PREVALENCE OF PORCINE CYSTICERCOSIS AND TRICHINELLOSIS IN SLAUGHTER PIGS OF CAMBODIA

THAN SOVYRA

MASTER OF SCIENCE

IN VETERINARY PUBLIC HEALTH

CHIANG MAI UNIVERSITY AND FREIE UNIVERSITÄT BERLIN

SEPTEMBER 2005

ISBN 974-9887-78-6

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A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY AND FREIE UNIVERSITÄT BERLIN IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH

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23 September 2005

Thesis title Prevalence of Porcine Cysticercosis and Trichinellosis in Slaughter Pigs of Cambodia

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ABSTRACT

Pig production and pork consumption have increased significantly in Cambodia during the last decade. Concurrent with the increases in smallholder pig keeping and pork consumption, there have been problems with zoonotic parasitic diseases, especially porcine cysticercosis, trichinellosis and others in some rural districts of Cambodia. Porcine cysticercosis caused by the human tapeworms *Taenia solium* and *T. asiatica* as well as trichinellosis caused by different *Trichinella* species and were ones of the most serious parasitic zoonoses leading additionally to a high economic loss. The objectives of this study were to determine prevalence and identify predilection sites of *T. solium* cysticercosis in pigs as well as to determine additional occurrence of *T. asiatica* cysticercosis (*viscerotropica*), determine sero-prevalence of trichinellosis using larval E/S antigen ELISA and retrospectively collect data on the occurrence of neurocysticercosis in humans in selected hospitals in Phnom Penh/ Cambodia. The study involved a slaughterhouse survey of 432 examined pigs for porcine cysticercosis and 440 pig serum samples for trichinellosis at four slaughterhouses in Phnom Penh, where pigs were delivered to slaughterhouses from 29 districts and 3 intensive farms in nine provinces. Lingual examination of live pigs and meat inspection of their carcasses used were as parameters to measure infection. Out of the 432 pigs examined at the slaughterhouses, 6.71 % (29/432), 95% CI (4.54-9.48) and 10.87 % (47/432), 95% CI (8.10-17.2) were found positive by lingual

examination and meat inspection, respectively. Moreover, out of 440 pigs examined at laboratory investigation for trichinellosis, 5 specimens [1.13%, 95% CI (0.36-2.63)] were sero-positive by AB-ELISA and 5 sera (1.13%) were suspected (doubtful). The questionnaire survey revealed poor pig husbandry practices, absence or missing of meat inspection and disease control, poor knowledge about diseases and poor sanitation in some rural districts of Cambodia. In addition, the degree of infection to harbour cysticerci by organs and muscles was determined in 47 positive pig carcasses and was categorized two types: low (Less 100 cysts per organ) and high (More than 100 cysts per organ). As a result, it was found that the contaminated organs of low the degree of infection ranged from 8.9% to 100% and these organs of high the degree of infection estimated at 24.3-91.1 %. Cysticerci were not detected in livers of infected pigs. Samples of cysticerci taken from other infected organs or muscles were identified microscopically as *T. solium* cysticerci. Retrospectively survey data on human neurocysticercosis in hospitals around Phnom Penh of Cambodia revealed that cases were not recorded due to lack of knowledge and diagnostic facilities.

It can be concluded that the study found porcine cysticercosis and trichinellosis were endemic in some rural districts of Cambodia. These findings have contributed to a better understanding of the epidemiology of these two diseases.

ชื่อเรื่องวิทยานิพนธ์	ความชุกของซิสติเซอร์โคซิสและทริคิเนลโลซิสในสุกรชำแหละในประเทศกัมพูชา
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บทคัดย่อ

การผลิตสุกรและการบริโภคเนื้อสุกรได้เพิ่มขึ้นอย่างสำคัญในกัมพูชาในทศวรรษที่ผ่านมา พร้อมกับการเพิ่มของผู้เลี้ยงสุกรรายย่อยและการบริโภคเนื้อสุกรก็มีปัญหาโรคปรสิตจากสัตว์สู่คน โดยเฉพาะซิสติเซอร์โคซิส ทริคิโนซิสในสุกรและอื่น ๆ ในอำเภอแถบชนบทในกัมพูชา ซิสติเซอร์โคซิสในสุกรเกิดจากพยาธิตัวตืดของคน *Taenia solium* และ *T. asiatica* เช่นเดียวกับทริคิโนซิสซึ่งมีสาเหตุจาก *Trichinella* ชนิดต่าง ๆ นับว่าเป็นโรคปรสิตจากสัตว์สู่คนที่ร้ายแรงนำไปสู่ความสูญเสียทางเศรษฐกิจ การศึกษานี้มีวัตถุประสงค์ที่จะหาความชุกและชี้ส่วนชอบอยู่อาศัยของซิสติเซอร์โคซิสสาเหตุจาก *T. solium* ในสุกรพร้อมกับหาความชุกของซิสติเซอร์โคซิสสาเหตุจาก *T. asiatica* (*viscerotropica*) หาความชุกของทริคิเนลโลซิสโดยใช้ E/S antigen ELISA และรวบรวมข้อมูลย้อนหลังเกี่ยวกับการเกิดซิสติเซอร์โคซิสของระบบประสาทในคนจากโรงพยาบาลบางแห่งในพนมเปญ กัมพูชา การศึกษาเกี่ยวกับการสำรวจสุกร 432 ตัวในโรงฆ่าสัตว์หาซิสติเซอร์โคซิส และตรวจตัวอย่างเซรุ่มจากสุกร 440 ตัว หาทริคิเนลโลซิสในโรงฆ่าสัตว์ 4 แห่งในพนมเปญซึ่งสุกรเหล่านี้ส่งมาจาก 29 อำเภอ และ ฟาร์มสุกรใหญ่ 3 แห่งใน 9 จังหวัด ตรวจวัดการติดเชื้อโดยใช้การตรวจลิ้นของสุกรมีชีวิต และการตรวจซากสุกรเป็นตัวแปรเสริมจากการตรวจสุกร 432 ตัวที่โรงฆ่าสัตว์ พบผลบวกร้อยละ 6.71 (29 จาก 432), 95% CI (4.54-9.48) และ ร้อยละ 10.87 (47 จาก 432), 95% CI (8.10-17.2) โดยการตรวจลิ้นและซากสุกรตามลำดับ เมื่อแยกสุกรตามต้นตอ พบว่าจากซากสุกร 220 ตัวที่เป็นพันธุ์พื้นเมืองเลี้ยงแบบปล่อยหรือผสม พบ 29 ตัว (ร้อยละ 13.2) ให้ผลบวก จากการตรวจลิ้น และ47 ตัว (ร้อยละ 21) ให้ผลบวกจากการตรวจเนื้อ ในขณะที่สุกรฟาร์มทั้ง

212 ตัว ให้ผลลบ นอกจากนั้น จากการตรวจสุกร 440 ตัวในห้องปฏิบัติการหาทริคิเนลโลซิส พบผลเซรุ่มเป็นบวกโดย AB-ELISA จำนวน 5 ตัวอย่าง [ร้อยละ 1.13, 95% CI (0.36-2.63)] และ ให้ผลน่าสงสัย 5 ตัวอย่าง (ร้อยละ 1.13) การสำรวจโดยใช้แบบสอบถามเผยให้เห็นถึงการเลี้ยงสุกรที่ไม่ค่อยดี ขาดการตรวจสอบเนื้อและการควบคุมโรค ความรู้เกี่ยวกับโรคยังน้อยและการสุขาภิบาลไม่ดีในบางอำเภอชนบทของกัมพูชา นอกจากนั้น เพื่อหาส่วนชอบอาศัยของซิสติเซอร์คัส อวัยวะต่อไปนี้พบว่าติดเชื้อในระดับมากกว่าและน้อยกว่าลดลงตามลำดับ: กล้ามเนื้อโครงร่าง เช่น ขาหลัง ขาหน้า กล้ามกราม หัวใจ ลิ้น หลอดอาหาร กะบังลม และพบน้อยในสมอง อวัยวะที่ตรวจไม่พบซิสติเซอร์คัส ได้แก่ ตับ ปอด ม้าม ไต และสำไส้ การบ่งชี้ทางกายรูปของซิสต์ทั้งหมดพบว่าเป็นซิสติเซอร์คัสของ *T. solium* จากตะขอของซิสต์ ข้อมูลจากการสำรวจย้อนหลังเกี่ยวกับซิสติเซอร์โคซิสของระบบประสาทในโรงพยาบาลรอบพนมเปญของกัมพูชาพบว่าไม่มีการบันทึกไว้เนื่องจากขาดความรู้และอุปกรณ์วินิจฉัย

จากการศึกษานี้สรุปได้ว่าพบทั้งซิสติเซอร์โคซิสและทริคิเนลโลซิสในสุกรเกิดประจำถิ่นในบางอำเภอของกัมพูชา ผลการศึกษาทำให้เกิดความเข้าในในวิทยาการระบาดของสองโรคนี้

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AB-ELISA = Antibody enzyme linked immunosorbent assay
ABTS = 2,2'azino-di-(3 ethylbenzthiazoline sulfonic acid)
Ag-ELISA = Antigen-capture enzyme linked immunosorbent assay
BfR = Federal Institute for Risk Assessment Berlin, Germany
CNS = Central nervous system
CMU = Chiang Mai University
CAC = Codex Alimentarius Commission
CFT = Complement fixation test
CI = Confidence intervals
CT = Computerized Tomography Scan
^{o}C = Degree Celsius
DAAD = Deutscher Akademischer Austausch-Dienst (German Academic Exchange Services)
DAHP = Department of Animal Health and Production
DNA = Deoxyribonucleic Acid
EEC/ EC = European Communities
EITB = Enzyme Linked Immunoelectrotransfer Blot
E/S antigen = Excretory/ secretory antigen
Format. = Formation
FAO = Food and Agriculture Organization of United Nations
FUB = Freie Universität Berlin
^{o}F = Degree Fahrenheit
GDP = Gross Domestic Products
HCl = Hydrochloric acid
HAT = Haemogglutination test
KW = Kruskal-Wallis
H = High or Heavy

HE = Hematoxylin-Eosin
ID = Identification
IFAT = Immunofluorescence antibody test
Insp Code = Inspection code
KCl = Potassium chloride
KH_2PO_4 = Potassium dihydrogen phosphate
Kg = Kilogram
LPG = Larvae per gram
L = Low or light
MRI = Magnetic Resonance Imaging
MNE = Mean netto extinction
MAFF = Ministry of Agriculture, Forestry and Fisheries
MS Excel = Microsoft Excel
mg = milligram
ml = milliliter
µl = microliter
mm = millimeter
µm = micrometer
nm = nanometer
NAHPIC = National Animal Health and Production Investigation Centre
NCC = Neurocysticercosis
NF = National Formulary (United States)
NGOs = Non-Government Organizations
NIS = National Institute of Statistics of Cambodia
N = Negative
NE = Netto extinction
Na_2HPO_4 = Disodium hydrogen phosphate
NaCl = Sodium chloride
No. = Number

OIE	= Office International des Epizooties (World Organization for Animal Health)
OD	= Optical density
PCR	= Polymerase Chain Reaction
PCR-REA	= Polymerase Chain Reaction Restriction Enzyme Analysis
PBS	= Phosphate buffered saline
PBS-T	= Phosphate buffered saline, containing 0.05% Tween 20
P, P- value	= Probability value
Preval.	= Prevalence
QAS	= Quality assurance system
RCP	= Recommended International Code for ante and post mortem of slaughter animals
R., Rostel.	= Rostellum
Rudiment.	= Rudimentary
Sl.	= Slaughterhouse
T.	= *Taenia*
USA	= United States of American
UVMA	= University of Veterinary Medicine, Austria
VPH	= Veterinary Public Health
WHO	= World Health Organization
WBA	= Western blot analysis
χ^2	= Chi- square
+/-	= Questionable (doubtful)
+++	= Positive

1. INTRODUCTION AND OBJECTIVES

1.1 General overview

The Kingdom of Cambodia, one of developing countries in South-East Asia, occupies a territory of some 181.035 square kilometers (km^2) and is home to a rapidly growing (growth rate: 3.8%) population in excess of more 13 million (NIS Census, 1998, FAO, 2003). It shares its frontier with Thailand, Laos and Vietnam, with the Gulf of Thailand serving the other boundary. The country is divided administratively into 24 provinces including Phnom Penh municipality. It is a country that is highly varied in economic development, living condition (standards) and social benefits. Moreover, Cambodia is poorest and least developed in agricultural industries, especially in livestock production and veterinary services (FAO, 1999).

Cambodia's climate- like that of the rest of Southeast Asia is dominated by the monsoons, which are known as tropical wet and dry because of the distinctly marked seasonal differences. This climate is governed by two monsoons: the cool, dry northeastern monsoon from November to March, and the humid southwestern monsoon from May to October. The monsoon airflows are caused by annually alternating high pressure and low pressure over the Central Asian landmass. In summer, moisture-laden air, the southwest monsoon, is drawn landward from the Indian Ocean. The flow is reversed during the winter, and the northeast monsoon sends back dry air. Temperatures are fairly uniform throughout the Tonle Sap Basin area, with only small variations from the average annual mean of 25°C. The maximum mean is about 28°C while the minimum mean is about 22°C. Maximum temperatures of higher than 32°C, however, are common and, just before the start of the rainy season, they may rise to more than 38°C. Minimum temperatures rarely fall below 10°C. January is the coldest month, and often reaching 40 ^{o}C, April is the hottest month (FAO, 1999).

The total annual rainfall average is between 100 and 150 centimeters, and the heaviest amounts fall in the southeast. The relative humidity is high at night throughout the year; usually it exceeds 90 percent. During the daytime in the dry season, humidity averages about 50 percent or slightly lower, but it may remain about 60 percent in the rainy period (FAO, 1999; FAO 2003).

1.2 Livestock production and pork demands in Cambodia

Cambodia is characterized by its agricultural domain, which employs over 80 % of the population. Around 46.4 % of the agricultural Gross Domestic Products (GDP) and 14.4 % of the total GDP are estimated to be from livestock production. But its role in national economy is far above the figures suggested (Census 2001, MAFF).

In reference to the Department of Animal Health and Livestock Production Census 2002, it is estimated that the country has 2.9 million cattle, 0.6 million buffalo, and 2.1 million pigs (Census of DAHP, 2002). The animal production is only plays an important role for supplying domestic demands. The annual growth rate per species: cattle 2.4 %, pig 2.5 % and poultry 5.6 % (Census, MAFF, 2000; FAO, 2003).

Over 80 % of Cambodian farms are in rural areas and raise pigs, cattle, buffalo and poultry for draught power, food consumption and generating income (Census, MAFF, 2000).

The annual production of meat and percentage of growth rate are estimated approximately: beef 69,900 metric tons (6.1%), pork 105,000 tons (5.7%), poultry 20,400 tons (1.8%) and milk 25100 tons (4.4%). Moreover, annual consumption and percentage of growth rate are: beef 60,100 tons (4.8%), pork 102,500 tons (4.5), poultry 24,700 tons (3.9%) and milk 47,200 tons (10.6%), (FAO, 2003).

Livestock production in Cambodia is mostly marginal due to free ranging conditions, feed shortages for the greater part of the year, widespread occurrence of

animal diseases, low educated people, poor genetic potential, hygienic and husbandry practices as well as underdeveloped infrastructure and marketing systems. In addition, slaughter slabs and slaughterhouses in Cambodia have not been “standardized” due to a lack of hygienic concerns, missing or improper disease control measures, inspection and prevention, and underdevelopment (Report of MAFF, 2003).

Among the many prevalent animal diseases, parasitism represents major problems. Of the various parasitic diseases, porcine cysticercosis and trichinellosis are considered to be two of the most important zoonosis (Flisser *et al*., 2004; La Rosa *et al*., 1998).

1.3 Porcine cysticercosis and trichinellosis

Porcine cysticercosis caused by the human tapeworms, *Taenia solium* and *T. asiatica* as well as trichinellosis which is caused by nematode in the genus *Trichinella* were two of the most serious parasitic zoonosis leading to a high economic loss. These two helminthic zoonoses are widespread over the world, occurring mainly in rural areas of underdeveloped countries where the hygienic conditions and health education are low. The prevalence is fostered when pigs are reared traditionally and have access to free running areas outside (Dorny *et al*., 2004; Garcia *et al*., 2005). Porcine cysticercosis and human taeniosis are mainly known in regions where pork is consumed predominantly. Main focuses are in Central America, Africa and Asia. In the Indochina region many investigations on this subject have been done in the recent past, whereas in Cambodia no detailed data concerning porcine cysticercosis and trichinellosis and their zoonotic risk have been published in recent times. Consequently, the epidemiological and veterinary public health aspects of the diseases are poorly worked out in most countries of Asia such as Cambodia. Data on the prevalence of porcine cysticercosis, *Taenia asiatica* and trichinellosis in most countries of Asia are not available or are of questionable reliability. Moreover, reliably estimated prevalence of *Taenia solium* cysticercosis by overview is 10% in Cambodia, 8% in Vietnam, 11% in Thailand and 10% in Laos (Singh *et al.*, 2002). In

China, the sero-prevalence of trichinellosis in humans by AB-ELISA has been found to be approximately 4.01% and sero-prevalence in pigs is around 7.3% (Liu *et al.*, 2002).

Therefore, the main goals were to identify recent prevalence of porcine cysticercosis and trichinellosis in pigs in different areas of Cambodia using several methods such as tongue palpation, visual meat inspection, as well as *Trichinella* larval E/S antigen ELISA test.

1.4 Objectives of the study

As a basis of assessing these problems, basic survey data is required. Such data are necessary to carry out for the design and possible recommendation of control measures and prevention. Therefore the important objectives of the present study are to:

1. Determine prevalence and identify predilection sites of *T. solium* cysticercosis in pigs by *ante* and *post mortem* examination as well as determine additional occurrence of *T. asiatica* cysticercosis (*viscerotropica*).

2. Determine sero-prevalence of trichinellosis using *Trichinella* larval ES antigens AB- ELISA.

3. Retrospectively collect data on the occurrence of neurocysticercosis in humans in selected hospitals in Phnom Penh / Cambodia.

2. LITERATURE REVIEW

2.1 *Taenia solium* complex

2.1.1 General overview

Porcine cysticercosis is a parasitic zoonotic disease in pigs caused by the larval form of the tapeworm, *Taenia solium* that is transmitted between pigs and humans as well as among human being (Ngowi *et al.*, 2004).

Man is the only natural definitive host while pigs are the natural intermediate hosts. When humans eat the larval stage in raw or inadequately cooked infected pork, an adult tapeworm develops in the small intestine resulting into a condition known as taeniosis. Gravid proglottids or worm eggs are released and come out with feces from tapeworm carriers and may contaminate environments in case of uncontrolled fecal disposal. When the pig ingests infective eggs, the oncospheres hatch and develop into the larval stage (*Cysticercus cellulosae*) in muscles and organs, completing the life cycle of the infection stages for humans, resulting in a condition known as human cysticercosis (Soulsby, 1982).

The tapeworm eggs are also infective if ingested by humans as in pigs; they develop into cysts, which are found in the muscles and in nervous tissue. The cysticerci may lodge in the brain causing cerebral cysticercosis (neurocysticercosis), a very serious zoonosis-causing headache, epileptic seizures, epilepsy, mental disturbance and death (Garcia *et al*., 1997; White, 2000). Neurocysticercosis is recognized as a serious zoonosis of public health concern because it causes disability of the infected persons and may be fatal if left untreated (WHO, 1979). Studies from countries where neurocysticercosis is endemic have shown that up to 50% of all cases of adult-onset epilepsy cases globally are due to neurocysticercosis and that the prevalence is rising (Tsang and Wilson, 1995). In addition, porcine cysticercosis represents one of the most important constraints to increased pig production in the

developing world, especially affecting the economic and nutritional well being of the rural poor and veterinary public health importance. *T. solium* causes great economic losses in the pig industry due to condemnation of infected carcasses. A very conservative and rough economic estimate indicates that the annual losses due to porcine cysticercosis in developing countries are millions of Euros per year (Zoli *et al.*, 2003).

The transmission of *T. solium* is associated mostly with environments with low socio-economic or low sanitary hygienic concerns, deficient sanitary facilities, poor meat inspection and control or poor pig husbandry practices where pigs can gain access to human feces (Phiri *et al.*, 2002).

Infection with *Taenia solium* is widely prevalent in humans and swine hosts and most common in many developing countries of Latin America, Africa, and Asia (Plancarte *et al.*, 1999). However, with tourism and increasing migration of people harboring tapeworms, human cysticercosis is now transmitted worldwide and it is considered as an emerging disease in the United States of America (Flisser *et al.*, 1998; Schantz *et al*., 1998). Human cysticercosis has been reported in non-pork consuming communities including an Islamic community and an Orthodox Jewish community (Schantz *et al*., 1992). In India, more than 95% of patients with neurocysticercosis are vegetarians or do not consume pork (Rajshekhar *et al*., 2003).

Small-scale producers in rural areas raise most pigs on free ranges. Organizations, private companies and schools within an intensive system, however, raise a small fraction. Land scarcities, rapid turnover of the pigs, and increased pork consumption in some urban areas of the country have contributed to the increase in the rural pig production. Most of the pigs in small-scale farming are allowed to scavenge, looking for their foods especially when there are no crops in the field. The majority of these pigs only spend the night in poorly constructed houses. This practice predisposes pigs to malnutrition, intake of human feces, and various infections.

2.1.2 Morphology

An adult cestode always lives as a single in the middle of the small intestine. The adult tapeworm grows up to 5 meters in length. The head or scolex is armed with four suckers and rostellar hooks, an elongated neck and strobila. The strobila is segmented in proglottids containing male and female reproductive organs. The immature, mature and gravid proglottids differ from each other in size, shape and stage of development. The mature proglottids are almost rectangular up to 12 mm in length, located towards the very distal end of the strobila and each is packed with an uterus full of eggs (Singh *et al.*, 2002). The gravid segments have 7-13 lateral uterus branches and they do not usually leave the host spontaneously, but leave passively in chains with the feces, *i.e.*, gravid proglottids detach from the strobila by apolysis. The ovary has three lobes, there is no vaginal sphincter muscle and the cirrus sac extends to the excretory vessels (OIE, 2004). *Taeniid* eggs are round or sub-spherical in form, measure approximately 30-45 µm in diameter and are characterized by a thick brownish shell containing an oncoshere 30x20 µm in size, bearing 3 pairs of hooklets. The adult tapeworm can shed up to 3,000,000 eggs daily. Each gravid proglottid of the tapeworm has approximately 40,000 eggs (De-Bittencourt *et al.*, 1996; Hoberg, 2002)

2.1.3 Life Cycle

The life cycle of *Taenia solium* includes different developmental stages:

(a) Pre-adult: a stage after ingesting the cysticercus, and growing up to mature (pre-patent period)

(b) Adult: the reproductive stage with mature proglotids (patent period).

(c) Egg: a small embryo covered by an embryophore and a thick shell, a stage responsible for dissemination to the external environment surviving up to one year.

(d) Oncosphere: a hexacanth embryo, which migrates from the intestine to internal tissues or organs within the intermediate host.

(e) Post-oncosperal form: the migrating intermediate stage between an oncosphere in the tissues and fully developed cysticercus.

(f) Cysticercus: a bladder metacestode form with one invaginated scolex that parasitizes tissues of the intermediate host, mainly pigs and humans (Singh *et al*., 2002).

T. solium requires two hosts to complete its life cycle.

Development in pig: A human infected with an adult tapeworm excretes eggs or gravide proglottids into the environment through feces, and the eggs can survive outside for several months up to one year. Pigs become infected by ingesting the eggs or proglottids via contaminated food or water. The oncospheres hatch in the small intestine under influence of gastric and intestinal juices, penetrate the intestinal wall into the blood, venous vessels or lymph system, and then are distributed by circulation to the muscle via liver, lung, heart and CNS where they grow up to cysticerci. Cysts have different sizes depending on the period of infection, for example 20 days-pinhead, 60 days-pea with the head visible and up to 2-3 months the cysts containing a translucent bladder with the invaginated scolex, surrounded by a capsule of host connective tissue (Joseph *et al*., 1999).

Development in humans: Humans can become infected with the adult tapeworm by eating raw or uncooked pork containing cysticerci or infected with the larval tapeworm by accidentally ingesting *T. solium* eggs from the environment or contaminated food and water, which develop into a single adult worm inhabiting in the small intestine. After ingestion of cysticercus, the scolex evaginates and attaches to the mucosa of the small intestine. Proglottids develop from the base of the neck. The mature proglottids are 1 cm wide, 1.2 cm long, and 2–3 mm thick. Eggs and/ or proglottids are shed intermittently in the stool. Tapeworm carriers usually note few symptoms other than observing proglottids passed with stool. Excretion is intermittent, thus stool examinations for ova can be negative. Human cysticercosis, however, results from ingestion of ova shed by a human tapeworm carrier. Close personal contact with or perhaps food preparation by a tapeworm carrier is noted in

most cases. Autoinfection may also occur. In the intestines the larvae hatch from *Taenia* eggs, penetrate the intestinal mucosa, enter the blood stream, migrate to the tissues, and develop into cysticerci in muscles and also in brain (White, 2000).

Cysticercus can develop in muscles and in the brain. In the latter organ, different types of cysticerci exist in CNS called neurocysticercos: sterile (unfertile), fertile, or cluster-shaped cysts, which are called Cysticercus racemosus. Sometimes, they reach infectivity also in other organs such as the eyes and subcutaneous tissues (Botero *et al.*, 1998).

2.1.4 Pathogenicity and clinic

(a) Symptoms in pigs with cysticercosis: Infected pigs are usually asymptomatic except in heavy infection pigs may have muscular stiffness and possible loss of condition.

(b) Symptoms in humans with cysticercosis: Symptoms may appear months to years after infection, usually when the cysts are in the process of dying and calcifying. Symptoms will depend on the location and number of cysticercus at various sites of body.

Cysticerci in muscles: Humans can be affected directly by the eggs of *T. solium* from environment or autoinfection by tapeworm carriers growing to pea like cysts in different muscles or subcutaneous tissues, called muscle cysticercosis. Humans with cysticercus in muscles do not have symptoms of infection.

Cysticerci in the eyes: Eye infection can cause blurry or distributed vision in the cyst stage. Infection may also cause swelling or detachment of the retina (Cardenas *et al.*, 1992).

Cysticerci in the brain and spinal cord: Neurocysticercosis is a severe disease. Symptoms depend upon where and how many cysticerci are found in the brain.

Seizures and headaches are the most common symptoms. When these happen, the brain can swell. The pressure caused by swelling is what causes most of the symptoms of neurocysticercosis. However, confusion, lack of attention to people and surroundings, difficulty with balance, swelling of the brain (called hydrocephalus) may also occur. Death can occur suddenly in the case of epilepsy-like symptoms in heavy infections (white, 2000). Inactive: is located in parenchymal calcification enhancement and chronic hydrocephalus. A typical symptom is seizures and symptoms of increased intracranial pressure (White, 2000). Active: is located in parenchymal, ventricular, subarachnoid (cisternal), ocular, spinal subarachnoid radiculopathy or myelopathy and spinal intramedullary. Typical symptoms are seizures, hydrocephalus, stroke, visual changes and myelopathy (White, 2000).

(c) Adult tapeworm infection in man: The first symptom is itching around the anus due to the migrating proglottids. Other clinical signs include abdominal pain, digestive disturbances, diarrhoea, constipation, nervousness, nausea and vomiting as well as loss of weight (Garcia *et al.*, 1999).

2.1.5 Epidemiology

Porcine cysticercosis is worldwide in distribution. *Taenia* eggs are very highly resistant and can long live in the environment (Schantz, 2002). The main source of infection of pigs is pollution of the environment by sewage and uncontrolled distribution of human feces. Free-range pigs with the disposal of infected human feces particularly in rural and suburban areas, the poor hygiene, low education and the lack of or improperly conducted meat inspection in urban areas lead to a high prevalence (Martin *et al.*, 1987; Singh *et al.*, 2002).

Autoinfection in humans: Cysticercosis in man may also be acquired by direct transfer of *T.solium* eggs from the feces of individuals harboring an adult worm through internal and external autoinfection. External autoinfection implies fecal-oral infection with *T. solium* eggs in an individual with intestinal taeniosis. The reason is neglect of hygienic standards such as washing hands after defecation and before

consuming meals. Internal autoinfection implies infection with eggs through reverse peristalsis and appears improbable since eggs are required to pass through a brief period of peptic digestion that is necessary for disintegration of the embryophores before being invasive to human tissues. Autoinfection is also caused vomiting and swallowing proglottids of *Taenia solium* carriers (Garcia-Noval *et al*., 1996).

Taenia solium cysticercosis has long been recognized as highly endemic in Latin America. However, in the past few years more data have become available from Asia and Africa, which reveal that the prevalence of *T. solium* in these continents is as high as or higher than those in Latin America (Geerts *et al*., 2002; Ito *et al*., 2002; Singh *et al*., 2002)

Transmission may occur in the highly endemic rural areas in Asia, where pigs usually get infected by food or roaming in areas contaminated by human feces (which can come from sewage water or direct pollution). It has been stressed that *T. solium* cysticercosis in Asia manifests as both neurocysticercosis and subcutaneous cysticercosis. In Japan and South Korea as well as Central Europe, where hygiene is good, *T. solium* has been eradicated (Pawlowski, 2002; Gilman *et al*., 1999).

Occurrence of neurocysticercosis*:* Neurocysticercosis is highly prevalent in developing countries, where it constitutes a serious public health problem. Millions of people are affected by *Taenia solium*/cysticercosis in Latin America, Asia and Africa, where the disease is a factor in the relatively high prevalence rate of epilepsy (Preux *et al*., 1996). Neurocysticercosis is commonly found in India, China, Central and South America and Mexico as well as Southeast Asia countries. Local people live with pigs, which scavenge and are therefore infected with cysticerci of *T. solium* (Gemmell *et al*., 1983; De-Aluja *et al*., 1998). Eating dog meat is still not rare in Asia, especially in Korea, China and Vietnam, some parts of Indonesia, including North Sumatra, Java, Sulawesi, and Papua. Therefore the life cycle of *T. solium* could be maintained through the dog-human cycle as well as the pig-human cycle because exceptionally, *cysticercus cellulosae* can develop in dogs and cats (Gonzalez *et al*., 1994). The situation with *T. solium* taeniosis/cysticercosis in other islands in

neighbouring countries, including East Timor and Papua New Guinea, needs to be studied (Geerts *et al*., 2002).

2.1.6 Diagnosis

Diagnostic methods of confirmation focus on:

Human taeniosis: The specimens usually required for diagnosis are feces or bloods.

Adult tapeworm infection: Adult cestodes can be expelled from infected humans using an antihelminthic followed by a purgative and are identified on the basis of a single proglottid or chains of segments migrating through the anus. In addition, DNA probes and polymerase chain reactions (PCR) by using *Taenia* material in feces can differentiate human *Taenia* spp. Today, several methods have been used to identify *Taenia* eggs for the diagnosis of *Taenia solium*:

(a) Detection of eggs: Eggs or embryophores with a thick, striated, brownish shell can be detected using different methods. The simplest procedure is the flotation method with a saturated salt solution to detect the eggs in stool specimens or excreted proglottids (Allan *et al.,* 1996; Rodriguez-Canul *et al*., 1999). *Taenia solium* eggs, however, can not be distinguished from the eggs of other *Taenia* species, *i.e., T. saginata* and *T. asiatica*.

(b) Identification of proglottids: Worms can be obtained from egg-positive humans by giving anthelminthic drugs followed by a purgative. Alternatively, proglottids sometimes appear in feces. Species identification is done by injecting India ink into the uterus of gravid proglottids and counting the number of lateral uterine branches. For mature proglottids, carmine stain reveals the number of ovarian lobes (Morakote *et al.,* 2000).

(c) Copro-antigen detection: Adult *Taenia* infections in humans can be recognized by detection of *Taenia* antigen in human feces using antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA), but the test does not differentiate species (OIE, 2004). This method greatly improved convenience and sensitivity in comparison with the detection of eggs in stool specimens. On the other hand, this method is very costly and is not commercially available.

(d) Antibody detection: Immunoblot assay for the detection of antibodies against *Taenia solium* has been developed (Wilkins *et al*., 1999).

Currently, both the copro-antigen test and immunoblot assay are very useful for the detection of taeniosis patients, with confirmation of expelled worms by morphology as well as DNA analysis recommended.

Human cysticercosis: Imaging techniques and biopsy of the tissues containing parasites provide direct diagnosis of cysticercosis. Alternatively, Ag-ELISA, antibody AB-ELISA, and immunoblot provide indirect diagnosis.

(a) Tissue biopsy: specimens of subcutaneous or brain tissues are sometimes removed surgically. Tissue section and stain in pathological laboratory reveals scolex with hooks typical of *T. solium* cysticercus.

(b) Imaging techniques: usually, the diagnosis of neurocysticercosis can be determined on the presence of cysts or typical calcifications by magnetic resonance imaging (MRI) or computerized tomography (CT) scan. In the clinical practices, computerized tomography (CT) scan and magnetic resonance imaging (MRI) are used to detect the exact locations and viability of *T. solium* metacestodes. Calcified cysts are also detected by radiography (White, 2000).

(c) Serological detection: several serological techniques are used for detection of anti-cysticercus antibodies such as ELISA and immunoblot. However, not all cysticercosis patients are positive. For neurocysticercosis, the enzyme-linked immunoelectrotransfer blot (EITB) or immunoblot assay is highly specific and commercially available (OIE, 2004).

(d) Molecular technique: while PCR tests have been used largely for the differentiation of adult taeniids in humans, they could be usefully applied to identify species of metacestode infection too.

Porcine cysticercosis: Diagnosis of cysticercosis in pigs can be difficult due to short life and absence of symptoms and may require several testing methods as follows:

(a) Ante mortem procedure: Palpation of tongues in living animals, particularly for the presence of cysts *T. solium*, still remains widely used in surveys, but the most common diagnosis of cysticercosis in pigs has been meat inspection.

(b) Post mortem procedure: in slaughter animals visual meat inspection searching for cysts in whole carcasses including organs is employed. Cysts are essentially found in the following muscles: hearts, tongue, esophagi, masseters and diaphragm, shoulder muscles, intercostals muscles and livers (Evans *et al.*, 1997).

(c) Serological Test: the serum antibody detection such as ELISA is not used for the diagnosis of cysticercosis due to lack of commercial availability on the market. On the other hand serology is too expensive for routine, particularly because of several false positives (Sciutto *et al*., 1998).

(d) Molecular detection: commonly, PCR can be used to differentiate cysticercus species, but the kits are not available commercially (Sciutto *et al.*, 1998).

2.1.7 Therapy and prevention:

In humans: Therapy exists for humans when diagnosis is made in time.

(a) Adult tapeworm infection: For treatment of individuals with *T. solium,* intestinal taeniosis, the drug of choice is praziquantel at dose 5–10 mg/kg body weight (Sarti *et al*., 2000). A single dose of praziquantel or niclosamide is sufficient to expel adult worms of *T. asiatica,* or even *T. solium* (Allan *et al*., 1997). Moreover, adult cestodes can be expelled from humans using an anthelmintic followed by a purgative, in the case of niclosamide praziquatel worms are expelled immediately.

(b) Larval tapeworm infection: In the case of neurocysticercosis, albendazole is the treatment of choice, although praziquantel may also be useful (Garcia, 2003). They are treated with albendazole at a dosage of 15 mg/kg/day for 2 weeks and praziquantel at a dosage of 100 mg/kg/day for 2 weeks additionally. To decrease inflammation in active disease, immunosuppressive agents may be used such as corticosteroids (except pregnant women), but prolonged treatment with steroids is not recommended (Evans *et al*., 1997). In this case efficacies are up to 80% complete disappearance of the cyst, 10% decrease in the size of the cyst and 10% failure. In heavy infection, surgery is sometimes necessary for treatment, such as ocular cysticercosis (Gonzalez *et al*., 1999).

According to OIE guidelines for meat inspection: The infected carcasses or meats containing living cysts are inactivated by cooking or heating at 60°C for 15 to 20 minutes and prevention is done through hygiene and proper meat inspection at the slaughterhouse. Deep-freezing at -20°C for 4 days can destroy cysts. In cases of moderate infestation, the meat has been processed using one of the methods provided in the "Recommended International Code of Practice for ante and post mortem judgment of slaughter animals and meat", namely: freezing or heat treatment at 60°C (140°F) (FAO/WHO - CAC/RCP 34-1985; OIE, 2004; Hillwig, 1987). No vaccines have been developed so far. Many investigations have been done to vaccinate against cysticercosis in pig, but unfortunately no commercial vaccines is on the market.

The prevention and control of *T. solium* cysticercosis can be applied through health education and better sanitation. In the high risk areas there are two strategies for health education of people free of taeniosis. First, people in endemic areas do not eat uncooked or undercooked meat containing viscera (cysts). The second is to keep pigs indoors without contact with human feces (Lightowlers, 1999). Cutting off the life cycle of *T solium* depends on sustainable public-health education, such as washing hands with soap and water after using the toilet and before handling food; not touching contaminated raw meats; cleaning and peeling all raw vegetables and fruits before eating; drinking only bottled or boiled water or carbonated (bubbly) drinks in

cans or bottles, and not drinking fountain water. Another way to make water safe is by filtering it through a filter and dissolving iodine into it (Sarti *et al*., 1997).

Porcine cysticercosis: the therapy of cysticercus stage in pigs is not available (economical or unfit) and vaccination is not completely safe as well, as it is not yet commercially available.

2.2 *Taenia asiatica* complex

A new taeniid species named *Taenia asiatica* is closely related to but genetically distinguishable from *T. saginata* (Eom and Rim *et al*., 1993). The adult worm in humans has an ovary, vaginal sphincter muscle and cirrus sac like those of *T. saginata*, but Asian *Taenia* does have a rudimentary rostellum with rudimentary hooklets, posterior protuberances on segments, and total length and number of proglotids less than *T. saginata saginata. T. asiatica* is 5-8 meters in length, proglottids and side branches of the uterus seem similar to *T. solium*. The metacestodes are small and sometime have two rows of primitive hooks. Otherwise, *T. asiatica* metacestode (Cysticercus *viscerotropica*) was different morphologically from *T. saginata* metacestode (Cysticercus bovis) in having wart-like formations on the external surface of the bladder wall (Eom *et al*., 1998). They occur in viscera, mainly in the liver of domesticated and wild pigs, occasionally in cattle, goats, and monkeys. Based on the morphologic characteristics of adult and metacestodes of Asian *Taenia saginata*, the third kind of human taeniid tapeworm is known to spread in Asian countries, particularly Southeast Asian countries. The life cycle of *T. asiatica* appears to be completed in rather remote areas, where pigs roam with free access to human feces in many Southeast Asian countries, similar to the situation with *T solium* in Asia (Fan *et al*., 1995). *T. asiatica* has been also found in Taiwan, Korea, China, Vietnam, and Indonesia; at least where people eat uncooked pork or pig viscera after killing pigs at home. But it has been difficult to differentiate between *T. saginata* from beef and Asian *Taenia* from pork (Simanjuntak *et al*., 1997; Eom *et al*., 2002; Erhart *et al*., 2002). The life cycle of this cestode was also different from classical *T.*

saginata in its intermediate host animals as well as infected organs such as the liver, omentum serosa and lung of pigs in its larval stage, but CNS penetration is never recorded (Eom, 1993).

2.3 Differentiation of cysticercus species

The tapeworms of the genus *Taenia* that infect human beings are *T. solium*, *T. saginata* and *T. saginata asiatica*. *Taenia solium* and *T. saginata* exhibit unequivocal features that characterize them. In contrast, only recent DNA studies, morphological characteristics, and epidemiological and sanitary aspects indicate that *T. saginata asiatica* is a subspecies of *T. saginata*. These 3 tapeworms occur in humans in their adult stage, and the intermediate hosts are pigs for *T. solium* and *T. asiatica* and cows for *T. saginata*. Their identification is crucial considering the migratory increase from Asia to the Western Hemisphere and the fact that these tapeworms coexist in the same environment in Asia; furthermore, it is estimated that movement in both directions across the United States–Mexico border exceeds 200 million persons per year, and thus, opportunities for acquiring and transporting *T. solium* infections are multiplied. It is not easy to distinguish among these tapeworms; therefore, a comparative diagram of the 3 parasites is shown in this article, which will facilitate their identification. All morphological features, some of which allow for identification, are clear and can be easily distinguished among the 3 tapeworms (Flisser *et al*., 2004).

Today, difference among these parasites can be definitely demonstrated by using molecular study such as PCR. Species-specific identification of human tapeworm infections is important for public health purposes, because prompt identification of *Taenia solium* carriers may prevent further human cysticercosis infections (a major cause of acquired epilepsy). Two practical methods for the differentiation of cestode proglottids:

(a) Routine embedding, sectioning, and hematoxylin-eosin (HE) staining,

(b) PCR with restriction enzyme analysis (PCR-REA) (Mayta *et al*., 2000).

The morphological differentiation of three tapeworms is indicated in Table 1.

Table 1: Morphological differentiation between *T. solium, T. saginata and T. asiatica*

Characteristics	Morphological differentiation		
	T. solium	*T. saginata*	*T. asiatica*
Metacestodes			
Intermediate host	Pigs, humans, dogs and wild boars	Cattle, buffalo and reindeer	Pigs, cattle, goats and wild boars
Site of location	Brain, skin, eye, tongue and muscle	Muscles, viscera & brain	Viscera, mainly livers
Size (mm)	5.6-8.5 x 3.1-6.5	7-10 x 4-6	2 x 2
Scolex	Rostellum with hooklets	No rostellum, no hooks	Rostel. with no or rudiment. hooks
Bladder surface	Wart-like formation	Rugae	Wart-like format.
Adult tapeworms			
Scolex:	Rostel. with hooks	No rostel. hooks	R. with no hooks
Diameter (mm)	0.6-1.0	1.5-2	0.8
No. of suckers	4	4	4
Diameter of sucker	0.4-0.5	0.7-0.8	0.24-0.29
Rostellum	Present	Absent	Present
No. of hooks	22-32	Absent	Absent
Proglottides: N^{o}	700-1000	2000	200-1016
Length (mm)	10-16	4-13	5-9.5
Maximal breadth	7-10	8-11	3-5
Mature of progl.: No.	375-575 (testes)	800-1200	868-904
Ovary	Three lobes	Two lobes	Two lobes
Vaginal sphincter	Absent	Present	Present
Gravid proglottides: No. of uterine branch	7-12	15-32	16-21
Branching pattern	Dendritic	Dichotonous	Dichotonous
Expulsion from host	Mainly in group passively	Single, actively and spontaneously	Single, actively and spontaneously

R., Rostel.= Rostellum; Rudiment.=Rudimentary; Format.= Formation

2.4 *Trichinella* complex

2.4.1 General overview

Trichinellosis is mainly important, parasitically and zoonotically, because of its public health significance for more than 150 years (Ljungstrom *et al.*, 1998). The *Trichinella* species was first reported in man in 1835 and was first recorded in the United States in 1846. It is a globally distributed zoonotic disease caused by the ingestion of raw or undercooked meat harboring larval muscle parasites of the genus *Trichinella* (Gamble, 1997). The adults of *Trichinella* are at least 3-5 mm in length and may be found in the small intestine of humans, pigs, rats, bears and many other flesh-eating mammals, but may also occur in horses that have eaten fodder containing dead infected rodents (Dupouy-Camt, 1997). *Trichinella* larvae have low host specificity and are capable of infecting a broad range of carnivores and omnivores i.e. at least all vertebrates including birds and reptiles (Kapel *et al*., 1998).

The discovery of *Trichinella zimbabwensis* in farm crocodiles of Zimbabwe has opened up a new frontier in the epidemiology of the *Trichinella* genus (Murrell *et al*., 2000). There have been ten *Trichinella* genotypes described so far, eight of which are considered to warrant valid species status:

(a) *Trichinella spiralis* (T-1) belongs to the domesticated cycle and is found in temperate regions worldwide and is commonly associated with domestic pigs and rats living in food competition. It is one of the main zoonotic helminthosis and is highly infective for pigs, mice and rats, as well as man.

(b) *Trichinella nativa* (T-2) is a cold-climate-adapted species and belongs to the sylvatic cycle. It has limited infectivity for pigs, but is commonly found in wild canids, bear, walrus and wild pig-like carriers, only in remote areas far from the influence of human civilization. It is further distinguished its resistance to freezing (Kapel *et al*., 1997).

(c) *Trichinella britovi* (T-3) belongs to the sylvatic cycle and is found predominantly in wild animals, although it may occasionally be found in pigs or horses. It occurs in the temperate regions of Europe and Asia. *Trichinella britovi* has some of the intermediate characteristics of other species; including some resistance to freezing, moderate infectivity for swine and slow capsule formation (larvae have been confused with non-encapsulating species in some cases).

(d) *Trichinella* T-8 is an isolate from Africa that is similar to *T. britovi* and *Trichinella* T-9 from Japan, but is found to differ by molecular analysis.

(e) *Trichinella pseudospiralis* (T-4) does not form a capsule in muscle, is cosmopolitan in distribution, and has been recovered from raptorial birds, wild carnivores, rats and marsupials in Asia, North America and the Australian subcontinent (Raque *et al*., 2000).

(f) *Trichinella murrelli* (T-5) is a North American species found in wildlife and occasionally horses and humans. It has low infectivity for domestic pigs, but poses a risk to humans who eat game meat.

(g) *Trichinella papuae* (T-6 or T10) is a very small 2-4 mm long hair-like worm, dwelling deep in the mucus membranes of the small intestine and does not form a capsule in muscle. To date, it has only been reported from Papua New Guinea. *Trichinella papuae* is found in North America. It is resistant to freezing, has low infectivity for pigs, is found in a variety of wild mammals and has been implicated in human disease. But all species may infect man (Pozio *et al*., 1999).

(h) *Trichinella nelsoni* (T-7) has been isolated sporadically from wildlife in Africa. It is characterized by greater resistance to elevated temperatures as compared with other species of *Trichinella.*

(i) *Trichinlla zimbabwensis* adult worms were collected from the intestine and larvae from the muscles of reptile species (OIE, 2004; Dick *et al.*, 2001).

2.4.2 Morphology

Trichinella species belong to the family *Trichuridae* and mainly measure approximately 1-7 mm in length, are unsegmented, cylindrical and tapered at both ends; the esophagus is extremely elongated surrounded by stichosoma, living deeply pierced in the small intestinal wall. Male worms are about 1.4 - 1.6 mm long with an almost terminal anus, they have no spicules, but they have twin terminal appendages and papillae. Moreover, they have a stichosome with a short muscular esophagus and die soon after copulation. Females are about twice the length of males with a similarly located anus. The vulva is located about half way along the pharynx. The single uterus is filled with developing eggs in its posterior region. The females are viviparous, laying first larvae and then dying shortly after the completion of oviposition (Corwin *et al*., 1999).

2.4.3 Life Cycle

Trichinella species are not very host specific. They infect a very broad range of host species including vertebrates. The definitive host becomes infected when raw or poorly cooked meat containing the infectious stage of the muscle larvae (first-stage larva) is eaten. Larvae are encysted in the muscle fibers. On passage through the stomach of the host, the larvae are released from the cyst. In the small intestine the larvae rapidly penetrate the mucus membrane where they undergo successive moultings to become young adults, within 30 hrs post-infection. In 5-6 days they moult 4 times to become adult worms and shed larvae. The adults mate deep within the mucus membrane where they are regarded as being intracellular parasites, lying within a serial row of host cells. The female worms produce about 1,500 larvae over 4-6 weeks. Males die soon after copulation and the females die shortly after the completion of larvae-position. Newborn larvae enter the blood or lymph vessels, flood into all organs by way of the circulatory system, and after 3 weeks spread throughout the body. Finally the larvae reach striated muscle where they penetrate the individual muscle fiber. They have predilection sites for highly active muscles such as the tongue, masticatory muscles, intercostal muscles, diaphragm, eye muscles and the

muscles of the arms and legs. The larvae absorb nutrients from the muscle cells and increase their length to about 1 mm. They finally coil and remain dormant until eaten and enter the digestive system of the next host. The larvae for nourishment require the muscle cell, and the worm induces changes in muscle cell structures so the larvae stay alive as long as the muscle fiber does not degenerate. The changing muscle fibers are called “nurse cells”, enclosed by the hyaline capsule. In the late phase the cyst wall becomes gradually thicker and eventually calcified (Kapel, 2000; Jospeh *et al.*, 1999).

2.4.4 Pathogenicity and clinic

Trichinellosis is rarely detected clinically in animals.
In humans the pathogenicity of all the different species of *Trichinella* has not yet been totally explored. For the most common species, *T. spiralis*, clinical signs are well documented.

Intestinal phase: the intestinal phase is characterized by a self-limiting bout of abdominal pain and diarrhea with expulsion of mature worms, anorexia, fever, weakness and myositis causing unwillingness to move.

Parasitaemical phase: The newborn larvae enter circulation systems, spread to different organs and invade host cells causing lesions. The symptoms observed include heart attacks, inflammation of the brain, and heart failure. Life-threatening complications include myocarditis, central nervous system involvement, and pneumonitis. Deaths are common (up to 40%) either due to anaphylactic chock or due to the consequences of the myocarditis. Symptoms such as edema around the eyes, muscle pain, fever, itchiness in the skin, and lesions of the skin have been described. More serious cases in humans have caused breathing difficulties as a result of an infected diaphragm.

Muscle phase: Larvae are able to penetrate any cell in various tissues in the body resulting in cell death and associated inflammation and subsequent granuloma formation (Despommier, 1999). Later on they penetrate the cells of the skeleton

muscles and have the ability to become encapsulated. The muscle phase is very complex due to the penetration of *Trichinella* larvae into striated skeletal muscle cells and their permanent residence there (Zarlenga, *et al.*, 2001).

2.4.5 Epidemiology

*Trichinella*s are some of the most widespread parasites infecting people and other mammals all over the world, regardless of climate. The global prevalence of the disease is difficult to evaluate, but worldwide trichinallosis is estimated to affect at least 11 millions people with different epidemiological patterns (Dupuoy-Camet, 2000).

The International Commission reported more than 10,000 cases of human trichinellosis on trichinellosis from 1995 to June 1997 and about 10,000 porcine infections were reported by the Office International des Epizooties in 1998. The present global status of trichinellosis is determined as a worldwide zoonosis. In contrast to animals where *Trichinella* infection proceeds without clinical symptoms, food borne infection in man usually entails typical trichinellosis with the threat to human health (Marinculic *et al*., 2001).

A variety of patterns exist which determine the way of *Trichinella* along the food chain to human beings. Two main cycles, *i.e*., the sylvatic and the domestic cycles have been recognized in the epidemiology of trichinellosis (Campbell, 1988). The main agent is transmitted among domestic pigs by infected pock scraps or infected rats. The natural cycle occurs in sylvatic carnivores and omnivorous animals, mainly in those with cannibalistic and scavenger behaviors (Pozio, 1998). There are natural and artificial factors, which contribute to the maintenance of *Trichinella* within the domestic and sylvatic cycles.

Natural factors which influence the *Trichinella* cycle: the sylvatic cycle is regularly completed by cannibalism and the scavenger behavior of wild animals (Pozio, 2001). Domestic animals, which are infected with these species, represent a

dead end for the sylvatic *Trichinella* species. Another aspect is that they may be transmitted to the sylvatic, where wild animals have other food sources such as garbage dumps or carcasses of domestic animals. Further on, the population density plays an important role for the spread of *Trichinella* in the sylvatic cycle.

Otherwise, domestic pigs and synanthropic rats play an essential role for *Trichinella* infection in the domestic habitat. Infections in domestic habitats occur where domestic animals have access to the environment, and thus may be in contact with synanthropic or others (Pozio, 1998). Rats might play an important role in the flow of *Trichinella* infection between domestic and sylvatic cycles (Pozio, 2000).

Artificial factors which influence the *Trichinella* cycle: there is no doubt that human behavior strongly influences the transmission routes within domestic and sylvatic cycles (Pozio, 2000). The prevalence of trichinellosis is strongly related to hunting practices due to the improper disposal of fox carcasses, which may be a new infection source of other wild carnivores (Kapel *et al*., 1997). On the other side, domestic animals like pigs and dogs are fed with the remains of sylvatic animals, or domestic pigs are kept under outdoor conditions where close contact to the sylvatic *Trichinella* cycle may exist.

Infection sources in human trichinellosis: in comparison to domestic and sylvatic animals, which may show a variation in susceptibility for different species and genotypes, all *Trichinella* species are pathogenic for human (Ramisz *et al*., 2001; Kapel *et al*., 1995; Dupouy-Camet *et al*., 2002). The trichinellosis cases in man were traced back to the following main food sources: pork consumed or other raw products played the most important role as the source of infection (Cuperlovic *et al*., 2001; Marinculic *et al*., 2001). Wild boar meat was detected as another important source for human trichinellosis in many countries (Ramisz *et al*., 2001) and horse meat was firstly discovered as emerging *Trichinella* food-borne infection in Italy in 1975 (Boireau *et al*., 2000). Finally, trichinellosis cases due to infection acquired abroad in third countries or imported food products, which may harbor *Trichinella* larvae, have to be considered.

Risk factors for human trichinellosis: from all the aspects mentioned above, different levels have to be considered for the specification of risk in acquiring a *Trichinella* infection: first of all it must be clear if a sylvatic or domestic cycle or both of them are present in the defined region or not. If the *Trichinella* species is present in the habitat of the kind of species, its characteristics (freezing tolerance, encapsulated or non-encapsulated forms) and presence in domestic and sylvatic host animals have to be identified (Pozio, 2000). In this respect the kind of pig farming has to be specified (indoor and outdoor). Sylvatic and domestic animals kept under poor hygienic conditions, *i.e.* feeding of untreated food, no rodent control or extensive husbandry allowing uncontrolled contact to sylvatic animals, will pose a high risk (Pozio, 2001). Every pig and wild boar has to be examined for trichinellosis. If the meat inspection is not applied at all or if it is not performed properly, trichinellosis can be introduced in the food chain and pose a risk for consumers. After the slaughter or hunting of animals, the kind of meat preparation may imply a risk. This especially relates to all raw or insufficiently cooked products (Gamble *et al.*, 2000). The consumers expose themselves to the risk of trichinellosis by the consumption of raw or insufficiently treated meat from improperly examined carriers of the *Trichinella* species (Noeckler *et al.*, 2003). Moreover, cultural behaviors such as hunter meals with undercooked roasted ribs from wild boars favored human infections (European Commission, 2001).

Consequently, a very high risk for human trichinellosis will exist if *Trichinella* species are endemic in the domestic and /or sylvatic cycle, if meat inspection is not at all or improperly performed and if the meat is consumed raw or under insufficiently processed conditions. The risk will significantly decrease when every infected carcass can be removed from the food chain by an efficient meat inspection (Gamble *et al.*, 2000; Nöckler *et al.*, 2000).

Trichinellosis is more common in temperate regions than in tropical regions. It occurs in North America, South America (Argentina and Chile), northern and Eastern Europe, Kenya, Egypt, Lebanon, China, Nepal, Thailand and Indonesia. Three species are important in Southeast Asia and the Pacific region, *T. spiralis, T. pseudospiralis*

and *T. papuae.* In the Pacific region, serological evidences of *Trichinella* spp. have been found in Fiji, Kiribati, Palau and Samoa, Solomon Islands. In Southeast Asia *T. spiralis* and *T. pseudospiralis* are found principally in China, Japan and Thailand (Takahashi *et al*., 2000). For example the outbreak that occurred in Thailand, which affected 59 individuals who ate raw pork from a wild pig, was particularly severe with one death and individuals showed clinical signs of myalgia, muscular swelling and aesthesia that persisted for more than 4 months (Jongwutiwes *et al*., 1998). The newest non-encapsulated species of *Trichinella, T. papuae*, was isolated from a wild pig in a remote part of southwestern Papua New Guinea and described in 1998 (Pozio *et al.*, 1999). Infection was identified in approximately 8.8% of village and wild pigs in the area (Owen *et al*., 2000).

2.4.6 Diagnosis

The main sources of infection in humans are eaten pork, horse meat, bears and small wild carnivores. According to the "Manual of Standards for Diagnostic Tests and Vaccines" published by OIE (2004), two main methods are recommended for the diagnosis of trichinellosis: direct detection of first-stage larvae encysted or free in striated muscle tissue, and indirect detection of parasitism by tests for specific antibodies (Gamble, 1996).

Direct detection:

(a) Applications of the methods

The direct detection of *Trichinella* larvae in muscle samples is usually done at post-mortem inspection. In order to prevent human trichinellosis in many countries, the examination of muscle samples of pigs and of all other animal species, *e.g*., horses, wild boars, etc., that may potentially serve as a source of this food-borne infection, is a part of routine slaughter inspection (Gamble, 1996). Direct detection is useful for epidemiological studies in wildlife, in which indicator animals, *e.g*., foxes and raccoon dogs are examined for the presence of this nematode in order to

investigate the reservoir competence of the host and to evaluate its importance within the sylvatic and domestic cycles. Indicator animals provide an estimation of the prevalence of *Trichinella* in the environment (Forbes *et al*., 1998).

(b) Factors important for direct detection

Direct methods for the detection of *Trichinella* larvae in muscle samples are designed to provide maximum sensitivity, but have limitations. Methods suitable for routine meat inspection are designed primarily to prevent clinical trichinellosis in humans and do not have the capacity to prevent infection entirely. The efficiency of the direct detection of *Trichinella* larvae depends on the methods used, the site sampled and the sample size. The correct choice of a suitable diagnostic method is necessary in order to obtain reliable results. *T. spiralis, T. nativa, T. britovi, T. nelsoni* and *T. murrelli* induce the formation of a nurse cell in the striated muscles of the host, whereas the non-encapsulating species *T. pseudospiralis* and *T. papuae* are characterized by the lack of a capsule around the muscle larva (Murrell *et al*., 2000). Larvae of non-encapsulating species would be more difficult to detect by trichinoscopy. Therefore, all samples should be examined in conjunction with or by the digestion method because trichinoscopy cannot ensure the detection of all *Trichinella* species.

(c) Sample location

After the internal and migratory phases, larvae of *T. spiralis* reach the striated muscles where they become infective for a new host as early as 17 days post-infection. Larvae prefer sites in muscle tissues that are well supplied with blood. Predilection sites differ among animal species and may be dependent on the specific mobility behavior of the species (Kapel *et al*., 1995). Identification of the predilection sites in an animal species will determine the choice of muscle to be tested for *Trichinella* larvae. In domestic pigs, the three main predilection sites for *T. spiralis* are the diaphragm, the tongue and the masseter muscle (Gamble, 1996; Forbes and Gajadhar, 1999). Wild boars infected with *T. spiralis*, *T. nativa*, *T. britovi*, *T.*

pseudospiralis, *T. murrelli*, *Trichinella T6* and *T. nelsoni* harbored most larvae in the diaphragm and the tongue (Kapel, 2000). In horses infected with *T. spiralis*, the tongue and masseter were found to be typical predilection sites (Gamble *et al.*, 1996). In a naturally infected horse, most larvae of *T. spiralis* were detected in different muscles of the head. Infections with *T. pseudospiralis* in poultry (cock-broilers) demonstrated that the muscles of the head (*e.g.*, masseter and the neck) were typical predilection sites (Britov *et al.*, 1997).

(d) Sample size

The amount of sample to be used for the detection of *Trichinella* larvae must be chosen to provide an adequate level of sensitivity and an acceptable cost-benefit relationship. It is generally accepted that for routine meat inspection, to prevent clinical trichinellosis in humans, it is necessary to ensure a sensitivity of approximately 1–3 larvae/g (LPG) of tissue taken from the predilection site. Theoretically, a 1 g sample would be enough for the detection of at least 1 LPG of tissue, on the condition that there is a homogenous distribution of larvae in the tissue investigated. In practice, this is true for high larval densities, but in cases of a low level of infection, larvae are not distributed homogeneously.

For the routine slaughter inspection of pig carcasses and game meats, using the pooled sample digestion method, a minimum of a 1 g sample of tissue from a predilection site is recommended. For the same purposes, a minimum of 0.5 g sample and preferably more may be used for the inspection of individual pig carcasses by trichinoscopy (Gamble *et al.*, 2000). To ensure high sensitivity, horse meat is examined with the pooled sample digestion method using 5 g or preferably 10 g samples. If the muscles from predilection sites are not available for inspection, carcasses should be tested using larger amounts (up to 100 g samples) in order to achieve adequate sensitivity (Gamble *et al.*, 2000).

Concerning epidemiological studies in reservoir animals, the sample size should be adjusted upward to achieve a sensitivity of less than 1 LPG. Low larval densities

occur in the muscle tissues of wild carnivores infected with *Trichinella*. For this reason, the samples to be tested in such studies should have a weight of at least 5 g or more.

(e) Main characteristics and performance of direct methods of detection

Trichinoscopy is a simple but tedious method for the inspection of individual carcasses and requiring much time and labor. In contrast, the pooled sample digestion method allows testing of up to about 100 carcasses at the same time. The digestion method requires more technical equipment than trichinoscopy, but is cheaper and has become the method of choice for routine slaughter inspection in most industrialized countries. Because of the enhanced sensitivity of digestion tests, the use of trichinoscopy as a standard method of control is discouraged. OIE guidelines for tests of trichinellosis are available for examining pork, as well as wild boar and horse meat for muscle larvae, and should be adequate for preventing clinical trichinellosis in humans (Gamble, 1996, 1998). To ensure that tests are performed properly, all authorities conducting routine slaughter inspection should introduce and maintain a suitable quality control system.

A documented quality assurance system (QAS) that meets international standards will soon be essential for any test used in domestic or international trade. Complete data for the validation of a digestion test for pigs and horses are available (Forbes and Gajadhar, 1999).

International regulation of direct detection for meat inspection includes:

(f) Trichinoscopy (Compression method)

According to the OIE “Manual of Standards for Diagnostic Tests and Vaccines”, 28 small pieces of muscle of about 2 mm x 10 mm in size, with a total weight of about 0.5 g, should be taken from prescribed predilection sites (Gamble, 1996). The small

spindle shaped pieces along the muscle fibers are cut with a scissors. Then the muscle pieces are compressed between two glass plates until they become translucent, and then examined individually for *Trichinella* larvae, using a trichinoscope or a conventional stereo-microscope (15–40x magnification). All the samples are recovered for processing by artificial digestion test (Nöckler *et al*., 2000).

(g) Artificial digestion (Pepsin fermentation)

The magnetic stirrer method described as follows:

Homogenize a maximum of 100 g of samples of muscle tissue 1 gram each from the prescribed predilection sites of the animals under inspection are pooled. The sample pool is digested using an artificial digestive fluid consisting of 2 liters of tap water, 10 g of 1% pepsin (1:10.000 NF= US National Formulary), and 16 ml of 25% HCl.

The digest is stirred for 30 min at a temperature of 44–46 ^{o}C in a 3-liter glass beaker using a hot plate magnetic stirrer. During this process, the trichinae are released from the muscle. The digestion fluid is then poured through a sieve (mesh size 180 mm), which keeps back any undigested tissues, but allows the passage of *Trichinella* larvae, into a 2-liter separation funnel. Larvae are allowed to settle for 30 min, and then a 40ml sample is quickly released into a 50 ml tube. After a further 10 minutes of sedimentation to clarify the suspension, 30 ml of supernatant is withdrawn. The remaining 10 ml of sediment is poured into a gridded petri dish. The 50 ml tube is rinsed with 10 ml of tap water, which is added to the petri dish. Subsequently, the sample in the petri dish is examined by a trichinoscope or stereomicroscope (15–40x magnification) for the presence of *Trichinella* larvae (Gamble *et al*., 2000).

Indirect detection including serological and molecular studies

Serological study: Part of this review is directed at sero-diagnostic methods for the detection of *Trichinella* specific antibodies in different animal species, *i.e*., antibody detection with larval E/S antigen AB-ELISA test. Classical methods of sero-

diagnosis such as the complement fixation test and immuno-fluorescence antibody test are reviewed and the characteristics and performance of the AB-ELISA are discussed. Factors dependent upon the animal species being tested or on components of the AB-ELISA test system are considered. Using serology, it has become possible to perform additional *Trichinella* control measures (Directive on Zoonoses EEC /117/92) to ensure consumer protection.

(a) Applications and characteristics of the methods

Serological methods are used mainly for ante-mortem and post-mortem examination of blood serum samples for *Trichinella*-specific antibodies, and under some conditions may have a higher sensitivity than methods of direct detection. Other uses include *in vivo* studies on immune responses in long-term infection and surveillance of live caught wild animals. Because serological methods allow testing of samples from living pigs as well as of samples obtained post-mortem, they may be useful for establishing *Trichinella*-free areas and reducing restrictions in international animal trade. The tissue fluids (meat juice) from slaughtered pigs or from hunted or other dead animals (*e.g.*, wild boars) may be suitable for serologic examinations using ELISA (Gamble and Patrascu, 1996; Gamble, 1999; Kapel *et al*., 1998).

(b) Conventional serological methods

The immunofluorescence antibody test (IFAT), western blot analysis (WBA), complement fixation test (CFT) and haemagglutination test (HAT) are examples of conventional serological methods that are labor intensive and can not be used in an automated system. As a result, these methods are more expensive in comparison to the enzyme-linked immunosorbent assay (AB-ELISA) and are preferentially used in human medicine for the examination of individual samples (Nöckler *et al*., 2000).

Enzyme-linked immunosorbent assay (AB-ELISA)

In comparison to the CFT, HAT, WBA and IFAT, the AB-ELISA is easy to conduct, can be automated and detects infection levels as low as one larva/100 g of tissue (Gamble, 1996). The AB-ELISA method is recommended for herd surveillance programs and is useful for detecting ongoing transmission of *Trichinella* at the farm level (Gamble, 1996). However, the AB-ELISA may fail to detect infected pigs during both the early and the very late phases of infection. It is for this reason that this serological method can not be used to replace digestion testing for the detection of *Trichinella* larvae at slaughter inspection, but it can be recommended for practical use in herd surveillance in pigs (Nöckler *et al*., 1995; Gamble, 1996). The use of enzyme-linked immunosorbent assay (AB-ELISA) to detect the presence of parasite-specific antibodies provides a rapid method that can be performed on blood serum and tissue juices collected before or after slaughter. Several antigen preparations have been developed that provide a high degree of specificity for *Trichinella* infection in pigs and horses. In slaughterhouse testing, the AB-ELISA yielded less than 0.3% false-positive results and was nearly 100% sensitive in detecting infected pigs with more than one larva/gram of tissue. The *T. spiralis* excretory and secretory (E/S) antigens used in the AB-ELISA are conserved in all species/types of *Trichinella,* and therefore infection may be detected in pigs or other animals harbouring any of the seven species or types. Serological tests other than AB-ELISA (*e.g.*, indirect immunofluorescence tests) lack specificity and are not sensitive enough for detection of *Trichinella* infection.

Molecular studies

This method includes the PCR test, which enables differentiation between the different species of *Trichinella.* However such tests require the development and amplification of specific DNA-probes. DNA sequences have been identified and are specific for *T. spiralis* and other *Trichinella* species as well. This new generation of PCR test is not available, *i.e.*, specific primers are very costly and uneconomical in the diagnosis of meat in the human food chain.

2.4.7 Therapy and prevention

Therapy exists for humans if diagnosed in time. When the life of the patients is threatened by overwhelming infection, intensive care treatment with all available supportive therapies is mandated, *i.e.*, fluid replacement, steroids, treatment for shock and toxemia as well as circulatory and cardiac failure. Specific therapy for the parasites with various benzimidazoles (mebendazole or albendazole) is also necessary. Immunosuppressions due to steroids, although often a life-saving procedure, prolong the life of the adult parasites as well and result in further production of newborn larvae if unchecked. As already mentioned, patients may harbor adults shedding newborn larvae for several weeks during the acute phase of infection. Mebendazole (200mg/day for 5 days) or albendazole (400mg/day for 3 days) should be given to adults, as well as to children (5 mg/ kg of body weight per day for 4 days). Prednisolone at 40-60mg/ day alleviates the fever and the side effects of inflammation due to the cell damage that results from larval penetration into the tissues. They can also use engimidazoles over 14 days, effective in the muscle phase (Kociecka, 2000).

These symptoms usually disappear within days after the initial dose is given. Prolonged treatment with steroids is not recommended, although symptoms may recur when treatment is suspended. Long-lasting sequels must be treated symptomatically as they arise.

To prevent trichinosis in pigs at the farm level (main host at risk for human health in the region), measures involve the environmental control (requirement for *Trichinella*- free pig production) such as architectural and environmental barriers, feed and feed storage, rodent control and farm hygiene (dead animals are disposed of within 24 hours) and by sanitation means as well as no garbage dumps present within a 2 km radius of the farm (Craig *et al.,* 2002; EC, 2000).

No vaccines have been developed so far for the prevention of trichinellosis.

Prevention of trichinellosis for consumers or humans can be done by avoiding the consumption of raw or contaminated meat from game animals or pigs raised in situations that favor the existence of rodent population. These animals are the most frequent source of infection by any *Trichinella* species. In addition, trichinellosis can be prevented by either cooking meat thoroughly at 58.5^{o}C for 10 min. or by deep-freezing it at -20 ^{o}C for 3 days. Consumer food safety education programs include cooking to an internal temperature of 71 ^{o}C (160^{o}F), freezing solid (-15 ^{o}C or less) for 3 weeks (cut up to15 cm in thickness) and freezing solid (-15 ^{o}C or less) for 4 weeks (cut up to 69 cm in thickness). Cooking using microwaves, curing, drying or smoking are not recommended (Gamble *et al*., 2000; Bessonov *et al.*, 2000).

According to the OIE guideline and EC regulations for trichinellosis, the gold standard are subjected to a testing procedure for trichinellosis with negative results or have been processed to ensure the destruction of all the larvae of the parasite (OIE, 2004; Anonymous, 1994; Herenda *et al.*, 2000).

2.5 Global importance of porcine cysticercosis and trichinellosis

2.5.1 Porcine cysticercosis

Infection with *T. solium* cysticercosis is widely prevalent in human and pig hosts in many developing countries of Latin America, Africa, and Asia (Sarti *et al*., 1992).

Asia: several reports of patients with cysticercosis from many countries in Asia such as India, China, Indonesia, Thailand, Korea, Taiwan and Nepal are a clear indicator of the wide prevalence of *T. solium* cysticercosis and taeniosis in these and other Asian countries. However, epidemiological data from community-based studies are sparse and available only for a few countries in Asia. Cysticercosis is the cause of epilepsy in up to 50% of Indian patients showing partial seizures. It is also a major cause of epilepsy in Bali (Indonesia), Vietnam and possibly China and Nepal. Sero-

prevalence studies indicate high rates of exposure to the parasite in several countries (Vietnam, China, Korea and Bali (Indonesia)) with rates ranging from 0.02 to 12.6%. Rates of taeniosis, as determined by stool examination for ova, have also been reported to range between 0.1 and 6% in the communities in India, Vietnam, China, and Bali (Indonesia) (Rajshekhar *et al*., 2003).

Africa: in West Africa, *T. solium* cysticercosis in both pigs and man has been reported in Benin, Burkina-Faso, Ghana, Ivory Coast, Senegal and Togo, and although official data are lacking, *T. solium* is anticipated to be present in most of the pig-raising regions of other West African countries as well. In some regions of Nigeria, the prevalence of porcine cysticercosis and human taeniosis is quite high (20.5 and 8.6%, respectively). Surprisingly, however, no cases of human cysticercosis have been reported, although epilepsy is very common. Large epidemiological surveys have only been carried out in Togo and Benin, where the prevalence of human cysticercosis was 2.4 and 1.3%, respectively. In Central Africa, porcine and human cysticercoses are (hyper)-endemic in Rwanda, Burundi, the Democratic Republic of Congo and Cameroon. The parasite also has been reported in pigs in Chad and Angola. Cysticercosis has been shown to be one of the major causes of epilepsy in Cameroon with figures as high as 44.6% (Zoli *et al.*, 2003).

Europe: *T. solium* cysticercosis has been eradicated from European countries except for a few areas where sporadic human cases are reported (Overbosch *et al*., 2002). However, it is unclear if these cases are due to the European *T. solium* or to foreign *T. solium* re-introduced by immigrants or travelers from other areas. It is possible that some isolates of European origin might remain in Spain/Portugal, Eastern Europe and Russia or the northern part of Mongolia where *T. solium* cysticercosis still exists (Ito *et al*., 2003).

Latin America: During the last century information was based on autopsy findings, which clearly pointed to those countries where cysticercosis was considered afterwards a priority health problem: Brazil, Colombia, Mexico and Peru (Del-Brutto, 2000). Recent reports demonstrate the presence of *T. solium* also in other countries,

such as Guatemala (Flisser *et al*., 2003; Allan *et al*., 1996). The prevalence data of these countries are summarized in Table 2.

Table 2. Global prevalence data on porcine cysticercosis, taeniosis and human cysticercosis in some countries of the world

Countries	Human cysticercosis %	Taeniosis %	Porcine cysticercosis %	References
China	3-4	0.06-19	5.4 (0.8-40)	Rajshekhar *et al*., 2003
Cambodia	NA	NA	10	Singh *et al*., 2002
Vietnam	5-7	0.5-6	0.04-0.9	Rajshekhar *et al*., 2003
India	NA	2	9.3	Rajshekhar *et al*., 2003
Nepal	NA	10-50	32.5	Rajshekhar *et al*., 2003
Tanzania	NA	NA	4.5-26.9	Boa, 2002
Zambia	NA	NA	8.2-20.8	Phiri *et al*., 2002
South Africa	NA	NA	0-9.1	Phiri *et al*., 2003
West Africa	1.3-2.4	NA	0.6-20.5	Zoli *et al*., 2003
Latin America	3.7-22.6	0.3-2.8	1-38.9	Flisser *et al*., 2003

NB: NA= No data available.

2.5.2 Trichinellosis

Human trichinellosis, a parasitic nematode infection, represents the most frequent widespread food-transmitted helminth zoonosis with worldwide distribution with both sylvatic and domestic spreading, and is caused by tissue-dwelling roundworms of the genus *Trichinella* (Pozio, 1998). At present, human and animal trichinosis is considered as an emerging infection due to the increase in its prevalence

in countries such as Bulgaria, Rumania, Yugoslavia, Croatia, Lithuania, Russia, China, Argentina, and Mexico (Pozio, 2001). It is also considered to be a re-emerging infection since epidemiological and biological research on its life cycle in the wild life have led to the description of new species in various regions with new patterns of transmission, which increase the risk of human infection from consumption of the meat of wild animals (*e.g.,* in Canada, United States, Russia), or domestic animals such as the horse (in France and Italy) or dog (in China) (García *et al.*, 2005; Pozio, 2001).

Unlike other parasite infections, it has been the main public health problem in advanced countries where there is a great amount of meat consumption such as European countries and USA. This nematode infection has been reported in all of the continents except Australia. Due to the wide spread of trichinosis in many Asian countries including China and Japan, it has been suspected to be prevalent in Korea. However, trichinosis had not been reported in Korea until 1997. In December 1997, the first human infection with *T. spiralis* confirmed by detecting encysted larvae in the biopsied muscle (Sohn *et al.*, 2000)

Trichinellosis study is introduced the presence of *Trichinella* spp. in Asia, especially historical review of *Trichinella* in Japan, the epidemiology of trichinellosis in China and in Thailand. There have been numerous outbreaks of trichinellosis in continental Asia including countries such as China and Thailand, but there have been very few records of outbreaks in the island countries. These island countries are geographically isolated from the Asian continent, which is known to have *Trichinella*.

Although, no data on *Trichinella* are available for Taiwan, the Philippines, Malaysia and Cambodia, it is likely that high rates of trichinellosis occur in various parts of Southeast Asia. It is known, for example, that the aborigines of Taiwan in the Wulai district commonly eat raw flesh and internal organs of pigs and other wild animals (Takahashi *et al.*, 2000).

Many provinces of Thailand near the Laotian and Cambodian borders were reported as foci of trichinellosis, but the number of cases was low, consisting of 1.4% of the total number of reported cases in Thailand. Likewise, only 0.6% of the cases were reported in the central part of Thailand (Takahashi *et al*., 2000). The prevalence of trichinellosis in some countries is addressed in the Table 3.

Table 3. Global Prevalence data on trichinellosis in some countries of the world

Countries	Human cases/ year	Human trichinellosis %	Animal trichinellosis %	Reference
China	NA	4.01	0.021-7.3 (Pigs)	Liu *et al.*, 2002
Thailand	NA	NA	0.02 (Pigs) 11.4 (hilltibe pigs)	Takahashi *et al.*, 2000
North America	38 (91-96)	NA	0.013 (Pigs)	Mooread *et al.*, 1999
Mexico	1-19(90-97)	NA	1-2.5 (Pigs)	La Rosa *et al.*, 1998

NB: NA= No data available.

3. MATERIALS AND METHODS

3.1 Justification for the selection of study locations

Regarding the objectives of the thesis, the first field studies were conducted at 4 slaughterhouses around Phnom Penh city, where slaughter pigs were delivered to slaughterhouses from different areas (provinces) of Cambodia. Based on the district administrative map, slaughterhouses were randomly selected from different regions surrounding Phnom Penh city (Figure 1). At the same time, a visit was made to the selected hospitals to look for data of neurocysticercosis in humans caused by *T. solium* cysticercosis. According to the duration of the research activity, the work schedule was divided into two periods: the first investigation started from November 2004 to February 2005 and the second period started from the end of February to the end of April 2005. Beside places mentioned above, some parts of the laboratory work were conducted at the NAHPIC of DAHP/ Cambodia and another part was performed at the diagnostic laboratory/ CMU (Thailand).

3.2 Slaughterhouse and slaughter pigs

There are two categories of pig slaughter places in Cambodia: slaughter slabs and slaughterhouses. Around Phnom Penh municipality, there are 4 pig slaughter slabs and 4 pig slaughterhouses. The capacity of slaughter pigs at each slaughter slab is 1-50 pigs per day and in each slaughterhouse 100-600 heads per day. Slaughtering was done weekly. The pig inspection was first conducted by tongue examination during the evening and carcass examination in the early morning. The slaughterhouses were:

(a) Slaughterhouse 1 (Beungsalang) is located in the Tuol Kok District, Central part of Phnom Penh city (Figure 1). The slaughterhouse 1 belongs to a private owner, is not registered and consists of the slaughtering of pigs and cattle at the same time,

but in two separate places. The average number of slaughter pigs per day is estimated at about 225 head, and cattle 40-60 head. The environmental condition of this slaughterhouse is very dirty (no hygienic standard).

(b) Slaughterhouse 2 (Domnak Thom I) is located in the Mean Chey District, in the southern part of Phnom Penh city (Figure 1). Slaughterhouse 2 is not registered and belongs to a private owner. The slaughter pigs are on average 550 head per day.

(c) Slaughterhouse 3 (Phreash Phonlear) is located in the Mean Chey District, southeastern part of Phnom Penh municipality (Figure 1). Slaughterhouse 3 is private, not registered. The average number of slaughter pigs is about 275 head per day.

(d) Slaughterhouse 4 (Ruessiekaev) is located in the Ruessiekaev District, in the northern part of the city (Figure 1). The average number of slaughter pigs is estimated around 250 head per day. Moreover, this slaughterhouse is not registered and also belongs to a private company.

3.3 Description of study design

The study design was a cross-sectional study carried out at the pig slaughterhouses to establish the prevalence of cysticercosis as well as sero-prevalence of trichinellosis in the pig slaughtering line. Between November 2004 and April 2005, 432 pigs were examined at the four slaughterhouses in Phnom Penh for cysticercosis and more than 440 serum samples for trichinellosis. The materials presented were selected from all pigs on the days of sampling. Tongue palpation was routinely done before slaughter and meat inspection was conducted after slaughter.

The four slaughterhouses were conveniently selected. The slaughter pigs were separated on the basis of breeds and the study pigs were then randomly selected from each breed. The sampling was done 3-4 times per week.

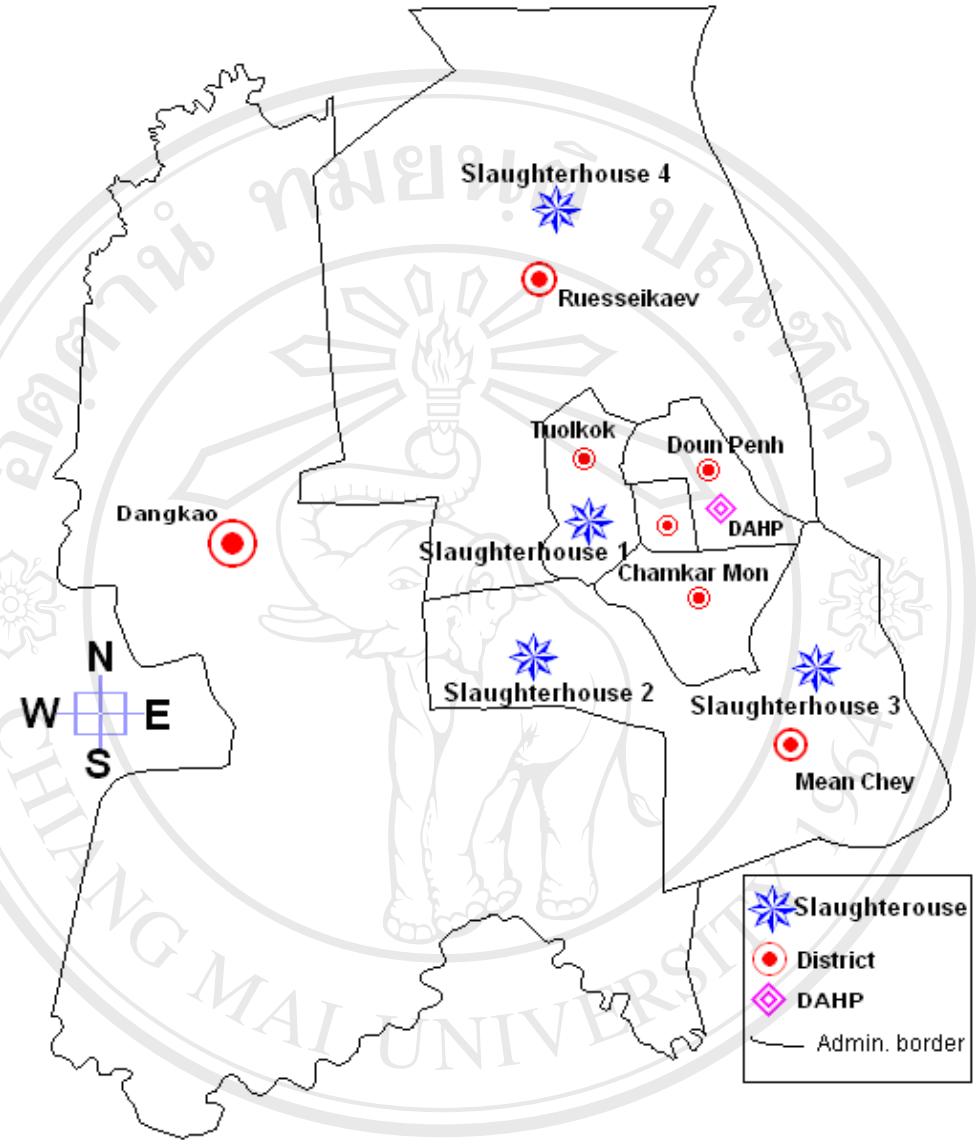

Figure 1. Phnom Penh Map showing slaughterhouses located in different districts around the Phnom Penh Municipality.

3.4 General description of study setting areas

The origin of slaughter pigs was later traced back to farms. Based on a slaughterhouse questionnaire survey and district administrative map, nine provinces, twenty-nine districts and three intensive farms were selected for this study, where pigs have always been delivered to these four slaughterhouses for slaughter. The twenty-nine districts were selected from these nine provinces. These provinces are located in different parts of the country such as central, western, northwestern, southern and southeastern as well as around Phnom Penh (Figure 2). Some provinces were selected such as Takaev (1), Prey Veaeng (2), Svay Rieng (3), Kampong Cham (4), Pousat (5), Kampong Spueu (6), Kandal (7), Kampot (8) and Banteay Mean Chey (9). Out of 432 pigs slaughtered in slaughterhouses during the study, it was of 18.5 %, 7.64 %, 13.42%, 4.62 %, 2.54%, 6.25%, 38.88%, 4.62% and 4.39% from province 1, 2, 3, 4, 5, 6, 7, 8 and 9 respectively. In these provinces, pigs are mainly used for commercial reasons and local consumption. A household keeps one pig to a hundred depending on the production system, which is mostly a fattening system.

3.5 Pig raising and pig breeds in Cambodia

The pig production (farming) system in Cambodia is comprised of 3 categories:

(a) Household raising (as extensive or outdoor): they keep from 1 to10 pigs per household. The pigs are free-range-outdoors all the time.

(b) Semi-intensive system is practiced by small and medium scale (farms): The number of pigs per household ranges from 10 to 200 heads per household or farm. Most of them are free roaming at the daytime and kept in the pens at night time, especially, piglets aged 2-4 months.

(c) Intensive production farms (Indoor): the number of pigs is more than 300 head per farm. The pigs are kept in the pens or piggery all the time (Census, MAFF, 2000).

The pig breeds in Cambodia are divided into 3 types:

(a) Local breeds (native): are slow growers and weigh an average of 60 to 80 kg. These breeds require fewer facilities and easily adapt to local feed and conditions. In addition, their body contains more fats than red muscles.

(b) Exotic breeds: like Landrace, Duroc, Large White, and Petrain. These breeds have been exported from different countries. They are characterized by fast growth and an average weight of 70- 120 kg.

(c) Crossed breeds: characterized by a strong body, aver. weight of 70-150 kg.

3.6 Description of study population

From November 2004 to April 2005, 48 working days were taken to conduct this study in the four slaughterhouses. A total of 62,400 slaughter pigs, 10,800 pigs were slaughtered in slaughterhouse 1, 26,400 pigs in slaughterhouse 2, 13,200 in slaughterhouse 3 and 12,000 in slaughterhouse 4.

3.7 Sampling procedure and biological sample collection

3.7.1 Sample size determination

The sample sizes used in this study were as follows:

(a) Ante mortem inspection 220 (Tongue)

(b) Post mortem inspection: 432 (included 220 tongue inspections)

(c) Blood samples: 440

These sample sizes were calculated using the Win Episcope program as below:

(a) Pig population estimated in Cambodia of 2,000,000.

(b) An estimate of disease prevalence of 50%

(c) An error rate of 5% and 95% confidence.

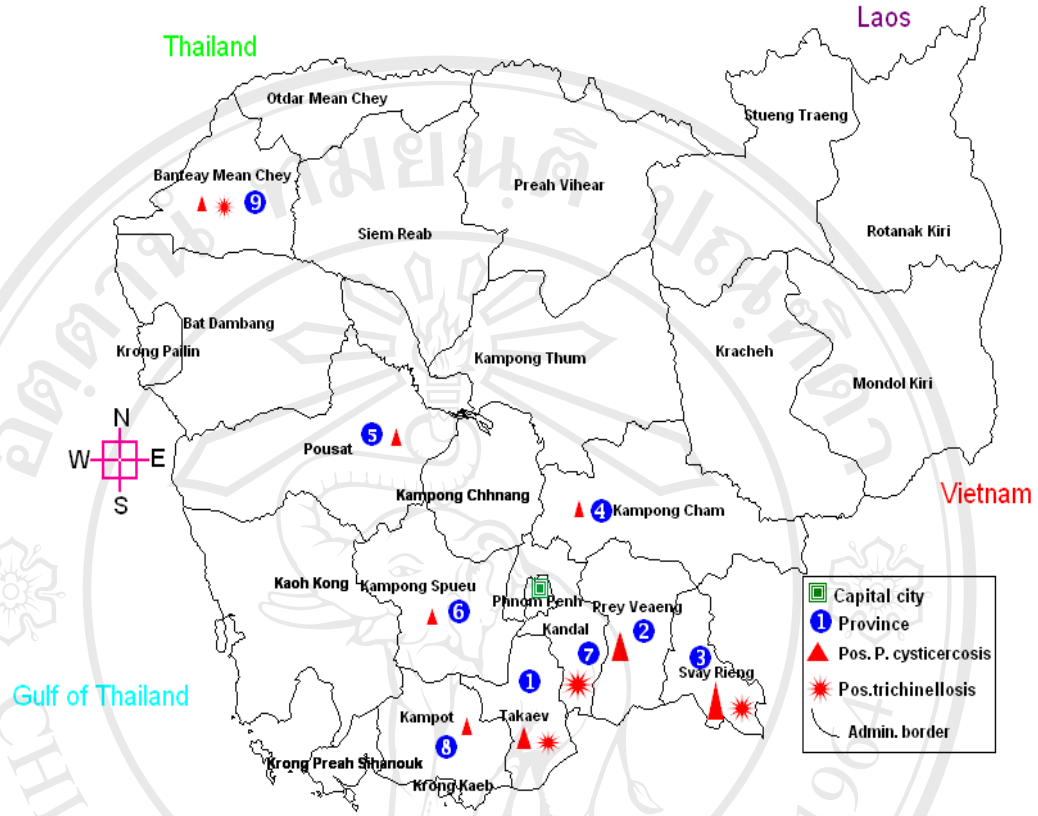

Figure 2. Map of Cambodia showing provinces where slaughter pigs were

3.7.2 Collection of biological samples

3.7.2.1 Cyst collection

In the slaughterhouse, pieces of meat and (or whole) organs with cysts were collected in plastic bags, labeled and transferred to the laboratory. The cysts were harvested, placed in separate plastic bags and kept in deep freeze until required for laboratory analysis.

3.7.2.2 Blood collection and preparation of sera

Blood samples taken at the slaughter process were transferred to the laboratory, kept in the laboratory at room temperature for 6-8 hours and then sera were collected and placed into numbered serum vials and kept in deep freeze for laboratory investigation.

3.8 Ante and post mortem examination for porcine cysticercosis

In the four study slaughterhouses with, a total of 432 pigs, 220 pigs were examined by tongue palpation, and after slaughter, 432 pigs by meat inspection for *T. solium* cysts respectively. The age, breed, sex, weight, and an approximate source of origin of each pig were recorded. The protocols of ante and post mortem examination were as follows:

3.8.1 Ante- mortem examination (Tongue palpation)

It was to conduct examination of the tongue for cysts in the upper and under the base of the tongues of local breed pigs. This was the traditional method for identifying cyst nodules in the tongue muscles. Mature viable *T. solium* larvae are oval (5-8 mm),

fluid-filled, and with a central whitish spot, which is the scolex (Gonzalez, 1994). Briefly the procedure was follows:

A pig was restrained, laterally and recumbent, and the head was stabilized or fixed by the use of a snare. The mouth was opened by the aid of a wooden rod, twisted across the upper and lower jaw and the tongue was gently pulled out using a piece of a cotton cloth. The under-surface of the tongue was thoroughly examined for the presence of cysts of *Taenia solium* larvae (Ngowi *et al*., 2004).

3.8.2 Post-mortem examination (meat inspection) and predilection sites

General provisions for the post-mortem inspection of pig carcasses for cysticerci of *T. solium* were followed which include long and parallel incisions into the external masseter muscles on both sides of the face in an upward direction to completely sever the parotid gland below the ear. Similar incisions were made in the internal masseters. The tongue was detached from the hyoid bone, viewed, palpated and cysts under the surface counted. A deep longitudinal incision covering about three-quarters the thickness of the tongue and covering the whole length of the tongue, diaphragm, esophagus, eyeball, conjunctiva, sexual organs and lymphatic glands was made to examine for cysts. After opening the pericardium, the heart was also visually examined for the presence of cysts. The heart was cut open and a deep (3/4 the thickness of septum) incision into the septum was made to expose any metacestodes. Three equidistant incisions were made in the triceps brachii muscle proximal to the elbow joint. Cysts that were encountered on incisional and intact surfaces were classified and enumerated as either viable (translucent, fluid-filled with invaginated whitish scolices visible) or degenerated (black, sand-like or powdery contents) (Boa *et al.*, 2002). Additionally, the following organs were inspected: lung, kidney, liver, spleen and brain. All organs and muscles with cysts were sliced in such a way that all fully developed cysts could be observed and noted (Dorny, 2004). The Distribution of cysts for those organs and muscle groups where cysts were counted and evaluated criteria as below:

Negative= 0 or no cyst in the carcasses

Low ≤ 1-100 cysts (light infection)

High > 100 cysts (Heavy cases)

3.9 Isolation, identification and characteristics of cysts of porcine cysticercosis

In the laboratory investigation in CMU, microscopic examination and serological test were conducted as indirect non-competitive ELISA test and endpoint titration. The protocols were described as follows:

3.9.1 Morphological examination of *T. solium* cysticerci

A total of 235 cysts were microscopically examined and randomly selected cysts (5 cysts per 1 infected pig) were used to measure the length of the hooks and their morphology.

The procedure of microscopic examination was as follows:

(a) Required specimens were cysts, collected from different sites or organs of infected pigs.

(b) Prepared 10% HCl solution.

(c) The free cysts from muscles put into 10% HCl in a Petri dish for 3-5 minutes

(d) Cyst wall was opened with forceps and scissor

(e) Then the invaginated scoleces were examined by 400 x (ocular 10 x, objective 40 x) magnification, looking for morphology and the presence of rostellum and hooks.

3.9.2 Indirect non-competitive enzyme linked immunosorbent (AB-ELISA)

The total of 440 blood serum samples were first screened by indirect non-competitive AB-ELISA test and doubtful results were re-examined. Test procedure is described as in the instructions below:

The AB-ELISA technique is according to the standard method used in the National Reference Laboratory for trichinellosis, Federal Institute for Risk Assessment (BfR) Berlin Germany. The same institute also provides the ELISA kits. The procedure is the indirect AB-ELISA and serum samples are tested for the specific anti-*Trichinella*-IgG.

An ELISA kit consists of

(a) Microtitre plates coated with *Trichinella* antigen (excretory-secretory antigen of *Trichinella spiralis*) contained 50µl *Trichinella*-E/S- antigen per well, storage at 4-8^{o}C

(b) *Trichinella*- positive control serum (1 ml, lyophilized), storage at –20^{o}C

(c) *Trichinella* negative control serum (1ml, lyophilized), storage at –20^{o}C Additionally, buffers and reagents:

(d) PBS buffer (not included, to be prepared according to protocol)

(e) Anti-pig IgG-peroxidase conjugate pre-diluted 1:10, (1.0 ml), storage at –20^{o}C (SIGMA, product N^{o}. A5670)

(f) ABTS buffer, dry matter from Boehringer, storage at 4-8 ^{o}C

(g) Tablets chromogen ABTS, storage at 4-8 ^{o}C

Test Procedure for AB-ELISA

(a) Preparation of PBS- Tween 20/dilution of prepared PBS- Tween 20 2000 ml of PBS- Tween 20 (pH= 7.2-7.4) consists of:

KH_2PO_4	0.4g
Na_2HPO_4* 12 H_2O	5.8g
NaCl	16.0 g
KCl	0.4 g
Tween 20	1.0 ml
Distilled water	ad 2000 ml

(b) Washing (blocking) of microtiter plate 1 time with aqua dest and 3 times with PBS-T (150µl), (every for 3 min)

(c) Preparation of test and control sera diluted in PBS- Tween 20 (1:100): 1ml (1000µl) PBS, add 10µl test serum samples or control sera, then placed them into the wells (volume 50 µl)

Example: a coated microtiter plate with serum samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Nc1	FS1	FS5	FS9	FS13	FS17	FS21	FS25	FS29	FS33	FS37	FS41
B	Nc1	FS1	FS5	FS9	FS13	FS17	FS21	FS25	FS29	FS33	FS37	FS41
C	Nc1	FS2	FS6	FS10	FS14	FS18	FS22	FS26	FS30	FS34	FS38	FS42
D	Nc1	FS2	FS6	FS10	FS14	FS18	FS22	FS26	FS30	FS34	FS38	FS42
E	Pc1	FS3	FS7	FS11	FS15	FS19	FS23	FS27	FS31	FS35	FS39	FS43
F	Pc1	FS3	FS7	FS11	FS15	FS19	FS23	FS27	FS31	FS35	FS39	FS43
G	Pc1	FS4	FS8	FS12	FS16	FS20	FS24	FS28	FS32	FS36	FS40	PBS-T
H	Pc1	FS4	FS8	FS12	FS16	FS20	FS24	FS28	FS32	FS36	FS40	PBS-T

Nc1= negative control serum

Pc1 =positive control serum

FS =samples for field sera

PBS-T= only coated with PBS-Tween 20 (blank of microtiter plate)

(d) Incubation for 30 min at 37 ^{o}C. Then washing as mentioned in the point (b)

(e) Anti-pig IgG- peroxidase-conjugate (pre-diluted 1:10) at final dilution of 1:1200 in PBS Tween 20 (200µl conjugate +24 ml PBS) is added in 50 µl amounts to all wells.

(f) Incubation for 30 min at 37 ^{o}C. Washing and soaking as mentioned in the point (b) and finally, 1 time with aqua dest.

(g) Preparation of ABTS buffer

Separate dilution of prepared citric phosphate buffer (pH=3.4-3.6):

ABTS buffer: dry matter	1.67 g
Distilled water	ad 100 ml

Dilution of 2 tablets ABTS (100 mg) in 100 ml of prepared ABTS buffer. Then, add 50 µl freshly prepared ABTS (substrate indicator system) to all wells. Store the chromogen at 4-8 ^{o}C in the dark (storage is possible for a couple of weeks).

(h) Measurement of extinction of all wells with the reader at 405nm if the positive control serum has an extinction value (OD) of 1.300-1.400. To reach this OD value, an incubation period for about 20-40 min at room temperature is needed.

(i) Preparation of stop solution for stop reaction of AB-ELISA

Calculation and Evaluation of test results

The results are calculated according to the "reference standard methods", *i.e.*, OD values of samples are related to those of the positive control in % as ELISA-index in the following way:

(a) Calculation of netto extinction (NE) of each well:

NE = OD-OD blank

(b) Calculation of mean netto extinction (mNE) of positive and negative control and samples

(c) Calculation of ELISA-index. The mean extinction of the sample (mNE sample) is related to the mean extinction of the positive control (mNE pos). The positive control has an ELISA-index of 100%

$$\text{ELISA-index (\%)} = \frac{\text{mNE sample}}{\text{MNE pos}} \text{ X } 100\%$$

(d) Evaluation of test results:

"*Trichinella*-negative"	(-)	ELISA- index (%) < 8
"*Trichinella*-questionable"	(+/-)	8 ≤ ELISA- index(%) < 14
"Trichinella-positive"	(+)	ELISA- index (%) > 14 (Nöckler *et al.*, 1995)

3.9.3 Endpoint titration of serum samples positive by single dilution AB-ELISA

Positive and doubtful serum samples were examined using endpoint titration test for confirmation. The test protocol was addressed as below:

(a) The procedure is as indirect non-competitive AB-ELISA, but it is different by a dilution step: 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1: 640 and 1:1280.

(b) Fill 90µl PBS and 10µl test sera into each well of the first row (1:10).

(c) Fill 50µl PBS into each well of second row until the last rows

(d) In the first row (1:10) of microtiter plate suck 3 times and take out 50µl of solution, then move to next row of microtiter plate and do the same way until the last row. Other steps follow AB-ELISA procedure.

(e) Evaluation of test results:

"Endpoint titration-positive" (+)	ELISA- index (%) ≥ 70 (1:10) and border of titer ≥ 1:80
"Endpoint titration- negative" (-)	ELISA- index (%) < 40 (1:10) and border of titer < 1:80 (Nöckler *et al*., 1995)

3.10 Questionnaire surveys

3.10.1 Slaughterhouse and hospital

Questionnaire surveys were done in the four former slaughterhouses and in four-selected hospitals around the Phnom Penh municipality (Hospital data were not available during time of study). 22 questions for slaughterhouses and 13 questions for hospital survey were performed (Appendix 2, I, II).

This was confirmed by examining slaughterhouse records, interviewing pig traders, farm owners and local veterinarians or inspectors and also by the nature of the typical local breeds brought for slaughter.

3.10.2 Pig farm survey ("Trace back" questionnaire survey for pig farm owners)

In total, 132 people answered these questionnaires containing 34 questions at the farm levels of three provinces, where there have been high risks. These areas were selected because most of the pigs brought to slaughterhouses originated here and are more infected (positive) than those of others.

This was administered in an attempt to establish information links of veterinary public health significance between slaughterhouses and farm origins (Appendix2, III). The people interviewed were from different levels and interests within those chosen.

3.11 Data management and analysis

Raw data from the slaughterhouse, laboratory, and questionnaire survey were appropriately coded and entered into a database using the MS. Excel program. Any errors were rechecked also using the MS excel program. Analyses were performed using Win Episcope 2.0, Epical 2000, EpiInfo, PopMap, StataSE 8.0 and SPSS 12.0 programs. Based on the objectives of this study, the analyses generally centered on the following:

(a) Determination of distributions of the various data variables and descriptive statistics of each;

(b) Determination of prevalence and sero-prevalence of porcine cysticercosis and trichinellosis, respectively, by slaughterhouse, farm and province;

(c) Comparison of distribution of prevalence and sero-prevalence of porcine cysticercosis and trichinellosis, respectively, by slaughterhouse, farm, disticts and province. Kruskal Wallis test or Chi-square ($\chi 2$) test were used and the results evaluated using as the criterion.

(d) Assessment of agreement between tongue palpation and meat inspection. Kappa statistic test was used and the results evaluated using the criterion described in Dohoo *et al*. (2003). The criterion is categorized into the following:

<0.2: slight agreement, 0.2-0.4: fair agreement, 0.4-0.6: moderate, 0.6-0.8: substantial and >0.8: almost perfect

4. RESULTS

Results were divided into 3 parts in relation to the objectives of this study as follows:

4.1 Investigation on porcine cysticercosis

The first objective of this study was to establish the current prevalence status of porcine cysticercosis in slaughter pigs, which came from different areas of Cambodia, and to conduct studies on predilection sites as well as the microscopic examination of cysts for confirmation of cysticercus species.

4.1.1 Slaughterhouse survey results

Visits were made to 4 slaughterhouses in Phnom Penh during 2004-2005 where a total of 432 pigs were examined. Among them, 220 were local pigs and 212 were commercial pigs. They came from 29 districts in 9 provinces and from 3 intensive farms.

4.1.1.1 Distribution of prevalence for porcine cysticercosis by breeds of slaughter pigs in Cambodia

In 4 slaughterhouses, 220 local pigs were examined by tongue palpation. After slaughter, all 432 pigs were examined for the presence of cysticerci by meat inspection. The results are summarized in Table 4. It can be seen that ante mortem (tongue) inspection yielded 29 positive pigs out of 220 local pigs examined (13.2%), while the number of positive cases increases to 47 pigs (21%) by meat inspection. None of the pigs from intensive production farms was positive.

Table 4. Distribution of prevalence for porcine cysticercosis by breeds of slaughter pigs in Phnom Penh/ Cambodia, 2004-2005

Type of pigs	No. pigs	Tongue palpation		Meat inspection	
		No. examined	No. positive (%)	No. examined	No. positive (%)
Local	220	220	29(13.2%)	220	47 (21%)
Commercial	212	0		212	0 (0%)

4.1.1.2 Distribution of prevalence of porcine cysticercosis by slaughterhouses

The distribution of the prevalence of porcine cysticercosis by slaughterhouses is showed in Table 5. The overall prevalence of porcine cysticercosis in slaughterhouses was 6.7% by tongue palpation and 10.9% by meat inspection. Moreover, there were no significant differences (KW= 3.06, df=3, p=0.382) among slaughterhouse prevalence obtained by tongue palpation. Similarly, it was not significant (H= 4.27, df=3, p=0.233) among slaughterhouse prevalence by meat inspection.

Table 5. Prevalence of porcine cysticercosis by four slaughterhouses in Phnom Penh/ Cambodia, 2004-2005

Sl.*** code	Total examined	Tongue palpation			Meat inspection		
		No. of positive	Preval* (%)	95% CI**	No. of positive	Preval* (%)	95% CI**
1	108	11	10.18	5.19-17.5	17	15.74	9.44-24.0
2	108	7	6.48	2.64-12.9	12	11.11	5.87-18.6
3	108	6	5.55	2.06-11.7	8	7.40	3.25-14.07
4	108	5	4.62	1.52-10.5	10	9.25	4.52-16.36
Total	432	29	6.71	4.54-9.48	47	10.87	8.10-17.2

*Prevalence, ** Confidence interval, *** Slaughterhouse

The comparison between tongue palpation and overall meat inspection gave a Kappa statistic of 0.717 (71.7%). Thus, it showed substantial agreement (0.6-0.8; Dohoo *et al.*, 2003) between tongue and meat inspection. This Kappa value was statistically significantly better than that expected (0.06) due to chance. However, the confidence interval was wide, reflecting considerable uncertainty about the estimate.

4.1.1.3 Distribution of prevalence for porcine cysticercosis by farm types

Table 6 shows that the prevalence of porcine cysticercosis as determined by tongue palpation was 16.1% and 25.5% by meat inspection in carcasses of outdoor-reared pigs, while it was 8.43 % by tongue palpation and 14.45 % by meat inspection in carcasses of pigs raised in mixed types of farms. Between farm-type specific prevalences, by procedure of inspection, showed significant differences by tongue palpation (Kruskal Wallis: H = 34.75, df = 4, p = 0.0001) and by meat inspection (Kruskal Wallis: H = 57.37, df = 4, p = 0.0001). None of the indoor-raised pigs and their carcasses was found infected.

Table 6. Distribution of prevalence for porcine cysticercosis by farm types, Cambodia, 2004-2005

Types of farms	Total examined	Tongue palpation			Meat inspection		
		No. of positive	Preval* (%)	95% CI**	No. of positive	Preval* (%)	95% CI**
Outdoor	137	22	16.05	10.34-23.3	35	25.54	18.5-33.7
Mixed	83	7	8.43	3.45-16.6	12	14.45	7.69-23.93
Intensive	212	0	0	0	0	0	0

* Prevalence, ** confidence interval

4.1.1.4 Distribution of prevalence of porcine cysticercosis by districts of provinces

The detailed distribution of the prevalence of porcine cysticercosis was arranged by districts where pigs were. Positive cases were from 19 out of 29 districts as summarized in Table 7.

Table 7. Distribution of prevalence for porcine cysticercosis by districts/Cambodia, 2004-2005

Districts	Total examined	Tongue palpation			Meat inspection		
		No. of positive	Preval* (%)	95% CI**	No. of positive	Preval* (%)	95% CI**
1	13	1	7.69	0.19-36	4	30.76	9.09-61.44
2	8	2	25	3.18-65	2	25	3.18-65
3	10	1	10	0.25-44.5	3	30	6.67-65.24
6	7	3	42.85	9.89-81.59	3	42.85	9.89-81.59
7	7	1	14.28	0.36-57.87	2	28.57	3.66-70.95
8	7	1	14.28	0.36-57.87	2	28.57	3.66-70.95
9	10	2	20	2.52-55.6	4	40	12.15-73.76
10	9	3	33.33	7.48-70	3	33.33	7.48-70
11	10	3	30	6.67-65.24	4	40	12.15-73.76
12	19	3	15.78	3.38-39.57	7	36.84	16.28-61.64
13	22	2	9.09	1.12-29.16	2	9	1.12-29.16
15	6	1	16.66	0.42-64.12	2	33.33	4.32-77.72
16	7	0	0	0	1	14.28	0.36-57.87
17	3	1	33.33	0.84-90.57	1	33.33	0.84-90.57
18	8	2	25	3.18-65	2	25	3.18-65
20	2	1	50	12.57-98.74	1	50	1.25-98.74
22	4	0	0	0	1	25	0.63-80.58
27	5	1	20	0.5-71.64	2	40	5.27-85.33
28	9	1	11.11	0.28-48.24	1	11.11	0.28-48.24

* Prevalence, ** confidence interval

This table shows prevalences of porcine cysticercosis obtained by both tongue palpation and meat inspection. A prevalence range of 7.7% to 50% in 19 districts was recorded by tongue palpation and 9% to 50 % by meat inspection. Methodologically, the district-specific prevalences were compared and the outcomes showed that they were significantly (Kruskal Wallis: H= 80.93, df=31, P=0.0001) different by tongue palpation and by meat inspection (Kruskal Wallis: H= 105.30, df=31, P=0.0001).

4.1.1.5 Distribution of prevalence of porcine cysticercosis by provinces

The Distribution of prevalence of porcine cysticercosis by provinces is shown in Table 8.

Table 8. Distribution of prevalence for porcine cysticercosis by provinces/ Cambodia

Province codes	Total examined	Tongue palpation			Meat inspection		
		No. of positive	Preval* (%)	95% CI**	No. of positive	Preval* (%)	95% CI**
1	80	4	5	1.37-12.30	9	11.25	5.27-20.28
2	33	8	24.24	11.0-42.25	12	36.36	20.4-54.87
3	58	10	17.24	8.59-29.42	15	25.86	15.25-39.0
4	20	2	10	1.23-31.69	4	20	5.73-43.66
5	11	2	18.18	2.28-51.77	2	18.18	2.28-51.77
6	27	1	3.7	0.09-18.97	2	7.40	0.91-24.28
7	164	0	0	0	0	0	0
8	20	1	5	0.12-24.87	2	10	1.23-31.69
9	19	1	5.26	0.13-26.0	1	5.26	0.13-26.0

*Prevalence, ** confidence interval

It can be seen that infections were distributed in pigs from 8 provinces. Pigs from province 7 were negative by both tongue palpation and meat inspection. The highest prevalence was among pigs from province 2, which was 24.2%, and the lowest

prevalence was in province 6, which was 3.7%. The comparison of prevalence among nine provinces showed significant (H= 14.81, p=0.038) results obtain by tongue palpation as well as meat inspection (H= 17.82, p=0.021).

4.1.1.6 Predilection sites of cysts for porcine cysticercosis by meat inspection.

To find out which organ of pigs was the predilection for cysticerci, each organ or muscle of infected pig carcasses was sliced, and the degree of infection was recorded as negative, low and high (Appendix 3). Results of organ involvement (%) in infected pigs are summarized in Table 9.

Table 9. Organ and muscle involvement (%) of cysts in slaughter pigs in Phnom Penh / Cambodia, 2004-2005 (n= 47)

Muscles/ Organs	Total of positive by organs	Degree of infection					
		Low*			High**		
		No.	%	95%CI	No.	%	95%CI
Tongue	31	19	61.3	42.18-78.15	12	38.7	21.84-57.81
Esophagus	37	28	75.7	58.8-88.22	9	24.3	11.77-41.19
Heart	47	27	57.4	42.17-71.74	20	42.6	28.25-57.82
Diaphragm	47	29	61.7	46.37-75.49	18	38.3	24.5-53.62
Brain	3	3	100	29.24-100	0	0	0
External masseter	44	13	29.5	16.76-45.2	31	70.5	54.79-83.23
Internal masseter	42	12	28.6	15.71-44.58	30	71.4	55.41-84.28
Triceps brachii	47	19	40.4	26.36-55.73	28	59.6	44.26-73.63
Fore limb	44	6	13.6	5.27-27.35	38	86.4	72.64-94.82
Hind limb	45	4	8.9	24.75-21.22	41	91.1	78.77-97.52

* ≤ 1-100 cysts per organ, **>100 cysts per organ

This table shows percent levels of infections categorized by either a low or high degree of infections from the examined muscles or organs as follows: brain, esophagus, diaphragm, tongue, heart, triceps brachii, external masseter, internal masseter, fore limb and hind limb muscles, respectively. The organs as lungs, spleen, intestine, kidney, liver were not detected to harbor cysticerci and, in a very few case, they were found in the brain.

4.1.2 Morphology of *T. solium* cysticerci hooks

In order to confirm the species of cysticerci, 235 cysts randomly were selected (5 cysts per sample) from tongue, esophagus, heart, muscle and others and examined microscopically for the presence of scolices and hooks. When present, the size of hooks was measured under the microscope at 100 and 400 magnifications and multiplied by 2.4 to yield micrometer (μm). The data are summarized in Table 10. Two hundred and thirty five (235) cysts were collected from 47 infected carcasses during meat inspection. Based on scolices and morphology of hooklets, all 235 cysts were identified as cysticerci of *Taenia solium*. The large rostellum hooks had a mean of 139 μm and a range of 120-156 μm, whereas the small rostellum hooks had a mean of 94 μm and a range of 84-108 μm.

Table 10. Morphology of *T. solium* cysticerci hooks obtained from pig carcasses

Type of hooks	Mean (μm)	SD* (μm)	Range of hook (μm)
Large rostellum hooks	139	12	120-156 (50-65)**
Small rostellum hooks	94	9	84-108 (35-45)

*Standard deviation, ** = Size was measured under 400 magnifications.

4.2 Investigation on trichinellosis

The second objective of this study was to establish the current seroprevalence status of trichinellosis in slaughter pigs from different areas of Cambodia. The 440 pigs examined were from 9 provinces, 29 districts and from the only three intensive (commercial) farms. Of these pigs, 224 pigs were of local breeds and 216 were commercial (exotic/crossbreeds) pigs.

4.2.1 Distribution of sero-positive samples for trichinellosis by breeds of slaughter pigs

Pig sera were tested for antibodies against *T. spiralis.* The results are presented in Table 11. This table shows that by AB-ELISA test and endpoint titration, 5 sera out of 440 serum samples positive for *Trichinella* antibodies. Two positive serum samples were from outdoor-raised pigs, 1 from mixed-raised pigs and 2 from indoor-raised pigs. Overall, five serum samples produced doubtful results.

Table 11. Distribution of sero-positive samples (by AB-ELISA) for trichinellosis by breeds of slaughter pigs in Cambodia, 2004-2005

Breeds of pigs	Total of samples	Serological examination			
		AB-ELISA		Endpoint titration	
		No. positive	No. doubtful	No. positive	No. doubtful
Local breeds (native)	224	3	3	3	3
Commercial breeds	216	2	2	2	2
Total	440	5	5	5	5

4.2.2 Summary results of AB-ELISA reading of Trichinella of slaughter pigs

Ten sera, 5 positive and 5 doubtful were re-examined by AB-ELISA. The results are shown in Table 12. This table shows that in the first AB-ELISA two samples were positive and 8 samples gave doubtful results (+/-). Of the two positive samples, one was from indoor-farmed pigs and the other from outdoor-raised pigs. The first of the two positive samples had OD^{a1}= 0.403 and an $Index^{a1}$ (cut-off) = 34% while, the second one had OD^{a8} =0.352 and an $Index^{a8}$ (cutoff) = 30%. The second AB-ELISA revealed three more positive sera with the following readings: OD^{b6} = 0.232, $Index^{b6}$ = 22%, OD^{b7}= 0.225, $Index^{b7}$ = 21%, OD^{b9} = 0.275 and $Index^{b9}$ = 25.6%. In this second test five serum samples gave doubtful results.

4.2.3 Endpoint titration of serum samples positive by single dilution AB-ELISA.

The positive samples were tested by endpoint titration for confirmation and the results of the test are shown in Table 13. This table shows that the five samples were confirmed to be positive by endpoint titration.

4.2.4 Sero-prevalence of trichinellosis of slaughter pigs by husbandry and geographical distribution

Distribution of sero-positive pigs and prevalence of sero-positivity are shown in Table 14. Table 14 shows that the overall sero-prevalence of trichinellosis in slaughtered pigs was found 1.13%. One or two positive pigs were found in each slaughterhouse.

By farm type, 1.40% of outdoor-raised pigs, 1.21% of mixed-reared and 0.92% of indoor-reared pigs were positive, respectively. The comparison between farm-types specific sero-prevalence, by AB-ELISA, showed no significant differences (Kruskal

Wallis: H = 7.16, df = 4, p = 0.127). Doubtful test results were 0.70% in outdoor-raised pigs, 2.43% in mixed-reared and 0.9% in indoor-raised farms.

Positive pigs were from four out of 29 district and 3 intensive farms. Sero-positive rates ranged from 2.66 to 16.66%. The district-specific prevalences were compared and the outcomes showed that they were not significantly (Kruskal Wallis: H= 10.97, df=31, P=0.268) different by AB-ELISA.

From 9 provinces, sero-positive pigs were distributed in 4 provinces. The percentage of positive ranged from 1.19% in province 9 to 5.26% in province 7. The doubtful test results ranged from 1.19% of pigs from province 7 to 5% in province 4. The comparison of prevalence among 9 provinces showed no significant (Kruskal Wallis: H= 14.81, df=8, p=0.592) results obtain by AB-ELISA.

Table 12. Summary results of AB-ELISA reading of *Trichinella* of slaughter pigs (n=440)

Specimens No.	First test							Second test			
	Slaughterhouse ID	Farm ID	Breed	Age month	OD[a]*	Index [a] (cut-off) (%)	Results	OD[b]*	Index[b] (cut-off) (%)	Border-line titer	Results
1	1	indoor	exotic	6	0.403	34	++++	-	-	-	-
2	4	mixed	local	6	0.164	13.7	+/-	0.115	11	1: 160	+/-
3	1	outdoor	local	7	0.105	8.8	+/-	0.141	13	1:160	+/-
4	2	indoor	exotic	6	0.272	17	+/-	0.112	10	1:80	+/-
5	2	indoor	exotic	6	0.227	14	+/-	0.140	12	1:80	+/-
6	3	outdoor	local	7	0.243	15	+/-	0.232	22	1:160	+++
7	4	mixed	local	6	0.175	11	+/-	0.225	21	1:320	+++
8	3	outdoor	local	7	0.352	30	+++	-	-	-	-
9	3	indoor	exotic	6	0.135	12	+/-	0.275	25.6	1:160	+++
10	3	mixed	local	6	0.263	14.8	+/-	0.143	12.9	1:80	+/-

*Optical density.

OD[a]* = Optical density of the first AB-ELISA, OD[b]* = Optical density of the second AB-ELISA.

Index [a] = cut off of point of the first AB-ELISA, Index[b] = cut off of point of the second AB-ELIS.

Table 13. Endpoint titration of *Trichinella* positives of slaughter pigs by single dilution AB-ELISA

Specimens No.	ELISA index % (1:10)	Border titer	Result
1	83	1:640	+++
6	78	1:160	++
7	76	1:320	++
8	73	1:320	++
9	81	1:320	+++

Table 14. Sero-prevalence of trichinellosis of slaughter pigs by husbandry and geographical distribution in Cambodia, 2004-2005

Items	Total examined	AB-ELISA				
		No. positive	Preval* (%)	95% CI**	No. doubtful	Doubtful (%)
At farm types						
Outdoor	142	2	1.40	0.17-4.99	1	0.70
Mixed	82	1	1.21	0.03-6.60	2	2.43
Indoor	216	2	0.92	0.11-3.30	2	0.9
At district level						
District 1	13	0	0	0	1	7.69
District 5	6	1	16.66	0.42-64.12	0	0
District 11	10	1	10	0.25-44.50	1	10
District 16	7	0	0	0	1	14.28
District 29	10	1	10	0.25-44.50	0	0
District 31	75	2	2.66	0.32-9.30	2	2.66
At province level						
Province 1	80	1	1.25	0.03-6.76	1	1.25
Province 3	62	1	1.61	0.04-8.66	1	1.61

Table 14 (Continued). Sero-prevalence of trichinellosis of slaughter pigs by husbandry and geographical distribution in Cambodia, 2004-2005

Items	Total examined	AB-ELISA				
		No. positive	Preval* (%)	95% CI**	No. doubtful	Doubtful (%)
Province 4	20	0	0	0	1	5
Province 7	168	2	1.19	0.14-4.23	2	1.19
Province 9	19	1	5.26	0.13-26.02	0	0
Total	440	5	1.13	0.36-2.63	5	1.13

* Prevalence ** Confidence interval

4.3 Results from the questionnaire surveys

The questionnaire surveys were administered to interviewees in three sites, namely four slaughterhouses, farms in three provinces and four hospitals around Phnom Penh city. All were conveniently selected. A summary of the responses is as follows:

4.3.1 Responses of slaughterhouse questionnaire survey

The responses are summarized in Appendix 4. Twenty-three percent of respondents were in slaughterhouse 1, 24% in slaughterhouse 2, 29% in slaughterhouse 3 and 24 % in slaughterhouse 4. Sixty percent of slaughterhouse workers had a low level of education. Eighty nine percent of respondents said that demand of pork in Phnom Penh was higher (average 500-2000 slaughter pigs per day) than that of other meats such as beef, fish and seafood. They indicated that the demand fluctuates (increase or decrease) by seasons. All (100%) the responses indicated that the environmental and hygienic conditions were poor. Most (40%) of the pigs delivered to the slaughterhouses were raised in a household environment while, 33% and 27% were produced at small and medium farms (intensive production farms) respectively.

Of all the interviewees, 36 % and 61% of respondents had not heard about porcine cysticercosis and trichinellosis, respectively. Thus, only 57% of porcine carcesses were inspected for cysticercosis using tongue palpation (13%), by meat inspection (31%) and by using both (tongue palpation and meat inspection) (56%). The answers further showed that 63 % of respondents found parasitic diseases, mainly porcine cysticercosis. Respondents indicated that they detected 1-3 infected carcasses per month. They (74 %) speculated that the infected carcasses were from pigs that were raised extensively. Concerning trichinellosis, they have never inspected pigs for the infection.

Respondents observed infected carcasses irrespective of age, i.e., young or old as either having heavy (57 %) or light (43 %) infection. Twenty two percent of the heavily infected carcasses were destroyed whereas, 64% were processed for animal feeds or for human consumption in special institutions and only 14 % (lightly infected cases) went straight for human consumption. Most people, 57%, prefer well-cooked food for consumption, while the remainders ate uncooked or both cooked and uncooked foods.

4.3.2 Responses to farm questionnaire survey

The responses are summarized in Appendix 5. Thirty percent of respondents were in province 1, 34% in province 2 and 36% in province 3. The appendix shows that seventy nine percent of farmers had a low level of education. Most (85%) of the pigs in these areas were reared in the household environment, while 15% were produced in the small and medium farms (intensive production farms). The stages in pig production were as follows: fattening 45%, piglets (31%) and both (24%) stages. These pigs were sold depending on family needs and income. For example, 32 % of pigs were at the age of 2-4 months, 22% at 6 months, 36 % at 7-12 months and 10 % at more than 12 months. But, it is worth noting that sixty five percent of the farms brought in piglets from outside. The pig breeds commonly found in the study provinces were local pigs (native) (89%), while 11% of the respondents reared exotic and cross breeds.

Fifty four percent of the respondents used traditional feeds, 17% used leftovers, 5% used formula feeds and 24% used mixed rations. In addition, 55 % of respondents produced their own feeds, while 45% of them bought from outside. Water sources for both humans and pigs were as follows: 66% from ponds, underground and 17% from river waters (17%). Ninety three percent of respondents indicated that they used the water from its sources directly without treatment.

Only 4% of respondents had concrete floors in pens. All the responses indicated that the environmental conditions and waste management were poor. Most (83%) of the people in the rural areas encountered parasitic diseases. Of all the interviewees, 40%, 54% and 83% had not heard about porcine cysticercosis, *Taenia solium* and trichinellosis, respectively. Of all live pigs, only 66% were inspected for cysticercosis using tongue palpation and the rest (34%) were examined using both (tongue palpation and meat inspection). Respondents (28%) indicated that they detected 1-3 infected pigs per month, while 18% reported more than 5 infected pigs per month. Most (61%) of respondents sent infected pigs for proper processing and only 14% referred infected pigs directly for human consumption. Proper food cooking was indicated by 59% of the respondents while, 35% preferred uncooked pork and 6% well cooked or mixed. In addition, 84% of respondents said that there were not enough communal toilets. Seventy nine percent of respondents stated that control and prevention were still very poor and still not applied to these areas due to underdeveloped public health infrastructures within the country.

4.3.3 Responses to hospital questionnaire survey

Health personnel in two hospitals, one public health institute and the Pasteur Institute in Phnom Penh, Cambodia, were visited and questionnaires administered to them. In all these institutions, there were no records of human neurocysticercosis and medical staff had very little knowledge of neurocysticercosis. None of the medical centres visited had the capacity for diagnosing neurocysticercosis.

5. DISCUSSION AND CONCLUSION

Porcine cysticercosis and trichinellosis are crucial zoonitic problems in a country like Cambodia with high pork consumption. In a period of six months, a total of 62,400 pigs were slaughtered in 4 slaughterhouses in Phnom Penh/Cambodia. Out of these numbers of animals, 432 were randomly selected for this study. The following discussion of the obtained results summarizes as follows:

5.1 Investigation on porcine cysticercosis

This was the first prevalence study of *T. solium* cysticercosis in slaughter pigs in Cambodia. Thus, no published prevalence studies existed in Cambodia prior to this work, except a short review by Singh *et al.* (2002). Hence, the following discussion of the obtained results has been compared and contrasted with reports from other countries.

In Cambodia, lingual examination has been used to identify pigs having *T. solium* cysts. This is performed for purposes of eliminating infected pigs so as not to lose money due to carcass condemnation during meat inspection.

As shown in the result, it was found that the prevalence of porcine cysticercosis by breeds was 13.2 % in local pig carcasses examined by ante-mortem inspection. This prevalence increased to 21% in the pig carcasses by meat inspection. On the other hand, the total distribution of prevalence for porcine cysticercosis in this study (n=432) was 6.7% by tongue palpation and 10.9 % by meat inspection. These findings agree with observations of a review by Singh *et al.* (2002) from Cambodia and different neighboring countries: 10% in Cambodia, 10% in Laos, 11% in Thailand and 8% in Vietnam. However, none of the pig carcasses from intensive production farms was found positive.

In the slaughterhouse study, the two methods were used, tongue palpation and meat inspection: The results differ from 6.7% by lingual examination and 10.9 % by meat inspection and result was determined by Kappa statistic. However, a sensitivity of lingual examination was about 62% to detect porcine cysticercosis, found by meat inspection. Thus tongue palpation is a very specific method to demonstrate cysticercosis in pigs, but it has a diagnostic sensitivity not exceeding 70%. These findings agree with some studies previously reported. In lightly infected pigs, both the sensitivity and the specificity are low (Sciutto *et al.*, 1998; Boa *et al*., 2002). The study in Nigeria found that lingual examination could detect 14.9% of truly infected pigs (Onah and Chiejina, 1995), while in Peru, Gonzalez and others found that 70% of the infected pigs were detected by lingual examination (Gonzalez *et al*., 1994). Dorny (2004) reported that the routine carcass inspection was slightly better than tongue inspection in detecting moderate to heavy infections, but was equally insensitive in light infections. Nevertheless, lingual examination is a relatively quick and inexpensive way of doing a rapid assessment of the presence and burden of porcine cysticercosis. Pig traders in the district of Cambodia have been using the lingual examination method to reject pigs that are recognized to have *T. solium* larvae under the tongue for fear of losing money due to carcass condemnation during meat inspection.

Knowing the predilection sites, *i.e*., organs, which harbor a large number of cysticerci helps to increase diagnostic efficacy. The organs where cysticerci were mostly found infected in a higher degree were: the skeleton muscles as hindlimb, forelimb, masseters and in a lower degree were: heart, tongue, esophagus, diaphragm and rarely brain. Organs in which cysticerci were not detected include liver, lungs, spleen, kidney and intestine. Thus, infected organs vary depending on the degree of infection. These findings agree with some studies previously reported. For examples, Mendez *et al*. (1986) found the predilection sites to be forelimb above the elbows (pork shoulder), tongue and heart. Similar findings made by Onah and Chiejina, (1995) and Boa *et al*. (2002) who found the masseters and triceps muscles to be predilection sites. Liver, lung, spleen, kidney and intestine were not infected. However, Ma *et al*. (1992) found cysticerci of *T. solium* from lungs, kidneys and

livers of pigs in China and they differentiated from other *Taenia* species by the morphology of the hooks. These contrasting results may be due to species differences of *Taenia* and/or pigs from Africa and Asia, which could affect the host–parasite relationship. Also, it might be due to infection by *T. asiatica* found only in Asia, which has predilection in the liver.

Types of farm management are important factors, which may influence the infection rate. The prevalence of porcine cysticercosis in outdoor-reared types was 16.05 % (tongue palpation) and 25.54 % (meat inspection), while in the mixed-raised type found positive cases of 8.43 % (tongue palpation) and 14.45 % (meat inspection) were found. None of the pigs reared indoors or on intensive production farms was infected. These findings indicated that farm types have an influence on the infection rates of porcine cysticercosis.

Concerning the districts where pigs were reared, positive animals were from 19 out of 29 districts. By ante-mortem examination, prevalence ranged from 7.69 % to 50 % in 19 districts, while meat inspection showed prevalence of 9 % to 50 %. The findings are comparable to those reported in different areas of countries as China (0.8-40 %), Mozambique (6.5-33.3 %), Tanzania (4.5-26.9%) and Latin America (4-38.9%), (Table 2, pp.36).

In this study, 235 cysts were randomly selected (5 cysts of the 47 positive carcasses) from tongue, esophagus, heart and muscles examined microscopically for the presence of armed scolices. When present, sizes of hooks were measured. All of the 235 scolices exhibited small and large hooks. Obviously, in this study cysts of *T. asiatica* were not found. This is in agreement with Morakote *et al.* (2000) who reported that adult worms recovered from infected villagers in Chiang Mai, Thailand, consist of only *T. saginata* and *T. solium*. These findings pose questions on the role of *T. asiatica* in Cambodia and Thailand. Further studies should be done on identifying species of the adult tapeworms obtained from local people in endemic areas of Cambodia.

5.2 Investigation on trichinellosis

Similar to the porcine cysticercosis study, the present survey of trichinellosis in pigs was the first done in Cambodia.

The present study was conducted in 4 slaughterhouses. 440 pig serum samples were examined for trichinellosis.

Sero-prevalence for trichinellosis of slaughter pigs by AB-ELISA and endpoint titration for confirmation of trichinellosis positives was conducted at CMU laboratories. Five specimens were sero-positive: two were outdoor-raised pigs, one pig was mixed-reared and two positives were from indoor-raised pigs. Beside 5 positives, 5 specimens gave doubtful results. An overall sero-prevalence of trichinellosis was of 1.13 %. Similar findings are reported from some studies that used similar test conditions. For example, sero-prevalences of trichinellosis were recoded up to 2.5% in Mexico (La Rosa *et al*., 1998) and in China ranged between 0.021-7.3 % (Liu *et al.*, 2002). However, sero-prevalences of trichinellosis were found under 0.02% in Northern of Thailand (Takahashi *et al*., 2000) as well as under 0.013 % in North America (Gamble, 2000). These contrasting results may be due to many risk factors such as poor farm management, feed contaminations, close contact with rodents and wild animals and other sources.

In this study, all sero-positives were found in the 3-differnt farm types. The positive rates were: 1.40% of outdoor-reared pigs, 1.21% of mixed raised and 0.92% of indoor-reared animals. The infection rates by farm management did not differ significantly. This is much in contrast to cysticercosis, which indoor pigs were not infected with. It is possible that some risk factors are different, especially the sources of infection, such as infected rodents and feedstuffs.

Some positives were found in some areas of Cambodia, even though the details about sources of infection are not included in the objective for study due to time

limitation. Generally, it can be explained with frequently roaming free-range pigs coming into contact with infected rodent carcasses, with contaminated pork, garbage, etc. Most farmers also feed pigs with swill feed and uncooked animal organs. All these factors were suspected to favor the occurrence of swine trichinellosis, and may lead to the outbreak of human trichinellosis. The high prevalence of trichinellosis in both humans and animals invariably leads to significant economic loss. The positivity of trichinellosis in pigs among provinces shows that there were no significant differences of sero-prevalence of trichinellosis.

The questionnaire surveys were performed on residents of provinces, slaughterhouse workers and hospital personnel around Phnom Penh. Out of 202 people interviewed, 70 people were conducted in four slaughterhouses and 132 in three provinces. Awareness of the *T. solium* disease condition was virtually absent. In districts of provinces, human carriers and epilepsy were not reported. Pig husbandry practices, sanitary conditions, hygienic control and slaughter processes are very poor. The infection of pigs could be found very easily, when compared with other diseases. Surveys of hospitals around Phnom Penh showed that the data on human cysticercosis were lacking. It can be concluded that the results from the questionnaire survey, together with those from the prevalence study, help to create a better understanding on the role of pig slaughtering and farming management systems on the parasite transmission and provide more information about epidemiological factors which is necessary for the health authorities to develop integrated measures for control and prevention of cysticercosis and trichinellosis in the near future.

5.3 Conclusion and recommendation

It can be concluded that the two parasitic zoonotic diseases like porcine cysticercosis and trichinellosis may pose major problems in some endemic districts of provinces in Cambodia. The present studies used for disease detection have greatly contributed to a better understanding of the prevalence and epidemiology of the

infection and also of the impact of these diseases related to pig production systems and human health.

In this study design, there were advantages and disadvantages. It is noted that this study obtained more clear and precise prevalence than the previous study (Singh, 2002). This is because of the detailed study design that was based on well-established sampling methods, sample size determination, and data collection methods as well as standardized laboratory techniques. However, time was the limiting factor of this cross-section design. For example there was not enough time to study isolation and identification of *Trichinella* species as well as potential risk factors.

For further study, better study designs e.g. cohort study are important in investigations applied to trichinellosis, using gold standard methods like digestion and PCR for isolation and identification of *Trichilla* species in Cambodia. This type of study design (cohort study) could enable researchers also to determine or identify risk factors associated with trichenellosis in Cambodia. Results from a study design would assist in planning and implementing cost-effective surveillance systems and strategic control/eradication programs.

It is recommended that the hygienic standard at the slaughterhouses in Cambodia must be improved with standard guidelines of meat inspection. Prevention, disease controls, disease surveillance, awareness and treatment or de-worming of human tapeworm carriers in endemic areas should be implemented, additionally; toilet facilities and pig husbandry practices should be improved, avoiding access of pigs to human feces in the rural areas.

Recent surveys showed that many people in the rural areas either ignore or are ignorant of the danger to which they expose themselves by eating infected meat. Most of them did not understand the association between the presence of cysticerci in the animal and the tapeworm infection in man. Thus the long-term strategy involves health education, modernization of pig farming, and rigorous inspection of pork in slaughterhouses, creation of hygienic and sanitary conditions in the community,

disease control measures and active epidemiological surveillance systems to identify tapeworm carriers and to activate potential eradication.

A recent study conducted in slaughterhouses found that the chance of detecting cysts of porcine cysticercosis in slaughter pigs is low under the current meat inspection regulations. However, due to the lack of well-organized meat inspection and the presence of illegal slaughtering, almost all infected carcasses are marketed and/or consumed. Usually, contaminated pig carcasses with cysticerci are sold at a decreased price. It can be also suggested that meat inspection is mandatory to all slaughterhouses and put into national law.

It is recommended that intensive national surveillance is essential for trichinellosis control in both domestic pigs and wild animals in Cambodia based on the OIE guideline. Consumers and producers of swine in endemic area should follow the recommendations of the International Commission on Trichinellosis for the control of *Trichinella* in domestic and wild animals intended for human consumption (Gamble *et al*., 2000). Enforcement of effective measures for trichinellosis control in Cambodia is strongly required. The easiest measure would be to educate and inform the public, insisting on the need to cook pork meat thoroughly. Then it would be to organize a network of laboratories specifically responsible for *Trichinella* monitoring and control in animals in slaughterhouses, as well as to use gold standard methods as recently indicated by the International Commission on trichinellosis (*i.e.*, the digestion test and ELISA test which are ten or a hundred times more sensitive than microscopy). It would also be useful to collect samples from wild animals, to evaluate more precisely the areas where intense control should be applied.

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7. APPENDICES

Appendix 1: Reagents and solutions

I. Reagents and solutions

1. Porcine cysticercosis:

Cysticerci examination: 10 % HCl, 10% formalin and 100% alcohol

2. Trichinellosis

Blood serum samples

Microtiter plates coated with *Trichinella* antigen (larval excretory-secretory antigen of *Trichinella spiralis*): 50 µl *Trichinella* E/S antigen per well.

Trichinella-positive control serum (1 ml lyophilized)

Trichinella-negative control serum (1 ml lyophilized)

PBS buffers (Physiological buffer solution

Sterile distilled waters (Asqua bitus)

Ati-pig IgG-peroxidase conjugates per diluted 1:10 (SIGMA, product N°. A5670)

ABTS buffer, dry matter from Boehringer

Stop solution

Tablets chromogen ABTS

2000 ml of PBS-Tween 20 (pH=7.2-7.4) consist of

KH_2PO_4	0.4	g
$Na_2HPO_4 \times 12H_2O$	5.8	g
NaCl	16.0	g
KCl	0.4	g
Tween 20	1.0	ml
Distilled water	ad 2000	ml

Appendix 2: Survey questionnaires

I. Hospital questionnaire survey of neurocysticercosis in humans in selected hospital

Date_____/_____/_____;

Name of the Hospital_____ Location_____

Name of the Doctor_____ Title _____

1. Patient's residence areas:_____
   _____
2. Number of the patients showing following clinical signs:
   a) Seizure_____ b) Violent behavior_____ c) Head ache_____
   d) Depression_____

e) Facial palsy____ Monoparesis or hemiplegia.

3. What is the method of diagnosis used in the hospital?
   a) CT scan____ b) ELISA____ c) MRI____ d) Other_____
4. How many epileptic cases are found for the period of:
   a) Year 2000_____ b) 2001_____ c) 2002_____ d) 2003_____
5. How many confirmed neurocysticercosis cases:
   a) Year 2000____ b) 2001____ c) 2002_____ d) 2003_____
6. Age groups:
   a) 0-14 year_____ b) 15-35 years____ c) About_____
7. Sexes:
   a) Male____ b) Female_____
8. Patient's socioeconomic status:
   a) Upper_____ b) Middle_____ c) Lower_____
9. Treatment:
   a) Surgery_____ b) Medical _____
10. Name of the drugs used:
    a)_____
    b)_____

c)_____

11. Is the treatment effective?

a) Yes____ b) No____

12. List the patients according to caste:

a) Brahmins_____ b) Non-brahmins_____

13. Other comments:_____

_____

II. Slaughterhouse questionnaire survey of porcine cysticercosis and trichinellosis

Date_____/_____/_____;

Interviewee name _____ Title _____

Interviewer name _____ Title _____

Name of slaughterhouse_____

Location_____

1. Educational level

a) Low___ b) Medium___ c) High___

2. Slaughter pigs for consumption per day:

a) 1-100___ b) 100-500___ c) 500-2000___ d) more than 2000

3. Average slaughter pigs per day in each slaughterhouse:

a) 50-200___ b) 200-300___ c) 300-600___ d) More than 600___

4. Environment and hygienic aspects in slaughterhouses

a) Good__ b) Poor___

5. Pig origins?

a) Household raising ___ b) Small and medium farm____

c) Intensive farm ___

6. Have you ever heard porcine cysticercosis?

a) Yes___ b) No___

7. Have you ever heard trichinellosis?

a) Yes___ b) No____

8. Inspected pigs for porcine cysticercosis?

a) Yes___ b) No___

9. Which method use for diagnosis?

a) Tongue palpation___ b) Meat inspection___ c) Both____

10. Have you ever found parasitic diseases of slaughter pigs?

a) Yes___ b) No____

11. Have you ever found infected cases of cysticercosis?

a) Yes___ b) No___

12. How many cases per month?

a) 1-3___ b) 3-5___ c) more than 5___

13. Most confirmed cases?

a) Household raising ___ b) Small and medium farms ___ c) Intensive farm ___

14. Inspected pigs for trichinellosis?

a) Yes___ b) No___

15. Ages of slaughter pigs

a) Young___ b) Medium___ c) Old___ d) All___

16. Pig breed for slaughtering?

a) Local___ b) Exotic__ c) Crossed bred___

17. Most ages of infected pigs

a) Young___ b) Medium___ c) Old___ d) All___

18. Most cases infected?

a) Heavy___ b) Light ___

19. Infected carcasses and organs use for:

a) Human consumption___ b) Processing___ c) Culling___

20. Food habits

a) Cooked___ b) Uncooked___ c) Mixed___

21. Disease control and prevention?

a) Yes___ b) No___

22. Other comments: _____

_____

III. Pig farm questionnaire survey of porcine cysticercosis and trichinellosis in 3 provinces

Date_____/_____/_____;

Interviewee name _____ Title _____

Interviewer name _____ Title _____

Name of farm or village_____

Name of owner_____

Location_____

Date of establishment_____/_____/_____

1. Educational level

   a) Low___ b) Medium___ c) High___

2. Sizes of pig production

   a) House hold raising___ b) Small & medium___ c) Intensive___

3. Pig production system?

   a) Free ranging ____ b) Mixed ____ c) Indoor ____

4. Target of pig production?

   a) Piglet___ b) Fattening ___ c) Mixed ___

5. Buying piglets from outside for raising?

   a) Yes___ b) No___

6. Age of pigs for selling:

   a) 2-4 month___ b) 6 month___ c) 7-12 month____

   d) More than 12 month___

7. Pig breed for raising:

   a) Local___ b) Exotic___ c) Crossed bred___

8. Do they have an identification number?

   a) Yes___ b) No___

9. Types of feed:

   a) Ratio feed ___ b) Leftovers ___ c) Mixing___ d) Traditional___

10. Getting feeds from outside?

11. Drinking water for pigs and human uses:

a) Tap water___ b) Underground water___ c) Pond water__ d) River___

12. Condition of water:

a) Direct use___ b) Treatment___

13. Condition of the holdings: Floor type

a) Concreted___ b) un-concreted___ c) Mixed

14. Environment of pig raising areas:

a) Clean___ b) Dirty___

15. Waste management:

a) Good____ b) Poor

16. Having problems with parasitic diseases

a) Yes___ b) No___

17. Have you ever heard porcine cysticercosis?

a) Yes___ b) No___

18. Have you ever heard of *Taenia solium*?

a) Yes___ b) No____

19. Have you ever heard of trichinellosis?

a) Yes___ b) No____

20. Investigate pigs for cysticercosis?

a) Yes___ b) No___

21. Which method use for diagnosis?

a) Tongue palpation___ b) Meat inspection___ c) No inspected____

22. Investigate pigs for trichinellosis?

a) Yes___ b) No___

23. Have ever confirmed porcine cysticercosis?

a) Yes___ b) No___

24. How many infected cases of cysticercosis per month?

a) 1-3 case___ b) 3-5___ c) More than 5___

25. Age of infected pig confirmed porcine cysticercisis?

a) 2-4 month___ b) 6 month___ c) 7-12 month____

d) More than 12 month___

26. Infected pigs sale for:

a) Human consumption___ b) Processing ___ c) Culling__

27. Food habits

a) Cooked___ b) Uncooked___ c) Mixed___

28. Are there sufficient toilets?

a) Yes___ b) No___

29. In the farms or villages, are there human cases of *Taenia solium or* neurocysticercosis?

a) Yes___ b) No___

30. Disease control and prevention?

a) Yes___ b) No___

31. Is the measure effective?

a) Yes____ b) No____

32. Treatment:

a) Conventional ____ b) Traditional ____c) Mixed___

33. Is the treatment effective?

a) Yes____ b) No____

34. Other comments:

_____

_____

Appendix 3. Degree of infection by cysticerci in organs and muscles (n=47)

Pig	Degree of infection by cysticerci in muscles and organs of carcasses													
N^{o}	To	Es	He	Di	Li	Lu	S	Ki	Br	Ext	Int	Tri	Fl	Hl
1	L	L	H	L	N	N	N	N	N	H	H	L	H	H
2	L	N	L	L	N	N	N	N	N	H	H	H	H	H
3	N	L	L	L	N	N	N	N	N	L	H	H	H	H
4	H	H	H	H	N	N	N	N	N	L	L	L	L	L
5	L	N	L	L	N	N	N	N	N	L	H	H	H	H
6	N	L	L	L	N	N	N	N	N	H	H	H	H	H
7	L	L	L	L	N	N	N	N	N	H	H	L	H	H
8	H	H	H	H	N	N	N	N	N	L	L	L	L	H
9	L	L	H	L	N	N	N	N	N	H	H	H	H	H
10	N	L	L	L	N	N	N	N	N	L	N	L	L	H
11	N	L	L	L	N	N	N	N	N	H	H	H	H	H
12	L	L	H	H	N	N	N	N	N	L	L	L	H	H
13	H	H	H	H	N	N	N	N	N	H	H	H	H	H
14	N	N	L	L	N	N	N	N	N	H	H	H	H	H
15	N	L	L	L	N	N	N	N	N	L	L	H	H	H
16	L	N	L	L	N	N	N	N	N	H	H	L	H	H
17	H	H	H	L	N	N	N	N	N	N	N	L	L	L
18	N	L	L	L	N	N	N	N	N	N	L	L	N	N
19	N	L	L	L	N	N	N	N	N	H	H	H	H	H
20	N	L	L	L	N	N	N	N	L	H	H	H	H	H
21	L	L	H	L	N	N	N	N	N	H	H	H	H	H
22	N	N	L	L	N	N	N	N	N	H	L	H	H	H
23	H	H	L	H	N	N	N	N	N	L	L	L	H	H
24	L	L	L	H	N	N	N	N	N	H	H	H	H	H
25	H	L	H	H	N	N	N	N	L	H	H	H	H	H
26	L	L	H	H	N	N	N	N	L	H	L	L	H	H

Appendix 3(continued). Degree of infection by cysticerci in organs and muscles (n=47)

Pig N^{o}	Degree of infection by cysticerci in muscles and organs of carcasses													
	To	Es	He	Di	Li	Lu	S	Ki	Br	Ext	Int	Tri	Fl	Hl
27	H	N	L	L	N	N	N	N	N	H	H	H	H	H
28	N	L	L	L	N	N	N	N	N	H	H	H	H	H
29	L	L	H	H	N	N	N	N	N	N	N	L	N	L
30	L	N	H	H	N	N	N	N	N	H	H	H	H	H
31	L	L	L	L	N	N	N	N	N	H	L	L	H	H
32	N	L	H	H	N	N	N	N	N	H	H	H	H	H
33	L	N	L	H	N	N	N	N	N	L	L	H	H	H
34	H	L	H	L	N	N	N	N	N	H	H	H	H	H
35	L	L	H	H	N	N	N	N	N	H	H	H	H	H
36	L	L	L	L	N	N	N	N	N	L	N	L	L	L
37	H	H	L	H	N	N	N	N	N	H	H	H	H	H
38	N	N	L	L	N	N	N	N	N	H	L	L	L	H
39	H	H	L	H	N	N	N	N	N	L	N	L	N	N
40	L	L	H	L	N	N	N	N	N	H	H	L	H	H
41	L	L	L	L	N	N	N	N	N	H	H	H	H	H
42	H	H	H	H	N	N	N	N	N	L	L	L	H	H
43	N	L	H	H	N	N	N	N	N	H	H	H	H	H
44	N	N	L	L	N	N	N	N	N	H	H	L	H	H
45	L	L	H	L	N	N	N	N	N	H	H	H	H	H
46	H	H	H	H	N	N	N	N	N	L	H	H	H	H
47	N	L	L	L	N	N	N	N	N	H	H	H	H	H
T.L	19	28	27	29	0	0	0	0	3	13	12	19	6	4
T.H	12	9	20	18	0	0	0	0	0	31	30	28	38	41

Note: N, negative = 0 or no cysts; L, light infection (Low) ≤ 1-100 cysts per organ; H, heavy infection (High) >100 cysts per organ; TL, total light infection; TH, total heavy infection. Key: To, tongue; Es, esophagus, He, heart; Di, Diaphragm; Li, liver; Lu, lungs; Ki; kidney; S, spleen; Br, brain; Int, internal masseter; Ext, external masseter; Tri, triceps bracii; Fl, forelimbs and Hi, hind limbs.

Appendix 4. Summary results of questionnaire surveys in pig slaughterhouses around Phnom Penh / Cambodia (n=70)

No.	Questions	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	Total No.
1	Educational level (1=Low, 2=Medium, 3=High)		60	31	9		70
2	Slaughter pigs for consumption per day (1= 1-100, 2=100-500, 3=500-2000, 4=more 2000)		0	3	89	8	70
3	Average slaughter pigs per day in each slaughterhouse: (1=50-200,2=200-300, 3=300-600 4=More than 600)		11	67	22	0	70
4	Environment and hygienic aspects in slaughterhouses (1=Good, 2=Poor)		0	100			70
5	Pig origins? (1=Household raising, 2= Small and medium farm, 3=Intensive farm)		40	33	27		70
6	Have ever heard of porcine cysticercosis? (1=Yes, 0=No)	36	64				70
7	Have ever heard of trichinellosis? (1=Yes, 0=No)	61	39				70
8	Inspected pigs for cysticercosis? (1=Yes, 0=No)	43	57				70
9	Which method? (1=Tongue palpation, 2=Meat inspection, 3=Both)		13	31	56		70
10	Find parasitic diseases of slaughter pigs? (1= Yes, 0=No)	37	63				70
11	Confirmed infected cases of cysticercosis? (1=Yes, 0=No)	37	63				70
12	How many cases per month? (1=1-3, 2=3-5, 3=more than 5)		63	31	6		70
13	Most confirmed cases? (1= Household raising, 2= Small and medium farms, 3= Intensive farm)		74	26	0		70

Appendix 4(continued). Summary results of questionnaire surveys in pig slaughterhouses around Phnom Penh/ Cambodia (n=70)

No.	Questions	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	Total No.
14	Inspected pigs for trichinellosis? (1=Yes, 0=No)	100	0				70
15	Ages of slaughter pigs (1=Young, 2=Medium, 3=Old, 4=All)		9	64	13	14	70
16	Pig breed? (1=Local, 2=Exotic, 3=Crossed bred)		50	24	26		70
17	Most ages of infected pigs (1=Young, 2=Medium, 3=Old and 4=All)		10	36	24	30	70
18	Most cases infected? (1= Heavy, 2=Light)		57	43			70
19	Infected carcasses and organs use for: (1=Human consumption, 2=Processing, 3=Culling)		14	64	22		70
20	Food habits (1=Cooked, 2=Uncooked, 3=Mixed)		57	16	27		70
21	Disease control and prevention? (1=Yes, 0=No)	76	24				70

Appendix 5. Summary results of questionnaire surveys of pig farms in 3 provinces / Cambodia, (n=132)

No.	Questions	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	Total No.
1	Educational level (1=Low, 2=Medium, 3=High)		79	16	5		132
2	Sizes of pig production (1= House hold raising, 2=Small &medium and 3=Investment)	=	85	15	0		132
3	Pig production system? (1= Free ranging, 2= Mixed, 3= Indoor)		86	11	3		132
4	Target of pig production? (1=Piglet, 2= Fattening, 3=Mixed)		31	45	24		132
5	Buying piglets from outside for raising? (1=Yes, 0=No)	35	65				132
6	Age of pigs for selling (1= 2-4 month, 2=6 month, 3=7-12 month, 4=More than 12 month)		32	22	36	10	132
7	Pig breed for raising (1=local, 2=Exotic, 3=Crossed bred)		89	3	8		132
8	Identification number (1=Yes, 0=No)	99	1				132
9	Types of feeds (1=Ratio feed, 2=Leftovers, 3=Mixing, 4=Traditional)		5	17	24	54	132
10	Getting feeds from outside? (1=Yes, 0=No)	55	45				132
11	Drinking water for pigs and human uses: (1=Tap water, 2=Underground, 3=Pond, 4=River)		0	17	66	17	132
12	Condition of water: (1=Direct use, 2=Treated)		93	7			132
13	Condition of holdings: floor type (1=Concreted, 2=Un-concreted 3=Mixed)		4	78	18		132
14	Environment of pig raising areas (1=Clean, 2=Dirty)		17	83			132
15	Waste management (1=Good, 2=Poor)		11	89			132
16	Having problems with parasitic diseases (1=Yes, 2=No)	17	83				132

Appendix 5 (continued). Summary results of questionnaire surveys of pig farms in 3 provinces / Cambodia, (n=132)

No.	Questions	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	Total No.
17	Have ever heard of porcine cysticercosis? (1=Yes, 0=No)	40	60				132
18	Have ever heard of *T.solium*? (1=Yes, 0=No)	54	46				132
19	Have ever heard of trichinellosis? (1=Yes, 0=No)	83	17				132
20	Investigate pigs for cysticercosis? (1=Yes, 0=No)	34	66				132
21	Which method? (1=tongue palpation, 2=Meat inspection, 3=No inspected)		66	0	34		132
22	Investigate pigs for trichinellosis? (1=Yes, 0=No)	100	0				132
23	Have ever confirmed porcine cysticercosis? (1=Yes, 0=No)	55	45				132
24	How many infected cases of cysticercosis per month (0= No case, 1=1-3, 2=3-5, 3=more than 5)	54	28	18			132
25	Age of infected pig confirmed porcine cysticercisis? (1=4 month, 2=6 month, 3=7-12 month, 4= More than 12 month.		24	26	40	10	132
26	Infected pigs sale for: (1=Human consumption, 2=Processing, 3=Culling)		14	61	25		132
27	Food habit (1=Cooked, 2=Uncooked, 3=Mixed)		59	6	35		132
28	Sufficient toilets? (1=Yes, 0=No)	84	16				132
29	Human cases of *Taenia solium*? (1=Yes, 0=No)	70	30				132
30	Disease control and prevention? (1=Yes, 0=No)	79	21				132
31	Is the measure effective? (1=Yes, 0=No	89	11				132
32	Treatment: (1=Conventional, 2=Traditional, 3= Mixed)		41	33	26		132
33	Is the treatment effective? (1=Yes, 0=No)	33	67				132

CURRICULUM VITAE

1. General background:

Family name:	THAN
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2. Education

Date from- to	Institution	Degree or Diploma
2000-2002	Pannasatra University of Cambodia, Phnom Penh, Cambodia	Diploma of English for Academic Purpose
1986-1993	Kharkov Zoo-Veterinary	DVM, MSc.vet.sc.

	Institute Kharkov, Ukraine	
1983-1986	Toul Kok High School Phnom Penh, Cambodia	Diploma of High School
1980-1986	Siem Reap Secondary School Siem Reap Province, Cambodia	Diploma of Secondary School

3. Professional Experience

Date from- to	Description
Oct. 2003-Sept.2005	Attended MSc. Course Program in Veterinary Public Health, Joint International Post-Graduate Studies in VPH between Faculties of Veterinary Medicine of Freie Universitat Berlin, Germany and Chiang Mai University, Thailand.
Mar.2003-Apr.2003	National Consultant of EC-project on Support to Veterinary and Livestock Services in Cambodia.
Aug.2002-Apr.2003	Member of National Consultation Committee (NCC) of FAO on Domesticated Animal genetic Resources.
May2002-June2003	Member of National Reformed Consultation Committee on Veterinary Sub-Degree for hygiene and inspection of animal meats and animal products in slaughterhouses, slaughtering sites and Markets.
Jan.2002-Feb. 2002	National Consultant of PRASAC- project on Assessment of nutrition and forage

	development in Cambodia.
-Jan.2001-Nov.2001	Supervisor of Bachelor Thesis's students from Royal University Agriculture of Cambodia
Jun.2001-to date	Chief of Livestock Division of Animal Production Office, Department of Animal Health and Production.
Jan.1997-Apr.1997	Supervisor of Thesis's students from Prek Leap Agriculture College
Oct.1996-Feb.1997	Chief of inspecting sector of Animal Quarantine Station.
1995-2001	Manager of own pig farm
1994-2001	Technical staff of Animal Production.
1993-2003	Manager of Small Animal and Pet Clinic in Phnom Penh.

DECLARATION

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in any University.

Name: THAN SOVYRA

Signature: 

Date of Submission: 20 September 2005