเนื้อ เมื่อหนูตายลง หมูกินเนื้อหมูเข้าไป หมูก็ติดโรคนี้ คือมีเชื้ออยู่ในกล้ามเนื้อของหมูและก๊วนเวียนกันอยู่เช่นนี้ตลอดไป เมื่อคนกินเนื้อหมูซึ่งมีเชื้อมีเชื้อนี้อยู่ โดยกินหมูดิบๆ สุกๆ เช่น แหนม ลาบ หลู้ เป็นต้น ในกล้ามเนื้อของหมูจะมีเชื้อซึ่งเป็น cyst อยู่ cyst นี้ จะถูกย่อยออกมาในกระเพาะ แต่ตัวอ่อนของพยาธิจะมีความทนทานต่อน้ำย่อยในกระเพาะของคน ดังนั้นตัวอ่อนของพยาธิจะเดินทางต่อไปเมื่อถึงลำไส้เล็กตัวอ่อนจะเข้าไปแทรกอยู่ใน columnar epithelium ของลำไส้เล็กและค่อยๆ เจริญเติบโต ลอกคราบ 4 ครั้ง กินเวลาประมาณ 36 ชั่วโมง และจะเป็นตัวแก่ อยู่ในเนื้อเยื่อของลำไส้เล็กในที่สุด พยาธิตัวแก่ทั้งตัวผู้และตัวเมียผสมพันธุ์กันและตัวเมียจะปล่อยพยาธิตัวเล็กๆ ออกมาซึ่งทั้งนี้กินเวลาประมาณ 7-4 วัน หลังจากเริ่มกินเชื้อเข้าไปและจะเริ่มมีลูกปล่อยพยาธิตัวเล็กๆ ออกมา การเกิดพยาธิตัวเล็กๆ นี้ อาจมีนานถึง 6-4 สัปดาห์ พยาธิตัวเล็กนี้จะเข้ามาทางหลอดเลือดและเข้าไปในกระแสเลือดสู่อวัยวะต่างๆ ไป โดยเฉพาะอย่างยิ่งจะเข้าไปอยู่ในกล้ามเนื้อลาย และเกิดเป็น cyst อยู่ในกล้ามเนื้อ พยาธินี้ไม่จำเป็นต้องอยู่ในหมูเท่านั้น อาจอยู่ในสัตว์ป่าต่างๆ เช่น เก้ง หมูป่า ม้า ก็เป็นได้ และ วัวควาย ได้ทั้งสิ้น อาการที่เกิดขึ้นจะมีไข้สูง เนื่องจากร่างกายต่อสู้เพราะมีสิ่งแปลกปลอมเข้าไปในร่างกาย มีท้องเสียเนื่องจากพยาธิเกิดไปผสมพันธุ์และออกลูกบริเวณลำไส้เล็ก มีอาการปวดตามกล้ามเนื้อทั่วๆ ไปเนื่องจากพยาธิจะเข้าไปอยู่ตามกล้ามเนื้อทั่วๆ ไป และเป็น cyst อยู่ในกล้ามเนื้อ อาจไปอยู่ที่หัวใจ ทำให้หัวใจวายได้ Eosinophilia จะขึ้นสูงกว่าปกติ อาการจะมากหรือน้อยขึ้นอยู่กับจำนวน cyst ซึ่งมีตัวอ่อนอยู่ ถ้ากินเข้าไปมาก อาการก็จะมากขึ้นตามจำนวนที่กินเข้าไป จะมีหนังตาบวม ระยะพักตัว 6-5 วัน และอาจจะยาวนานถึง -21 30 วัน ก็ได้ ถ้ากินเข้าไปน้อยอาการก็จะน้อย Trichinosis หรือ Trichinellosis โรคนี้เกิดจากพยาธิ genus *Trichinella* (Owen 1833) เกิดจากคนกินเนื้อของสัตว์ป่าหรือสัตว์เลี้ยง เช่น หมู เป็นต้น อาการก็จะเกิดดังได้กล่าวมาแล้ว

ในประเทศไทยได้มีการระบาดครั้งแรกที่อำเภอแม่สะเรียง จังหวัดแม่ฮ่องสอน ในปี 2505 ในการระบาดครั้งแรกมีผู้ป่วย 56 ราย ถึงแก่กรรม 11 ราย เหตุจากการกินเนื้อหมูที่ซื้อมาจากชาวเขาปรุงอาหารเป็นอาหารดิบๆ เช่น ลาบ หลู้ หรือแหนม และได้มีการระบาดเรื่อยๆ มา ทุกครั้งส่วนใหญ่จะเป็นการระบาดเนื่องจากกินหมูที่ซื้อมาจากชาวเขา และมาทำกินดิบๆ ได้

การระบาดที่จังหวัดเพชรบูรณ์ เมื่อเมษายน 2524 ชาวเขาได้มีส่วนในการสอบสวนโรคพบว่าได้มีชาวเขาเผ่าเผ่าเมว ได้อพยพมาจากภูเขาเนื่องจากทางการได้ให้ย้ายถิ่นฐานลงมาอยู่ข้างล่าง เพื่อมิให้ทำไร่เลื่อนลอยไปเรื่อยๆ ก็ได้นำหมูลงมาด้วยและนำมาขายในราคาตัวละ 800 บาท ชาวบ้านได้ซื้อและฆ่าและขายกัน และนำไปประกอบอาหารดิบๆ เช่น หมูส้ม แหนม หลู้ ลาบ หลังจากนั้น 8-7 วัน ก็มี อาการปวดท้องอย่างรุนแรง ท้องเสีย มีไข้ คลื่นไส้ อาเจียน มีการปวดตามกล้ามเนื้อและตามข้อ ตาบวม มีคนเป็นทั้งหมด 163 คน และถึงแก่กรรม 14 คน การระบาดเมื่อเร็วๆ นี้ จากการกินเนื้อแก่ง (จากจังหวัดหนองบัวลำภู และจังหวัดหนองคาย จากการรายงานฝ่ายรังทางระบาดวิทยา ประจำสัปดาห์ ฉบับที่ 22 วันที่ 4 มิถุนายน 2547 ของสำนักโรคระบาด

วิทยากรรมควบคุมโรค ที่จังหวัดหนองบัวลำภู พบคนไข้ชาย 27ราย หญิง 16ราย ที่จังหวัดหนองคาย พบ 18 ราย เป็นชาย 12ราย หญิง 6ราย อาการสำคัญทางคลินิก พบว่า ปวดตามกล้ามเนื้อ %100หนังตาบวม %93 ไข้ %88ถ่ายเหลวเป็นน้ำ %79หนาวสั่น %54เบื่ออาหาร %49ปวดท้อง %39จะเห็นว่า ปวดกล้ามเนื้อและหนังตาบวม เป็นอาการที่สำคัญช่วยในการวินิจฉัยในทางคลินิกมาก

Source: Department of Preventive Medicine, Siriraj Hospital, Mahidol University, Thailand

Appendix 11 Public education and alert of outbreak of *Streptococcus suis* infection in newspaper

ข่าวประจำวัน : กินหมูกระทะไม่สุกอันตรายขั้นหูหนวก

เล่าวัย 74กินหมูกระทะจนหูหนวก แพทย์เผยเชื้อ “สเตรปโตค็อกคัส ซูอิส” จากหมูเข้าสู่ร่างกาย ทำลายระบบสมองและประสาทหู เดือนผู้บริโภคนอกเหนือจากหมูดิบก่อนสายเกินแก้ ระบุสุดอันตรายถึงขั้นทำลายสมองจนเสียชีวิต

. 13)ย (อันตรายจากเนื้อหมูดิบจนถึงขั้นหูหนวกรายนี้ ได้รับการเปิดเผยจาก รศ.พญ.นิรมล นาวา เจริญ หัวหน้าหน่วยโสตประสาทวิทยา ภาควิชา โสต ศอ นาสิกวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ โรงพยาบาลมหาราชนครเชียงใหม่ ได้รับรักษาผู้ป่วยมีอาการหูหนวก อันเนื่องมาจากกินเนื้อหมูดิบ

รศ.พญ.นิรมล เปิดเผยว่า เมื่อวันที่ 3 กันยายน ที่ผ่านมา ได้รับตัวนายนายพร ลิงคะ อายุ 74ปี ราษฎร ในอ.หางดง จ.เชียงใหม่ เข้ารับการรักษาโดยมีการวินิจฉัยที่ระยะ เมื่อสอบถามประวัติทราบว่าเมื่อวันที่ 28 สิงหาคม ที่ผ่านมา นายพรได้ไปกินหมูกระทะที่ร้านแห่งหนึ่ง โดยกินเนื้อหมูและเครื่องในเข้าไป หลังจากนั้นได้มีอาการเวียนศีรษะ และมีไข้สูง ญาติได้นำตัวส่งโรงพยาบาลเอกชน แต่หลังจากนั้นอาการก็ไม่ดีขึ้น

จากนั้นญาติจึงนำตัวส่งมารักษาที่โรงพยาบาลมหาราชนครเชียงใหม่ โดยจากการสแกนสมอง (ซีที สแกน พบว่า เชื้อหุ้มสมองอักเสบและหลังจากนั้น ในวันที่ 6 กันยายน ผู้ป่วยมีอาการหูชาเสื่อมและเป็นอัมพฤกษ์ กระทั่งวันที่ 9 กันยายน ผู้ป่วยมีอาการสมองบวม หูชาเสื่อมรุนแรง หูซ้ายตึงเล็กน้อย จากการเพาะเชื้อจากน้ำที่เจาะจากไขสันหลังพบว่าในสมองมีเชื้อแบคทีเรีย “สเตรปโตค็อกคัส ซูอิส” (*Streptococcus suis*) ซึ่งเป็นเชื้อแบคทีเรียที่อยู่ในหมู ล่าสุดแพทย์ได้ให้ยารักษาจนอาการอัมพฤกษ์เริ่มดีขึ้น แต่อาการหูหนวกหูตึงไม่สามารถรักษาได้

รศ.พญ.นิรมล กล่าวว่ เชื้อแบคทีเรียดังกล่าวเป็นเชื้อที่อยู่ในโพรงจมูกและต่อมน้ำลายของหมู เชื้อดังกล่าวพบได้ในหมูทั่วไปจนกลายเป็นเชื้อประจำถิ่น เมื่อหมูอยู่ในภาวะเครียด เช่น อยู่ในที่แออัด อากาศชื้นหรือหนาวจากฝนตกหนัก ภูมิคุ้มกันของหมูจะลดลง เชื้อดังกล่าวจึงฉวยโอกาสเข้าสู่กระแสเลือดทำให้หมูป่วยหรือตาย ส่วนเชื้อดังกล่าวเข้าสู่คนได้ 2วิธี คือเมื่อร่างกายคนมีแผลไปจับต้องหมู และกินเนื้อหมูหรือเลือดสด

อาการของผู้ที่ได้รับเชื้อแบคทีเรียชนิดนี้ถือว่ามีความรุนแรงและน่ากลัว โดยเมื่อเชื้อเข้าสู่ร่างกายจะเข้าสู่กระแสเลือดและเข้าไปสู่เชื้อหุ้มสมองจนเกิดอาการเชื้อหุ้มสมองอักเสบอย่างรวดเร็วภายใน 3วันหลังจากผู้ป่วยได้รับเชื้อจะมีอาการไข้ ปวดศีรษะ ปวดกล้ามเนื้อ เนื่องจากเชื้อหุ้มสมองอยู่ใกล้กับปลายประสาทหูชั้นใน ทั้งสองข้าง เชื้อจึงลุกลามและทำให้เกิดหนองบริเวณปลายประสาทรับเสียงและประสาททรงตัว ทำให้หูตึงและหูหนวกร่วมกับอาการเวียนศีรษะ และเดินเซ อาการทั้งหมดนี้จะเกิดขึ้นภายใน 14วัน หลังจากเริ่มอาการไข้ อย่างไรก็ตาม ความน่ากลัวของเชื้อแบคทีเรีย “สเตรปโตค็อกคัส ซูอิส” ไม่เพียงแต่ทำให้หูหนวกและสูญเสียการทรงตัว แต่หากผู้ป่วยเข้ารับการรักษาช้าอาจทำให้เชื้อแบคทีเรียทำลายเชื้อหุ้มสมองจนถึงขั้นเสียชีวิตได้ โดยหากไม่ได้รับการรักษาผู้ป่วยจะเสียชีวิตภายใน 14วัน หลังจากรับเชื้อ

รศ.พญ.นิรมล กล่าวอีกว่า สาเหตุที่ต้องออกมาเปิดเผยเรื่องนี้เพราะต้องการให้ประชาชนได้ตระหนักถึงอันตรายของการบริโภคเนื้อหมูดิบ เพราะปัจจุบันยังมีประชาชนอีกเป็นจำนวนมาก นิยมบริโภคเนื้อหมูดิบ โดยเฉพาะลาบดิบและหู้เลือด หากต้องการกินลาบต้องทำให้สุกเพราะเชื้อดังกล่าวจะถูกทำลายด้วยการต้มสุกและการซื้อเนื้อหมูให้สังเกต หากมีสีคล้ำ หรือราคาผิดปกติควรหลีกเลี่ยง

ทั้งนี้ในระยะเวลา 6ปีที่ผ่านมา จากการเก็บข้อมูลของโรงพยาบาลมหาราชนครเชียงใหม่ พบผู้ป่วยติดเชื้อแบคทีเรียดังกล่าว จำนวน 74ราย มีตั้งแต่อายุ 74-17ปี ทั้งนี้การติดเชื้อขึ้นอยู่กับภูมิคุ้มกันของร่างกาย หากร่างกายอ่อนแอ โอกาสติดเชื้อก็มีมาก ขณะเดียวกันจากการสำรวจเชิงหมู 100แห่ง ใน จ.เชียงใหม่ พบเชื้อแบคทีเรีย “สเตรปโตค็อกคัส ซูอิส” ถึงร้อยละ 20

ด้านนายสมศักดิ์ พรหมมหาราช อายุ 46ปี ลูกจ้างประจำภาควิชา โสต ศอ นาสิกวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่หนึ่งในผู้ที่ติดเชื้อแบคทีเรีย “สเตรปโตค็อกคัส ซูอิส” จนหูหนวกถาวร ทั้งสองข้าง เล่าว่า ปกติเป็นคนชอบกินลาบหมูดิบเป็นประจำ กระทั่งเมื่อวันที่ 22มกราคม 2548กินลาบหมูดิบ

กับเพื่อนตามปกติ หลังจากนั้นเพียง 3 วัน มีอาการไข้สูง เวียนศีรษะ อาเจียน จนลุกไม่ขึ้น ญาติต้องหามส่ง
โรงพยาบาล แพทย์ผู้ให้การรักษาระบุว่าติดเชื้อ “สเตปโตคอกคัส ซูอิส” ในร่างกาย หลังจากนั้นอีก 5 วัน ในหู
มีเสียงดังตลอดเวลา และตรวจพบว่าทั้งสองข้างรับเสียงเกิน 90 เดซิเบลซึ่งหมายถึงหูหนวกถาวร

“ 1 ปี 8 เดือน หลังจากหูหนวก การใช้ชีวิตเป็นไปด้วยความยากลำบาก ในช่วงแรกเดินแทบไม่ได้
เพราะการทรงตัวไม่ปกติ เดินเหมือนคนเมา ขี่รถจักรยานยนต์ไม่ได้ ต้องเดือดร้อนเพื่อนที่ทำงานและญาติคอย
รับส่ง และระยะหลังเริ่มปรับตัวขี่จักรยานยนต์ได้แต่เหลือหลังไม่ได้ จึงอยากฝากเตือน ผู้ที่กินลาบหมูดิบหรือ
เนื้อหมูดิบให้เลิกก่อนจะสายไปกว่านี้และหูหนวกเหมือนกับผม” นายสมศักดิ์ กล่าว

Source: Komchadluek Newspaper, <http://www.komchadluek.net>
http://www.komchadluek.net/2006/09/13/a001_47015.php?news_id=47015

Appendix 12 Public alert about the outbreak of *Streptococcus suis* infection through newspaper

หนังสือพิมพ์แนวหน้า ออนไลน์ วันที่ 23 ตุลาคม 2551

คอลัมน์ สังคมชุมชนทั่วประเทศ ประจำวันที่ 6 ตุลาคม 2550

เดือนมาด้วยความหวังดีจาก นพ. ไพบูลย์ ธนเกียรติสกุล ให้ชาวจังหวัดพะเยา เผลาการกินเนื้อดิบๆ
สุกๆ ที่จะทำให้เกิดโรคสเตรปโตคอคคัสซูอิส เนื่องจากในอำเภอภูซางมีคนตาย และป่วยจำนวนมาก

Source: Naewna Newspaper, <http://www.naewna.com>

ระบาดวิทยาของโรค

ทริคิโนซิสเป็นโรคสัตว์ติดคนที่พบได้ทั่วโลก ในประเทศไทยส่วนมากพบในภาคเหนือเกือบทุกจังหวัด ที่จังหวัดชุมพรเคยมีรายงานพบผู้ป่วยโรคนี้เนื่องจากการกินเนื้อเต่าบกแบบสุก ๆ ดิบๆ อุบัติการณ์แตกต่างกันออกไปตามพฤติกรรมการบริโภคและการปรุงอาหาร โดยเฉพาะอาหารจากเนื้อสุกรและสัตว์ป่าที่ปรุงไม่สุก หรือสุก ๆ ดิบ ๆ การเกิดโรคมักเกิดระหว่างเดือนมกราคมถึงมิถุนายน เนื่องจากเป็นช่วงเวลาที่ชาวบ้านมีกิจกรรมร่วมกัน เช่น งานปีใหม่ งานเลี้ยงหลังการเก็บเกี่ยว งานมงคลสมรส และงานขึ้นบ้านใหม่ ซึ่งในงานดังกล่าวจะมีการฆ่าและสุกรมาปรุงเป็นอาหารในงาน ผู้ป่วยส่วนใหญ่อยู่ในเขตชนบทและอยู่ในวัยทำงาน โดยกลุ่มอายุที่มักพบคือ 25-40 ปี เมื่อพิจารณาตามเพศพบว่าเพศชายมากกว่าเพศหญิงเนื่องจากผู้ชายนิยมกินลาบดิบ ลูดิบ แหนมดิบกับสุรา จากอุปนิสัยการบริโภคของคนไทยในภาคเหนือที่นิยมบริโภคอาหารสุกๆ ดิบๆ ประเภทลาบ ลูหรือแหนม จึงมีโอกาสเกิดโรคนี้ได้สูง ประกอบกับโรคทริคิโนซิสในสุกรมักจะไม่แสดงอาการ จึงทำให้ประชาชนทั่วไปที่ประสงค์จะซื้อสุกรมีชีวิต เพื่อนำมาฆ่าและประกอบอาหารเองนั้น จะไม่มีทางทราบเลยว่าสุกรเป็นโรคหรือไม่ ดังนั้นจึงควรซื้อสุกรจากแหล่งที่มีการเลี้ยงอย่างถูกต้องตามหลักสุขาภิบาล หรือจากฟาร์มที่มีชื่อเสียง ควบคู่กับการปรับเปลี่ยนสุขนิสัยในการรับประทานอาหาร ก็จะทำให้ลดรายป่วยด้วยโรคทริคิโนซิสในประเทศไทยลดลงได้

การรักษาในสัตว์เศรษฐกิจ

ไม่นิยมรักษา ถ้าพบว่าสัตว์เป็นโรคทริคิโนซิส ให้ทำลาย และห้ามนำเนื้อนั้นไปเป็นอาหารของคนหรือสัตว์อื่น

การรักษา

การรักษาโรคทริคิโนซิสในคนประกอบด้วยการทำลายพยาธิตัวแก่ และการทำลายพยาธิตัวอ่อนในลำไส้ และตัวอ่อนในกล้ามเนื้อเนื้อที่ยังไม่มีซิสต์หุ้ม และตัวอ่อนที่มีซิสต์หุ้มแล้ว

การทำลายพยาธิตัวแก่ อาจเลือกใช้ยาทำลายพยาธิ เช่น ให้รับประทาน

1. Pyrantel ให้ขนาด 10 มก./กก./วัน ติดต่อกัน 5 วัน ,
2. Levamisole ให้ขนาด 2.5 มก./กก./วัน รวมไม่เกิน 150 มก./วัน นาน 3 วัน
3. Thiabendazole ให้ขนาด 25 มก./กก./วัน นาน 2-5 วัน

การทำลายพยาธิตัวอ่อน

ใช้ยาในกลุ่ม Benzimidazole ซึ่งปัจจุบันไม่แนะนำให้ใช้ยา Thiabendazole เนื่องจากมีผลข้างเคียง เช่น คลื่นไส้ เวียนศีรษะ ผื่นแพ้ตามตัว และระคายเคืองกระเพาะลำไส้สำหรับผู้ป่วยอาการน้อยถึงปานกลาง ให้ Mebendazole 5 มก./กก./วัน รวมไม่เกิน 200 มก./วัน แบ่งให้เข้าเย็น นาน 5 วัน หรือให้ Albendazole 400 มก. นาน 5 วัน ผู้ป่วยอาการน้อยไม่ควรให้ Corticosteroids เพราะจะยืดเวลากำจัดพยาธิตัวแก่ในลำไส้ ทำให้ผลผลิตตัวอ่อนได้มากขึ้น กรณีนี้ให้ antipyretic และ analgesic ก็เพียงพอ ผู้ป่วยอาการปานกลาง ให้ Prednisolone ขนาด 20-40 มก./วัน

สำหรับผู้ป่วยอาการรุนแรง

กรณีมีไข้สูง มักมีอาการแพ้รุนแรง มีเม็ดเลือดขาวสูง ฮีโมโกลินฟิลสูง ควรให้ Mebendazole 5 มก./กก./วัน แบ่งให้เข้าเย็น หรือเพิ่มเป็น 3 เวลา รวมไม่เกิน 400 มก./วัน โดยอาจให้ได้นานถึง 2 สัปดาห์ ควรให้ Corticosteroids เพื่อเป็น antiinflammatory, antiallergic และ antishock โดยใช้ Prednisolone 40-60 มก./วัน จนกว่าไข้และอาการแพ้จะหายไป ในรายที่มีอาการรุนแรงมากต้องเพิ่มขนาดยาเพื่อรักษาชีวิต จำเป็นต้องให้ออกซิเจนและให้ intensive care โดยเฉพาะรายที่มีอาการแทรกซ้อน ในรายที่รุนแรงอาจให้ Prednisolone 40 มก. เข้าหลอดเลือดดำทุก 6 ชั่วโมงจนกว่าอาการดีขึ้นแล้วตามด้วยการให้กิน Prednisolone 40-60 มก. นาน 10 วัน

การรักษาในผู้ป่วยบางกลุ่ม

1. เด็ก ต้องให้การดูแลรักษาใกล้ชิด โดยเฉพาะระยะเฉียบพลัน เนื่องจากมักที่ปฏิกิริยาทาง vascular และ hypersensitivity สูง
2. ผู้ป่วยที่มีอาการแทรกซ้อน ต้องดูแลรักษาใกล้ชิด

3. สตรีมีครรภ์ อาการมักรุนแรงน้อยกว่าผู้ป่วยกลุ่มอื่น อาจเนื่องมาจากการเพิ่มระดับ steroid และ histamine ไม่ควรให้ corticosteroids และไม่ควรรักษาในกลุ่ม benzimidazole นอกจากมีเหตุผลจำเป็น โดยเฉพาะอย่างยิ่งในช่วงไตรมาสแรกของครรภ์ เนื่องจากอาจจะมีผลต่อทารกในครรภ์

การตรวจทางห้องปฏิบัติการ

ประเทศไทยมีเครือข่ายห้องปฏิบัติการที่มีความสามารถตรวจโรคนี้ทางซีรัมอิมมูโนวิทยา การตรวจวินิจฉัย ได้แก่

1. ศูนย์ความร่วมมือนานาชาติ โรคทริคิโนซิส คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
2. ศูนย์วิจัยและชันสูตรโรคสัตว์ภาคเหนือ อ.ห้างฉัตร จ.ลำปาง
3. คณะเวชศาสตร์เขตร้อน และคณะสาธารณสุข มหาวิทยาลัยมหิดล

การศึกษาทางห้องปฏิบัติการ โดยการเจาะเลือดในผู้ป่วยพบเม็ดเลือดขาวชนิดอีโอซิโนฟิลเพิ่มสูงขึ้น 10-90% และ/หรือ Creatinine Kinase ในซีรัมสูงกว่าปกติ (มากกว่า 30 U/L) รวมทั้งตรวจพบตัวอ่อนของพยาธิที่กล้ามเนื้อ นอกจากนั้นแล้วให้น้ำเนื้อสัตว์ดิบหรือซากสัตว์ที่สงสัยซึ่งยังเหลืออยู่จากการบริโภค โดยเฉพาะให้ตัดบริเวณกล้ามเนื้อกะบังลม หรือเนื้อติดซี่โครง ให้นำมาตรวจด้วยวิธี Compression and Digestion เพื่อตรวจหาพยาธิในกล้ามเนื้อ โดยประสานงานกับปศุสัตว์ในพื้นที่

รายละเอียดของการเก็บตัวอย่างและการส่งตรวจทางห้องปฏิบัติการมีดังต่อไปนี้

1. การตรวจชิ้นเนื้อ: ควรทำหลังกินอาหารที่สงสัยตั้งแต่ 10 วันถึง 4 สัปดาห์ เป็นวิธีตรวจหาตัวอ่อนที่ยังไม่มีแคลเซียมมาเกาะ ควรตัดชิ้นเนื้อของบริเวณที่ผู้ป่วยรู้สึกปวดมากที่สุด เพื่อเพิ่มโอกาสการพบตัวอ่อน มักทำที่กล้ามเนื้อ deltoid, bicep หรือ gastrocnemius
2. การตรวจเลือด: จะพบเม็ดเลือดขาวเพิ่มขึ้น และพบเม็ดเลือดขาวชนิดอีโอซิโนฟิลเพิ่มสูงขึ้น 10-90% นอกจากนั้นอาจพบเม็ดเลือดแดงเพิ่มด้วย และจะพบระดับเอนไซม์สูงขึ้น เช่น CPK (Creatinine Phosphokinase)
3. การตรวจทางซีรัมวิทยา: อาจทำได้หลังจากสัปดาห์ที่ 3 และควรตรวจซ้ำ 2 ครั้ง ทำได้โดยวิธีต่าง ๆ เช่น Indirect fluorescent antibody test, Bentonite flocculation test , Latex agglutination test และ ELISA (Enzyme-Linked Immunosorbent Assay)

การเก็บตัวอย่างจากสัตว์

การวินิจฉัยโรคในสัตว์โดยดูอาการทำนั้นทำได้ยาก เนื่องจากสัตว์ไม่แสดงอาการ ยกเว้นมีตัวอ่อนเป็นจำนวนมาก ดังนั้นการวินิจฉัยจึงต้องอาศัยการตรวจทางห้องปฏิบัติการเป็นหลัก ซึ่งมีวิธีการตรวจได้หลายวิธี

1. การตรวจชิ้นเนื้อ: เก็บตัวอย่างชิ้นเนื้อซึ่งยังสดอยู่จากกล้ามเนื้อกระบังลม กล้ามเนื้อระหว่างซี่โครง กล้ามเนื้อน่อง หลัง ลิ้น แก้ม ขนาดประมาณ 100 กรัม นำไปตรวจสอบด้วยวิธีซึ่งจะกล่าวต่อไป หรือแช่ใน Glycerine ส่งห้องปฏิบัติการ

วิธี Compression technique: เลาะพังผืดออกจากเนื้อที่จะตรวจให้หมด แล้วใช้มีดผ่าตัดเจียนเนื้อให้บางที่สุดตามความยาวของกล้ามเนื้อ หรือใช้กรรไกรปลายงอนตัวแฉลบให้ได้ขนาดของชิ้นเนื้อกว้างประมาณ 3 มม. ยาวประมาณ 10 มม. นำชิ้นเนื้อที่ตัดออกนี้ไปบีบให้แบนระหว่างแผ่นสไลด์หรือกระจกหนาสองแผ่น บีบปลายสองด้านให้แน่นด้วยนิ้วหรือยางรัด ตรวจหาพยาธิตัวอ่อนในชิ้นเนื้อด้วยกล้องจุลทรรศน์กำลังขยาย x 25 หรือ Trichinoscope ในการตรวจควรตรวจชิ้นเนื้ออย่างน้อย 5 ชิ้น และถ้าหยดน้ำยา Lactophenol ลงบนชิ้นเนื้อจะทำให้เห็นพยาธิได้ชัดเจนยิ่งขึ้น

วิธี Digestive technique: เลาะพังผืดต่าง ๆ ออกจากเนื้อเช่นเดียวกัน แล้วไปบดด้วยเครื่องบดเนื้อ ผสมกับน้ำยาล่อยเนื้อในอัตราส่วน 1: 10 โดยน้ำยาล่อยมีส่วนผสมดังนี้

1. Pepsin 35.0 กรัม
2. HCL (conc.) 30.0 มล.
3. น้ำ 3,000.0 มล.

ผสมเนื้อกับน้ำยาย่อย คนให้เข้ากัน ตั้งไว้อย่างน้อยครึ่งชั่วโมงแล้วจึงนำเข้าตู้อบที่ อุณหภูมิ 37°C นาน 18 ชั่วโมง ดูดเอาส่วนข้างบนออกให้เหลือประมาณ 1/5 แล้วเติมน้ำเปล่าลงไปจนมีปริมาตรเท่าของเดิม ตั้งทิ้งไว้ครึ่งชั่วโมงแล้วดูดน้ำส่วนข้างบนออก นำ ส่วนที่เหลือเทใส่ petri dish ตรวจหาพยาธิตัวอ่อนด้วยกล้องจุลทรรศน์

2. การตรวจทางซีรัมวิทยา

เจาะเลือดจำนวน 3-5 มล. แล้วแยกซีรัมส่งห้องปฏิบัติการเพื่อตรวจต่อไป

มาตรการควบคุมโรคเบื้องต้นและจำเพาะ

มาตรการในระยะระบาด: ต้องสอบสวนโรคทางระบาดวิทยาเพื่อหาชนิดของอาหาร และ สัตว์ที่เป็นสาเหตุให้ได้ และการให้สุขศึกษาเพื่อปรับเปลี่ยนพฤติกรรมกรรมการบริโภคอาหาร หรือการปฏิบัติผิด ๆ ที่พบในการเลี้ยงสัตว์ และการผลิตอาหารจากเนื้อสัตว์ และแจ้งข่าว การระบาดไปยังหน่วยงานที่เกี่ยวข้องทั้งทางสาธารณสุขและปศุสัตว์ ควบคุมการ เคลื่อนย้ายสัตว์ไปยังต่างท้องที่ หากพบพยาธิในซากสัตว์ หรือสัตว์มีชีวิตต้องรักษาหรือ ทำลาย

มาตรการควบคุมผู้ป่วย ผู้สัมผัส และสิ่งแวดล้อม:

1. การแยกผู้ป่วย : ไม่จำเป็น
2. การทำลายเชื้อ : ไม่จำเป็น
3. การกักกัน : ไม่จำเป็น
4. การให้ภูมิคุ้มกันแก่ผู้สัมผัส : ไม่มี

มาตรการป้องกัน

1. ให้สุขศึกษาแก่ประชาชน รวมทั้งผู้ล่าสัตว์ให้ทราบถึงความจำเป็นที่จะต้องปรุง อาหารโดยเฉพาะเนื้อสุกรขาเขา สุกรป่า สัตว์ป่า ให้สุกด้วยความร้อนอย่างน้อย 77 องศาเซลเซียส หรือจนกระทั่งเนื้อเปลี่ยนไปจากสีชมพูเป็นสีเทาทุกส่วน
2. ให้สุขศึกษาแก่ผู้เลี้ยงสุกรให้เลี้ยงสุกรในคอกที่สุขลักษณะ และเลี้ยงด้วยอาหาร สำเร็จรูป หรือหากจำเป็นต้องใช้เศษอาหารต้องทำให้สุกก่อน และควรกำจัดนุ บริเวณคอกด้วย
3. ออกกฎหมายให้มีมาตรการตรวจเนื้อ เพื่อควบคุมคุณภาพผลิตภัณฑ์อาหาร ประเภทเนื้อสุกร ซึ่งอาจใช้การตรวจหาตัวอ่อนในเนื้อสัตว์โดยวิธีการย่อย
4. สืบหาโรคในสัตว์เพื่อประเมินความชุกของโรค และการกระจายตามพื้นที่ โดยทำ การตรวจทางอิมมูโนวิทยาด้วยวิธี ELISA ในสัตว์มีชีวิต เนื่องจากสัตว์ที่เป็นโรคนี้ มักไม่แสดงอาการ
5. ซากสัตว์ที่มีพยาธิตัวอ่อนทริคิเนลล่า ต้องทำลายตัวอ่อนก่อนอนุญาตให้จำหน่าย โดยการแช่แข็งขึ้นเนื้อหนาไม่เกิน 15 เซนติเมตร ที่อุณหภูมิ -15 องศาเซลเซียส นาน 30 วัน หรือที่ -25 องศาเซลเซียส นาน 10 วัน ขึ้นเนื้อที่หนาเกิน 15 เซนติเมตรต้องเก็บที่อุณหภูมิต่ำกว่านี้อย่างน้อย 20 วัน
6. แนะนำและตรวจสอบผลิตภัณฑ์อาหารสำเร็จรูปจากเนื้อสุกร เช่น แหนม เพื่อให้ ปลอดภัยจากพยาธิตัวอ่อน เช่น การฉายรังสีแกมมาเพื่อทำลายตัวอ่อน

เรียบเรียงโดย สัตว์แพทย์หญิงเสาวพักตร์ เหล่าศิริถาวร, สัตว์แพทย์ธีรศักดิ์ ชักนำ

Appendix 14 Fact sheet on *Streptococcus suis* infection published by Bureau of Epidemiology, Ministry of Public Health, Thailand

สำนักโรคระบาดวิทยา กรมควบคุมโรค กระทรวงสาธารณสุข

เชื้อก่อโรค

เกิดจากเชื้อแบคทีเรีย *Streptococcus suis* ในสกุล Streptococcus ในตระกูล Streptococcaeae ย้อมติดสีแกรม รูปกลม ถูกจัดอยู่ใน Lancefield กลุ่ม D, R หรือ S สามารถสร้างแคปซูลและสลายเม็ดเลือดแดง มีการจัดแบ่งเชื้อตามลักษณะของ Capsular Antigen เป็นซีโรไทป์ (Serotype) ต่าง ๆ ถึง 29 serotypes ปัจจัยที่มีผลต่อความรุนแรงในการก่อโรคของเชื้อแต่ละซีโรไทป์จะแตกต่างกัน โดยขึ้นอยู่กับสาร Muramidase-released protein (MRP) และ Extracellular protein (EP) ซึ่งพบว่าซีโรไทป์ที่มีความรุนแรงสูงในการก่อโรคในคนคือ serotype 2 และ serotype 1 ตามลำดับ

สัตว์รังโรค สุกกร

ระยะฟักตัวและอาการในสัตว์

โดยปกติสุกรที่มีการติดเชื้อมักไม่แสดงอาการ ส่วนใหญ่เชื้อจะอยู่บริเวณ ต่อมทอนซิล บริเวณเพดานปาก (palatine tonsil) และเยื่อเมือกในโพรงจมูก เมื่อสุกรอยู่ในภาวะเครียด เช่นเลี้ยงอย่างแออัดอยู่ สภาพอากาศเย็น และมีระบบการถ่ายเทอากาศไม่ดี ทำให้ร่างกายสัตว์อ่อนแอเชื้อจะฉวยโอกาสจนเป็นสาเหตุให้เกิดอาการสมองอักเสบ ซ้ออักเสบ แบบรุนแรง กล้ามเนื้อและเยื่อหุ้มหัวใจอักเสบ มีการติดเชื้อในกระแสโลหิตจนถึงขั้นเสียชีวิต กลุ่มสุกรที่เสี่ยงต่อการติดเชื้อเป็นสุกรหย่านม สุกรขุน โดยเฉพาะอย่างยิ่งสุกรที่อยู่ในช่วงอายุ 8-15 สัปดาห์

วิธีการติดต่อระหว่างสัตว์สู่คน

คนสามารถติดเชื้อจากการสัมผัสกับสุกรที่ติดโรค โดยเฉพาะอย่างยิ่งเกษตรกรผู้เลี้ยงสุกร คนทำงานในโรงฆ่าสัตว์ คนชำแหละเนื้อสุกร สัตวบาล และสัตวแพทย์ เชื้อจะเข้าสู่ร่างกายผ่านทางบาดแผล รอยถลอก หรือเยื่อบุตา รวมทั้งการบริโภคเนื้อสุกรที่ไม่ผ่านการปรุงสุกก็ทำให้เกิดการติดเชื้อนี้ได้

ระยะฟักตัวและอาการในคน

ระยะฟักตัวของโรคประมาณ 1-3 วัน อาการที่พบได้แก่ มีไข้ คลื่นเหียน ปวดศีรษะ เยื่อหุ้มสมองอักเสบ ซ้ออักเสบ ม่านตาอักเสบ ผู้ป่วยอาจสูญเสียการได้ยิน จนถึงขั้นหูหนวก ภายหลังจากหายจากอาการป่วยแล้วอาจมีความผิดปกติในการทรงตัว ในรายที่มีอาการรุนแรงอาจถึงขั้นเสียชีวิตเนื่องมาจากการติดเชื้อในกระแสโลหิต

การป้องกันและควบคุมโรค

ในสุกร เนื่องจากเชื้อนี้มีสุกรเป็นสัตว์พาหะนำโรคซึ่งมักไม่แสดงอาการป่วย ดังนั้นการเลี้ยงดูสุกรให้อยู่ในสภาวะสุขาภิบาลที่ดี เช่นไม่เลี้ยงให้อยู่กันอย่างแออัด อากาศในโรงเรือนถ่ายเทได้ดี สามารถป้องกันความหนาวเย็นขณะที่มีอากาศเปลี่ยนแปลงกะทันหันได้ สุกรจะมีร่างกายแข็งแรง เชื้อ *Streptococcus suis* ที่มีอยู่ในช่องปากและโพรงจมูกก็ไม่สามารถเพิ่มจำนวนและฉวยโอกาสก่อให้เกิดโรคในสุกรได้

ในคน หลีกเลี่ยงการบริโภคเนื้อสุกรและเลือดสุกรดิบ หรือสุก ๆ ดิบ ๆ

เรียบเรียงโดย นายสัตวแพทย์ธีรศักดิ์ ชักนำ

7 พฤษภาคม 2552

Appendix 15 Field work for focus group interview activities in communities in Nan Province

