PREVALENCE OF PORCINE CYSTICERCOSIS AND TRICHINELLOSIS IN SLAUGHTER PIGS IN KATHMANDU VALLEY, NEPAL

BUDDHI SAGAR SAPKOTA

MASTER OF SCIENCE
IN VETERINARY PUBLIC HEALTH

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22 September 2005

Thesis Title	Salmonella Isolation from Slaughter Pigs and Carcasses in a Slaughterhouse in Chiang Mai, Thailand Prevalence of Porcine Cysticercosis and Trichinellosis in Slaughter Pigs in Kathmandu Valley, Nepal
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ABSTRACT

A cross-sectional study was conducted to find the prevalence of porcine cysticercosis and trichinellosis in slaughter pigs, sampled from 4 major slaughter slabs in Kathmandu Valley, Nepal for the period of November 2004 to April 2005. In the same period, 8 main hospitals of the valley were surveyed by questionnaires to find the occurrence of human neurocysticercosis.

Of the 504 porcine carcasses inspected for cysticercosis five were found positive, the slaughter prevalence being 0.99% (95% CI: 0.32-2.29). All the sampled carcasses of indoor managed pigs were negative for cysticercosis while the carcasses of both mixed and outdoor-farmed pigs showed few positive results. There was a significant difference in the prevalences of cysticercosis among farming systems ($p = 0.001$). No significant difference was found between the four major areas of origin of pigs namely Kathmandu Valley, eastern Nepal, Terai and the adjoining districts of Kathmandu Valley ($p = 0.65$). Similarly, there was no significant difference between slaughter slabs ($p = 0.85$). Cysts being harboured by heart, other muscles and diaphragm were more than those by tongue and esophagus. No cysticercus was found in liver. The microscopic examination of the hook-armed rostellum of the cysts confirmed that all cysts were of *T. solium*.

During 2000 to 2004, neurocysticercosis (NCC) patients were found at an overall rate of 1.02 per 1,000 admission episodes in 6 hospitals. From the remaining two hospitals 1.5 NCC cases were found per 1,000 admission episodes and out patients' department (OPD) visits. The NCC rate in terms of epileptic admission episodes was 18.7% while; in terms of admission episodes and OPD visits of epilepsy, it was 43.2%. Survey data revealed that 32% (25/78) of the NCC cases were from Kathmandu Valley alone.

Of the 400 sera tested for *Trichinella* antibodies by ELISA using larval excretory –secretory (E/S) antigen, four were positive and one was doubtful. The seroprevalence of trichinellosis in slaughter pigs in Kathmandu Valley was 1% (95% CI: 0.27 - 2.54). Positive results were found only in Kathmandu Valley and adjoining areas. There was no significant difference in the prevalences between areas ($p = 0.43$). All four positive sera were from indoor managed pigs but there was no significant difference in the seroprevalences between farming systems ($p = 0.44$). Similarly, there was no significant difference in the seroprevalences of trichinellosis between slaughter slabs ($p = 0.56$).

The presence of cysticercosis in slaughter pigs of Kathmandu Valley and well-documented data of NCC cases in the hospitals showed that the conditions were conducive for transmission of *T. solium* infection in the valley. The low seroprevalence of trichinellosis determined in this study deserves the direct demonstration of the parasites for the proof of the presence of *Trichinella*.

ชื่อเรื่องวิทยานิพนธ์	ความชุกของซีสติเซอร์โคซีสและทริคิเนลโลซีสในสุกรที่โรงฆ่าในกาฐมาณฑุแวลลีย์ ประเทศเนปาล
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บทคัดย่อ

ได้ทำการศึกษาตัดขวางเพื่อที่จะหาค่าความชุกของการติดเชื้อซิสติเซอร์โคซีสและทริคิเนลโลซีสในซากสุกร ซึ่งสุ่มตรวจจากสถานที่ฆ่าชำแหละ 4 แห่งหลักในบริเวณหุบเขากาฐมาณฑุ ประเทศเนปาล ในระยะเวลาตั้งแต่เดือนพฤศจิกายน 2547 ถึง เมษายน 2548 ในช่วงเวลาเดียวกันนั้น ได้ทำการสำรวจโดยใช้แบบสอบถามในโรงพยาบาลใหญ่ 8 แห่ง เพื่อที่จะหาอุบัติการณ์ของโรคนิวโรซิสติเซอร์โคซิสในคน

จากซากสุกรชำแหละ 500 ตัว ตรวจพบซิสติเซอร์โคซีส 5 ตัว ได้ค่าความชุกที่โรงฆ่า 0.99% (ที่ช่วงความเชื่อมั่น 95% : 0.32 – 2.29) ตัวอย่างซากทั้งหมดที่มาจากการเลี้ยงในคอกตรวจไม่พบ ขณะที่ซากจากการเลี้ยงปล่อยตลอดเวลากับปล่อยบ้าง พบผลบวก 5 ตัวอย่าง มีความแตกต่างระหว่างความชุกของซิสติเซอร์โคซีสในการทำฟาร์มระบบต่างๆ อย่างมีนัยสำคัญทางสถิติ ($p = 0.001$) แต่ไม่มีความแตกต่างระหว่างพื้นที่แหล่งที่มาของตัวอย่าง 4 แห่งหลัก คือ หุบเขากาฐมาณฑุ เนปาล ตะวันออก เทไร และพื้นที่ติดต่อกับหุบเขากาฐมาณฑุ ($p = 0.65$) ในทำนองเดียวกันไม่มีความแตกต่างอย่างมีนัยสำคัญระหว่างสถานที่ฆ่าชำแหละ ($p = 0.85$) อวัยวะที่ตรวจพบซีสท์คือ หัวใจ กล้ามเนื้ออื่นและกระบังลม พบมากกว่าที่ลิ้นและหลอดอาหาร ไม่พบซิสติเซอร์โคซีสในตับ การตรวจลักษณะด้วยกล้องจุลทัศน์พบลักษณะโรสเทลลัมเป็นตะขอ ซึ่งยืนยันได้ว่า ซีสท์ทั้งหมดเป็นชนิด *T. solium*.

ระหว่างปี 2543 – 2547 พบคนไข้นิวโรซิสติเซอร์โคซีส ในอัตรารวมที่ 1.02 ต่อจำนวนการรับเข้ารักษา 1000 ครั้งในโรงพยาบาล 6 แห่ง โรงพยาบาล 2 แห่งที่เหลือพบในอัตรา 1.5 ต่อ 1000 การรับเข้ารักษาและผู้ป่วยนอก อัตราพบนิวโรซิสติเซอร์โคซีสต่อจำนวนการรับเข้ารักษาของผู้มีอาการชักเท่ากับ 18.7% ขณะที่หากรวมผู้ป่วยนอกที่มีอาการชักด้วย อัตราจะสูงขึ้นเป็น 43.2% ผลการสำรวจแสดงว่า 32% (25/78) ของกรณีนิวโรซิสติเซอร์โคซีสมีแหล่งที่มาจากหุบเขากาฐมาณฑุ

จากซีรั่ม 400 ตัวอย่าง ที่ตรวจหาแอนติบอดี้ต่อทริคิเนลล่า ด้วยวิธี ELISA ที่ใช้ อี/เอส แอนติเจน พบผลบวก 4 ตัวอย่าง สงสัย 1 ตัวอย่าง ค่าความชุกทางซีโรของทริคิเนลโลซีสในสุกรที่โรงฆ่าที่หุบเขากาฐมาณฑุ เป็น 1% (ช่วงความเชื่อมั่น 95% : 0.27 – 2.54) ตัวอย่างบวกพบเฉพาะจากพื้นที่หุบเขากาฐมาณฑุและเขตติดต่อ แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติในความชุกระหว่างพื้นที่ต่างๆ ($p = 0.43$) ตัวอย่างบวกทั้ง 4 มาจากสุกรที่เลี้ยงขังคอก แต่ก็ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติในระดับความชุกทางซีโรระหว่างระบบการเลี้ยงที่ต่างกัน ($p = 0.44$) ในทำนองเดียวกันไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างสถานที่ฆ่าชำแหละต่างๆ

การมีอยู่ของซิสติเซอร์โคซีสในสุกรที่โรงฆ่าในหุบเขากาฐมาณฑุและข้อมูลแสดงการป่วยด้วยนิวโรซิสติเซอร์โคซีสในโรงพยาบาล แสดงให้เห็นถึงวงจรการแพร่ติดต่อของการติดเชื้อ *T. solium* ในบริเวณหุบเขา การพบความชุกทางซีโรของทริคิเนลโลซีสในการศึกษานี้เป็นเครื่องบ่งชี้ให้มีการตรวจพิสูจน์ทางปรสิตถึงการมีอยู่ของพยาธิทริคิเนลล่าโดยตรง

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ABBREVIATIONS AND SYMBOLS

AG-ELISA	= Antigen Enzyme Linked Immunosorbent Assay
ABTS	= 2,2'-azino-di-(3 ethylbenzthiazoline sulfonic acid) buffer
AB-ELISA	= Antibody Enzyme Linked Immunosorbent Assay
aqua dest	= Distilled water
B&B	= Banskota and Baidhya
BfR	= Federal Institute for Risk Assessment
Brah	= Brahmins
CBS	= Central Bureau of Statistics
CFT	= Complement Fixation Test
CSF	= Cerebrospinal Fluid
ºC	= Degree Celsius
CI	= Confidence Interval
CLDP	= Community Livestock Development Project
CT	= Computerised Tomography
CVL	= Central Veterinary Laboratory
DAAD	= Deutscher Akademischer Autauschdienst
DLS	= Department of Livestock Services
DNA	= Deoxyribonucleic acid
EEC	= European Economic Commission
EITB	= Emzyme Linked Immunoelectrotransfer Blot
°F	= Degree Fahrenheit
E/S	= Excretory-secretory
FS	= Field sample
FAO	= Food and Agricultural Organization
g	= Gram
GDP	= Gross Domestic Product
HAT	= Haemagglutination test

HCL = Hydrochloric Acid
IFAT = Immunofluorescence Antibody Test
IgE = Immunoglobulin E
IgM = Immunoglobulin M
IgG = Immunoglobulin G
IHA = Indirect Haemagglutination
ILAE = International League Against Epilepsy
KCl = Potassium chloride
KH_2PO4 = Potassium hydrogen phosphate
m = Meter
km = Kilometer
KMC = Kathmandu Medical College
KTM = Kathmandu
mm = Millimeter
ml = Milliliter
mNE = Mean Netto Extinction
MRI = Magnetic Resonance Imaging
µl = Microliter
µm = Micrometer
NaCl = Sodium chloride
Na_2HPO_4 = Sodium Hydro-phosphate
Nc = Negative control
NCC = Neurocysticercosis
NE = Netto Extinction
Neg = Negative
NMC = Nepal Medical College
Norv = Norvic Escorts International Hospital
NonBra = Non Brahmins
Nonveg = Non vegetarians
OD = Optical Density

OIE	= Office International des Epizooties
OPD	= Out Patients' Department
PBS	= Physiological Buffer Saline
PBS-T	= Physiological Buffer Saline Tween
Pc	= Positive control
PCR	= Polymerase Chain Reaction
pos	= Positive
RIA	= Radioimmunoassay
TLDP	= Third Livestock Development Project
TUTH	= Tribhuwan University Teaching Hospital
US	= United States
Veg	= Vegetarians
WBA	= Western Blot Analysis
♂	= Male
♀	= Female
$	= Dollar

1. INTRODUCTION AND OBJECTIVES

Nepal is a landlocked mountainous country surrounded by China in the north and India in the east, west and south. It covers an area of 147,181 square kilometers. The length from east to west is 885 km, and from north to south is 192 km. The topography of Nepal is highly variable, ranging 60 meters above sea level in the southern plain to highest peak of the world, the Mount. Everest, which has an altitude of 8848m in the north. Geographically, Nepal has three regions. They are the mountainous region, the hilly region and the Terai. Fifteen percent of the total land remains covered by snow throughout the year (mountainous region), while the Terai covers 17% and the hills 68%. According to the latest census of 2001, Nepal's population was 23,151,423. The annual average growth rate of population during the last decade i.e. 1991-2001, was 2.25 % (CBS, 2002). The census also revealed that 49.95% of the total population was male, while the females comprised 50.05%.

Agriculture contributes to 39% of the gross domestic product (GDP) in Nepal, and livestock makes up 31% of the agricultural GDP. The livestock population in the country namely buffaloes, cattle, sheep, goats, pigs and poultry is (in millions) 3.7, 6.9, 0.8, 6.6, 0.9 and 21.3, respectively (CBS, 2002). The annual population growth rate is 1.12% in cattle, 1.93% in buffaloes, -0.82% in sheep, 2.03% in goats, 4.55% in pigs and 4.7% in poultry (CLDP, 2003). The distribution of pig population is 11% in the high mountainous region, 58% in mid-hills and 31% in the Terai (Sharma, 2003). Meat consumption per capita in Nepal is 8.39kg/per person/year. Pork occupies 6% of total meat consumption in the country; the highest being the buffalo meat 57.45% followed by goat 17.3% and poultry 15.8%. The production losses due to parasites and other diseases vary from one estimate to another. A modest loss reported from 1991 production (Lohani and Rasali, 1995) was about US $14.8 million. The loss was the highest (67.7%) in buffaloes, and in pigs, it was 12.1%. The hygiene and sanitation situation in the country is very poor. This is one of the major causes of parasitic diseases to both humans and livestock. The common practice of feeding offal

and kitchen waste, in free-ranged and back yard piggeries, potentially contributes to the transmission of parasitic diseases (Joshi *et al*., 2004b).

Porcine cysticercosis caused by the human tapeworms *Taenia solium* and *Taenia asiatica* as well as trichinellosis are among the most serious parasitic zoonoses. There is huge amount of economic loss in livestock sector due to these diseases (Zoli *et al*., 2003; Dupouy-Camet, 2000). The distribution of these diseases is cosmopolitan. The prevalence is fostered when pigs are reared traditionally and allowed to roam freely outside. These diseases are endemic in many underdeveloped countries, where hygienic situations are poor and people are uneducated (Ngowi *et al.*, 2004; Pozio, 2000).

Porcine cysticercosis and human taeniosis are found in regions where pork meat is consumed predominantly. The main areas are Central America, Africa and Asia (Roman *et al.*, 2000). In Asia, investigations on these subjects have been made in the recent past (Rajshekhar *et al.*, 2003). In Nepal, few reports are available about porcine cysticercosis, human taeniosis and cysticercosis (Joshi *et al*., 2004a).

Over the past decades, trichinellosis has been recognized in many parts of the world, in new hosts and new epidemiological contexts (Pozio, 2000, Pozio *et al.*, 2002). In Asia, many investigations have been made in China on *Trichinella* in humans as well as animals (Mingyuan and Pascal, 2002). India and Nepal show very few studies on this disease.

Porcine cysticercosis in China has been reported with variable percentages in different areas (Rajshekhar *et al.*, 2003). Porcine cysticercosis in certain part of pig farming community in India was found 26%, the human taeniosis at 38% and 9.7% of the taeniosis patients were showing the epileptic seizures (Prasad *et al.*, 2002). Joshi *et al.* (2004a) reported 14.28% of porcine cysticercosis in meat inspection in Kathmandu in 1997 and by tongue palpation 32% pigs were positive for cysticercosis at the Magar ethnic community of the Syanja District in 2000. The prevalence of human taeniosis in the Sarki and Magar communities of Syanja District was found

47.7% (Gaire, 2000). Out of total 23,402 general surgery specimens, 0.26% were diagnosed as cysticercosis by histopathology in Patan Hospital, Lalitpur (Amatya and Kimula, 1999). A research conducted at Model Hospital in Kathmandu showed 73% of epileptic patients were neurocysticercosis (NCC) cases (Dhakal *et al.*, 2005). However, more investigations are required to determine the prevalence and the importance of *T. solium* cysticercosis in Nepal.

The seroprevalence of porcine trichinellosis in China varied from 0.09% to 29.63% in 7 different provinces (Wang and Cui, 2001). Serological study has shown few sero-positive cases of trichinellosis in local pigs of Nepal (Joshi *et al.*, 2004b).

These data clearly indicate that cysticercosis and trichinellosis are prevalent in Nepal and neighboring countries. As Nepal is in the stage of implementing a meat act, the identification of zoonosis related problems in the country is important and should be brought to the attention of the veterinary and public health authorities. This would give the opportunity to draft new legislation allowing for statutory control measures to be put into effect.

Therefore, there is an urgent need to conduct research on these serious parasitic zoonoses in Nepal. The principal objectives of the present study are:

a) To determine the prevalence of *T. solium* cysticercosis in slaughter pigs in the Kathmandu Valley.
b) To determine the predilection sites of the cysts in pig carcasses.
c) Determination of additional occurrence of cysticercus asiaticus or vesicotropica, particularly in the liver.
d) Retrospectively establish the occurrence of neurocysticercosis by collecting data from human hospitals in Kathmandu Valley.
e) Seroprevalence of *Trichinella* in slaughter pigs.

2. LITERATURE REVIEW

2.1 *Taenia solium* complex

2.1.1 Morphology

The adult tapeworm dwells in the middle of the small intestine of the human being. It has different body parts e.g., scolex with rostellum and hooks, immature and mature proglottids. The scolex measures approximately one mm across (Bowman, 1999). There are four suckers. There is a presence of an armed (hooked) rostellum. Behind the scolex the neck or the proliferation zone develops the strobila with the segments. Each mature proglottid measures about 10-12 mm in length. The uterus of the gravid proglottid has 7 to 12 lateral branches on each side. Proglottids, which contain eggs, break off the distal end of the tapeworm, and these proglottids are either passed intact in the host's faeces or they dissolve in the host's intestine and eggs are passed in the faeces. Eggs are brownish and round. *Taenia* eggs have a thick, "striated" shell and have an embryo (oncosphere) with several larval hooks and an approximate size of 40µm (Urquhart, 1996). Eggs of all species of *Taenia* look similar. The cysticercus is the larval or metacestode form of the adult tapeworm. This develops in the intermediate host and consists of an invaginated scolex. A cysticercus measures approximately 5mm across.

2.1.2 Life cycle

The life cycle of *T. solium* involves two hosts, the definitive host (containing the adult, sexually-reproducing stage), the human, and an alternate or intermediate host, the pig. The adult tapeworm is lodged as a single, exclusively in the human intestine; it measures 1.8-4.8 meters long and consists of repetitive segments or proglottids. The last proglottids, which are shed with faeces, contain approximately 40,000 eggs each (Gracey *et al.*, 1999). When a pig ingests human faeces containing eggs or proglottids, eggs are released in the intestine; oncospheres (hexacanth embryos) hatch,

activate and cross the intestinal wall. The oncospheres travel in the blood (and probably the lymphatics) via liver and lungs in the circulation to the muscles and elsewhere throughout the body, where they transform into cysticerci, which are the larval stages or metacestodes. Lesions formed by larval stage consist of cysticerci in cysts, are 5-8mm by 3-6mm, translucid and filled with a brownish to pinkish liquid. Sometimes, the head of the metacestode can be seen as a white spot surrounded by the host's connective tissues. Cysts are essentially found in the following organs and muscles: heart, tongue, masseters and diaphragm muscles, shoulder and intercostals muscles and esophagus. More rarely cysts are found in lymph nodes, liver, spleen, lungs and the brain. Humans are accidental alternate intermediate host and, like the pig, may develop larval taeniosis. Humans are infected either by ingestion of food contaminated with faeces or by autoinfection. In the latter case, a human infected with adult *T. solium* can ingest eggs produced by that tapeworm; either through smear infections or possibly, from proglottids carried into the stomach by reverse peristalsis.

Once eggs are ingested, oncospheres hatch in the intestine, invade the intestinal wall, and flood to striated muscles as well as the brain, liver, and other tissues, where they develop into cysticerci. In humans, cysts can cause serious sequellae if they localize in the brain, resulting in neurocysticercosis. Occasionally, they reach up to two cm or more without a scolex in the brain of humans in a racemose form that may produce bullae not enveloped by connective tissues. The parasite's life cycle is completed, resulting in human tapeworm infection, when humans ingest undercooked pork containing cysticerci (Georgi, 1985). Cysts evaginate and attach to the small intestine by their scolex. Mature adult tapeworms develop after 8-10 weeks and reside in the small intestine for years.

2.1.3 Pathogenicity and clinic

Generally, the human looks more or less asymptomatic in the case of taeniosis except for itching around the anus and occasionally nausea and vomiting. Cysticercosis shows no clinical manifestations in pig even if the cysts are present in the brain. The metacestode at different body parts in the host produce the cysts. The

cysticerci of *T. soulim* can survive for a longer period. In general, cysts tend to die more rapidly in the predilection sites. It is suggested that this is due to greater blood circulation to these muscles. Conversely, the higher rate of activity in these muscles (which in itself accounts for the greater circulation) may damage the parasites, allowing leakage of fluid and perhaps disrupting the parasite's ability to evade the immune response. Cysts at different stages of viability and degeneration can be found in the same host (OIE, 2004). *T. solium* only causes disease to humans that harbor the larval stage, which occurs when they ingest *T. solium* eggs. The pathology associated with cysticercosis depends on which organs are infected and the number of cysticerci or state of the lesion activity and host immune response (Nash, 2003). An infection consisting of a few small cysticerci in the muscles would likely result in no overt pathology and go unnoticed.

A few cysticerci (perhaps only one), if located in a particularly sensitive area of the body, might result in irreparable damage. *T. solium* cysts survive for many years in the brain of humans, and frequently symptoms begin only as the cyst begins to degenerate. In brain, the parasite usually develops in the ventricles. Sometimes, the granulomatous lesions are found in the brain. The cysts may be single parenchymal or multiple parenchymal scattered throughout the brain parenchyma with secondary complications (Garcia and Del Brutto, 2003). Meningeal and intraventricular cysts block the cerebrospinal fluid pathways producing obstructive hydrocephalous manifesting headache, dementia and seizures. Cysticerci invasion of the brain induces inflammatory reaction to meninges, encephalon and vascular regions, resulting in meningo-encephalitis and vasculitis. A cysticercus in the eye might lead to blindness; a cysticercus in the spinal cord could lead to paralysis. Psychosis is a rare occurrence and may be seen in up to 5% of patients (Mahajan *et al.*, 2004).

2.1.4 Epidemiology

2.1.4.1 Porcine cysticercosis and human taeniosis

T. solium causes porcine cysticercosis and human neurocysticercosis (NCC). It is found principally in Central and South America, sub-Saharan Africa, China and non-Islamic countries of Asia where there are free-ranging, scavenging pigs (Roman *et al.*, 2000). Pigs may acquire massive infection because the gravid segments of *T. solium*, unlike those of *Taenia saginata,* are not active and may remain in and about the faeces, so that the eggs may be concentrated there (Gracey *et al.*, 1999). Pigs and wild boars are the main hosts for the metacestodes. Canine infections with cysticerci are rear and play little or no part in transmission (Ito *et al.*, 2002).

In the microscopic examination of faecal samples, the taeniosis is reported to be 0.11% to 50% in Asian countries. Porcine cysticercosis in Asia (method being the meat inspection or tongue palpation) varies from 0.02% to 32.5% (Rajshekhar *et al.*, 2003). In a review by Zoli *et al.* (2003), porcine cysticercosis in West Africa, is reported with the variation from 0.6% to 20.5% and in central Africa, from 0.1% to 39% in different regions, the methods being meat inspection or tongue palpation. In the same review, human taeniosis in different age groups of people in different parts of central and western African countries varies from 0.09% to 8.7% in stool sample examination. In Latin America, human taeniosis by microscopic examination of stool is reported at the range of 0.2% to 2.8% and by copro-antigen ELISA from 3.0% to 8.6%. In Latin America, porcine cysticercosis in tongue palpation is reported at the range of 1% to 38.9% while the survey by ELISA revealed the prevalence of 4% to 61% in different endemic areas (Flisser *et al.*, 2003).

Porcine cysticercosis in a pig farming community in India was found to be 26% and the human taeniosis 38% (Prasad *et al.*, 2002). In China, the infection in pigs is highly variable ranging from 0.84 to 15% and in some areas as high as 40% (Rajshekhar *et al.*, 2003). Human teaniosis in China is reported 0.28% with the

variable percentages in different regions. Tibet showed a 21.08% occurrence of taeniosis in the recent survey conducted by the Ministry of Health (Zhou, 2005).

Porcine cysticercosis in meat inspection was recorded at 14.28% (28/196) in Kathmandu, Nepal, in 1997. Thirty two percent (134/419) of the pigs were found positive for cysticercosis by tongue palpation at the Magar ethnic community of Syanja District, in 2000 (Joshi *et al*., 2004a). The prevalence of human taeniosis in the Sarki and Magar communities of the Syanja District was found at 47.7% (Gaire, 2000). Particularly in the Kathmandu Valley, the taeniosis in human was recorded at 1.42% (n = 211) in stool sample examination by the sedimentation method (Ghimire, 2002). In another study, (Karki, 2003) it was 1.48% (n = 217), the method being the same.

2.1.4.2 Occurrence of neurocysticercosis

In 1994, International League Against Epilepsy (ILAE) called neurocysticercosis a main cause of epilepsy worldwide. Over four-fifths of the 50 million people with epilepsy are thought to be in developing countries. The reported prevalence rates of active epilepsy in developing countries range from 5 to 10 per 1000 population (Robert *et al.*, 2001). In Nepal, it is reported 7.3 per 1,000 of the populations in a community-based survey in the Morang District (Rajbhandari, 2004). In India a community-based study with a comparison between urban and rural areas in Banglore city showed the prevalence of neurological disorder as 32 per 1000 population (Gourie *et al.*, 2004). As stated by Hui and Kwan (2004), in Chinese communities, the prevalence of epilepsy has been recorded as 3–7/1000, it is estimated that approximately 45,000 epileptic people are in the Hong Kong alone.

Worldwide, 50,000 people die from cysticercosis every year (Dhakal *et al.*, 2005). Studies from countries where neurocysticercosis is endemic have reported that up to 50% of all cases of adult-onset epilepsy are due to neurocysticercosis and that the prevalence is on the increase (Roman *et al.*, 2000). Neurocysticercosis is found to be associated with the one third of the cases of epilepsy in cysticercosis endemic

areas, Atahualpa, Ecuador (Del Brutto *et al.*, 2005). Neurocysticercosis is not the problem of western countries but *T. solium* carriers are extremely potent sources of contagion, and human (neuro)cysticercosis can also occur in small outbreaks around immigrant carriers in western countries (Roman *et al.*, 2000). Human cysticercosis has been a notifiable disease in Mexico since 1990, and in 1993 the incidence was 0.8 cases per 100,000. One of the highest (54.5 per 100,000) prevalences was found in Brazil in 1995. The disease has been notifiable in Brazil since 1992 (Roman *et al*., 2000). In Latin American countries, the prevalence of cysticercosis in humans by western blot and ELISA is reported in the range of 3.4% to 24% (Flisser *et al.*, 2003). In Asia, the prevalence data are available from China, Indonesia, Vietnam and Korea. The prevalence survey by ELISA and western blot showed cysticercosis from 1.7% to 13% in Asian countries. In Indonesia up to 50.6% (81/160) of human sera were found positive for cysticercosis by immunoblot (Rajshekahar *et al.*, 2003). The seroprevalence of human cysticercosis in central and western African countries has been recorded from 1.3% to 2.8% in the general population. But, in endemic parts of Cameroon, it is recorded as high as 44.6% by AB-ELISA (Zoli *et al.*, 2003).

Serological survey using Indirect Haemagglutination (IHA) (n = 1442 sera from apparently normal population) in people residing in and around Pondicherry, in the southern part of India, showed that 6.1% were positive for cysticercosis antibodies whereas among clinically suspected patients 22% (20/91) were found to be seropositive (Parija and Sahu, 2003). In the analysis of a total of 1026 of all kinds of seizure disorders in the hospital of north-west India, 34.6% were diagnosed with NCC (Singh *et al.*, 2005). More than 95% of NCC patients in India are vegetarians or do not consume pork (Rajshekhar *et al.*, 2003). To date, no case of human cysticercosis has been reported in Pakistan, Bangaladesh and Sri Lanka (Singh *et al*., 2005). In a report compiled by Rajshekhar *et al.* (2003), the seroprevalence of human cysticercosis in China, at five high endemic zones ranged from 3%-4%. They estimated that there were 3 million cysticercosis patients in China.

A diagnosis of cysticercosis was made in 0.01% (4/25033) of pathological specimens examined at Bir Hospital, Kathmandu Nepal from 1995 to 1997 (Joshi *et*

al., 2004a). In Patan hospital, Lalitpur District, 0.46% (62/23402) surgery specimens were diagnosed with cysticercosis (Amatya and Kimula, 1999). Seven of the eight epileptic Gurkha soldiers (Nepalese) serving at the British army in Hongkong were diagnosed as having neurocysticercosis (Heap, 1990). A research conducted at Model Hospital in Kathmandu showed 73% of positive cases of cysticercosis by EITB out of 121 patients showing focal seizures (Dhakal *et al.*, 2005). In the report of Rajbhandari (2004), from the total 300 epileptic cases in the Shree Birendra Military Hospital Kathmandu, Nepal, 47% were diagnosed with NCC by CT scan and MRI.

2.1.5 Diagnosis

Different methods can be applied for the diagnosis of taeniosis and cysticercosis. Coproscopy and the use of coproantigent in ELISA are for *Taenia* egg identification. The morphological study of proglottids is done by microscopy. Serological tests can be applied for antigen and antibody detection in humans as well as pigs, whereas molecular techniques like PCR and DNA probes are useful for the identification of *Taenia* eggs and cysticerci. X- rays are used for the detection of cysts in the muscles. CT scan and MRI are for the diagnosis of neurocysticercosis in humans (OIE, 2004).

2.1.5.1 Diagnosis in human

a) Adult worms

Adult *Taenia* infections in humans can be recognized by detection of *Taenia* coproantigen in faeces using antigen-capture enzyme-linked immunosorbent assay (AG-ELISA), but the test does not differentiate species. DNA probes and a polymerase chain reaction have been used experimentally to differentiate human *Taenia spp*. The visual examination and microscopy will be aimed at studying of following morphological characteristics. Adult *T. solium* has an armed rostellum bearing two rows of hooks. The ovary has three lobes, there is no vaginal sphincter

muscle and the cirrus sac extends to the excretory vessels. Gravid segments have 7-12 uterine branches (see Table 1 pp. 17).

b) Metacestodes

A number of immunological tests are under development for the diagnosis of cysticercosis, but remain experimental. An AG-ELISA using polyclonal or monoclonal antibody (used to detect antigen in cerebrospinal fluid) and enzyme-linked immunotransfer blot (EITB) assay (used to detect antibody in CSF) have had success. EITB showed specificity and sensitivity of 94% and 100%, respectively (OIE, 2004). For *T. solium* neurocysticercosis in humans, tests for serum antibody or serum antigen produce positive reactions that can be misleading in patients infected only in the musculature. The correlation between a positive serology and neurological symptoms and/or lesions indicative for the NCC on neuro-imaging techniques is poor to fair in most studies (Dorney *et al.*, 2003). There is no gold standard technique fully validated for the immunodiagnostic tools of cysticercosis. Recently, it has been reported that immunoblotting and ELISA showed a high correlation and both ELISA and immunoblotting assays are sufficiently sensitive to detect asymptomatic or symptomatic cysticercosis patients (Ikjima *et al.*, 2005).

The most common presenting sign is seizure in humans with *T. solium* neurocysticercosis. Ranges of efficient means of diagnosis are there, but application of these diagnostic facilities depends upon the number, location and viability or level of degeneration of the cysticerci. Computerized tomography (CT) scan and magnetic resonance imaging (MRI) are used to detect the exact locations and viability of *T. solium* metacestodes (Garcia and Del Brutto, 2003). But, these imaging techniques are sometimes limited by the small size of visualized lesions and atypical images, which are difficult to distinguish from abscesses or neoplasms (Sako *et al.*, 2005). Calcified muscle cysts are detected by radiography, while PCR tests could be applied to unambiguously identify an actual metacestode.

2.1.5.2 Diagnosis in pig

Metacestode i.e. cysticercus develops into a cyst in the muscles and organs of pigs. These are palpable in the tongue in living pigs at an anti-mortem examination and during meat inspection these can be seen visually and even palpated at different organs and muscles. Light infections are easily missed on tongue palpation (Sciutto *et al.*, 1998). Cysticercosis endemic areas have been using lingual examination to reject the pigs that are recognized having *T. solium* larvae under the tongue for fear of losing money due to carcass condemnation in meat inspection (Ngowi *et al.*, 2004). In the meat inspection, it was estimated that 10.6% of the cysts would be located at inspection sites if regulations of meat inspection were followed carefully while doing the meat inspection (Boa *et al.*, 2002). Serological tests for animals have not reached the stage where commercialization for individual diagnosis or large-scale detection of infected pigs is possible. In a prevalence study by Goenzalez *et al.* (1990), the prevalence percentages for three different techniques remained 23.4%, 31.2% and 37.7% in tongue palpation, meat inspection and serology respectively. Maternal antibodies transferred by colostrums from sow to piglet may persist for 7 months; this will overlook the situation in case of serology (Goenzalez *et al.*, 1999). All assays tested AG-ELISA, AB-ELISA, EITB and tongue inspection showed low sensitivity in rural pigs infected naturally with low levels of *T. solium* (Sciutto *et al.*, 1998). The antibody-based tests also detect nonviable cysts. So far, serological tests for animals are at the experimental stage.

2.1.6 Differential diagnosis

The cysts of the *Taenia solium* in the pig carcass should be differentiated from the cysticercus tenuicollis cyst of *Taenia hydatigena*, cystic echinococcosis (hydatid cysts) and *Sarcocystis* cysts.

Cysticercus tennucollis: Pigs are rarely infected with the cysticercus tennucollis. These cysts are found in the peritoneal cavity, omentum, mysentry and visceral surface of the abdominal organs. In liver, they are 2-6 cm round or elongated with

single scolex, long neck, slightly bigger than a pea but degenerated and undergo calcification rapidly (Gracey *et al.*, 1999).

Cystic echinococcosis: They are oval to spherical in shape and size may be similar in a size to a marble to small football. Generally, they look like geese eggs. They are predominantly found in the lungs and liver. Particularly in the liver, size and shape of the cysts may be variable according to the stage of development. Inside the cysts there are many scoleces (Gracey *et al.*, 1999).

Sarcocystis: There are two species of *Sarcocystis* infecting pigs. *Sarcocystis suihominis* and *Sarcocystis suicanis*. The cysts are formed by the merozoites predominantly found in the diaphragm, masseter and skeletal muscles and heart. They are microscopic cysts up-to the size of one mm with a banana shape (Kaufmann, 1996).

2.1.7 Prevention and therapy

The first approach for the control of cysticercosis should be mass treatment against human taeniosis in endemic areas where the risk of transmission is high. For the long run strategic plan, the things to be considered are health education, modernization of pig farming, rigorous inspection of pork in official slaughter houses, creation of hygienic and sanitary conditions in the community and active epidemiological surveillance system to identify tape worm carriers. Overall, pig vaccination, treatment of porcine cysticercosis, human mass treatment as well as health education campaigns are the possible measures of prevention and control of *T. solium* and cysticercosis (Sarti and Rajshekhar, 2003).

a) Pig vaccination

In searching for the best recombinant antigen to be included in the vaccine several studies have been made (Lightowlers *et al.*, 2000, Flisser and Lightowlers, 2001, Plancerte *et al.*, 1999). A recombinant vaccine effective against *T. solium*

cysticercosis in pigs has been described (Lightowlers, 1999). Vaccines from two different recombinant oncosphere antigens have shown to induce complete or near complete protection against experimental challenge in pigs (Lightowlers, 2004). But, there is no effective vaccine commercialized so far. Applicability of pig vaccination in field condition depends upon cost factor, how it is easy to administer in mass intervention campaigns, and subsidies from the government, as pig farmers are poor, etc.

b) Treatment of porcine cysticercosis

Oxfendazole has been reported to efficiently (100%) eliminate pig cysticercosis following treatment (Gonzalez *et al.*, 2001). The drug cures the disease after three months of its administration.

c) Human mass treatment

Praziquantal and niclosamide are two non-toxic drugs with the taenicidal efficacy of 95% and 85% respectively (Pawlowski, 2005). Five mg/kg body weight of praziquantal is prescribed for human mass treatment (Sarti *et al.*, 2000). It is recommended that mass therapy with these drugs be administered twice a year in endemic countries. Several rounds of such therapy have been recommended over the periods of 5 years.

d) Health education campaigns

Participation of the community and schools in maintaining hygiene has shown the effective results. A comparative study was undertaken in a rural community to evaluate the effect of health education in both the short and the long term (6 and 42 months), as an intervention measure against *T. solium* (Sarti and Rajshekhar, 2003). Four years after health education was implemented, no infected pigs could be identified in that community (before intervention swine cysticercosis 2.6%). Health

education requires the multidisciplinary input and active participation of the community.

e) Treatment of neurocysticercosis

Although it is known that larvicidal treatment kills viable cysts that commonly resolve or calcify, the clinical benefit of this treatment in the most common presentation is unproven (Theodore, 2003). For the long-term treatment by praziquantal, it requires higher dose i.e. 10 mg/kg body weight to reach the efficacy level of 100% (Sarti *et al.*, 2000). But at that level there is risk of inducing seizures in asymptomatic patients harboring live cysticerci (Flisser *et al.*, 1994). The treatment modalities for human cysticercosis include larvicidal drugs such as albendazole or praziquantal for the viable cysts, corticosteroids, antiseizure medications and surgical interventions. Recently, endoscopic surgery proved to be the optimal approach to ventricular cysts (Yancy *et al.*, 2005).

2.1.8 Carcass judgement

As per guidelines of FAO (Herenda *et al.*, 2000), for the meat inspection in developing countries, the carcass with cysticercosis would be judged as follows. Heavy infestation with cysticercus cellulosae calls for carcass condemnation e.g. measly pork. In light or moderate infestation, the carcass may be conditionally approved pending heat or freezing treatment. The freezing treatment includes -5ºC for 4 days, -20ºC for 12 hours (Gracey *et al.*, 1999).

2.2 *Taenia asiatica* complex

Taenia asiatica is a species of human tapeworm found in Asian countries. In the beginning *T. asiatica*, on the basis of its morphological structure was classified as the strain or subspecies of *Taenia saginata* (Bowles and McManus, 1994; Fan *et al.*, 1995). Zarlenga and George (1995), on the basis of their mitochondrial DNA findings, considered the parasite at a species level. *T. asiatica* like *T. saginata* is unable to cause human cysticercosis and to produce human intermediate infestation (Galan *et al.*, 2000).

The adult worm in humans has an ovary, vaginal sphincter muscle and cirrus sac like those of *T. saginata*. But, *T. asiatica* has unarmed rostellum on the scolex of adult. There exist posterior protuberances on segments and 16-21 uterine buds (Fan *et al.*, 1995). Segments are passed singly and often spontaneously. Metacestodes are small, about two mm, and have a rostellum and two rows of primitive hooks, those of the outer row being numerous and tiny. *T. asiatica* metacestode (cysticercus vesicotropica) was different morphologically from *T. saginata* metacestode (cysticercus bovis) in having wart-like formations on the external surface of the bladder wall (Eom and Rim, 1993). They occur mainly in the liver of domesticated and wild pigs, occasionally in cattle, goats, and monkeys. Metacestodes may be found on the omentum and, rarely on the lungs and colonic serosa. The characteristics of *T. solium*, *T. saginata* and *T. asiatica* are shown in Table 1.

Table 1; Characteristics of three human *Taenia* species (*T. solium, T. asiactica and T. saginata*).

Characteristics	***T. solium***	***T. asiatica***	***T. saginata***
Cysticercus			
Intermediate hosts	Pigs, human beings, dogs, wild boar	Pigs, cattle, goat, monkey, wild boar	Cattle, reindeer
Localisation	Muscle, brain, skin, eye, tongue	Viscera, mainly liver	Muscle, viscera, brain
Size (mm)	5–8×3–6	2×2	7–10×4–6
Scolex	Rostellum with hooklets	Rostellum with rudimentary hooks	No rostellum, no hook
Adult tapeworm			
Scolex	Rostellum with hooklets	Rostellum with no hooks	No rostellum, no hook
Number of uterine branches in gravid proglottids	7–12	16–21	18–32
Expulsion from human	Mainly in groups, passively	Single, actively	Single, actively

(Ito *et al.,* 2003)

2.3 *Trichinella* complex

2.3.1 Morphology and types of species

Trichinella is a genus of nematode parasite with many species, dwells deeply pierced in the small intestine, and belongs to the family, *Trichuridae*. The male is 1.4 to 1.6 mm long. It is more slender at the anterior than the posterior end. The anus has a large papilla on each side of it. A copulatory spicule is absent. Stichocytes are arranged in a row following a short muscular esophagus. The female is about twice the size of the male, also tapering towards the anterior end. The vulva is located near the middle of the esophagus, which is about a third the length of the body. The single uterus is filled with developing eggs in its posterior portion, whereas the anterior portion contains fully developed, hatching juveniles (Soulsby, 1982).

Seven species of *Trichinella* are recognized (OIE, 2000). These are 1. *Trichinella spiralis* (T-1), 2. *Trichinella nativa* (T-2), 3. *Trichinella britovi* (T-3), 4. *Trichinella pseudospiralis* (T-4), 5. *Trichinella murrelli* (T-5), 6. *Trichinella nelsoni* (T-7) and 7. *Trichinella papuae* (T-10).

Trichinella spiralis is very important species among all. It is commonly associated with domestic pigs therefore belongs to the domestic cycle. This species is distributed in temperate regions worldwide and is highly infective for pigs, mice and rats (Kapel, 2000). *Trichinella nativa* has limited infectivity for pigs. It exists in cold climates and is found in the sylvatic cycle in wild canids, bear and walrus. This species is resistant to freezing (Pozio, 2000). *Trichinella britovi* is found predominantly in wild animals, although it may occasionally be found in pigs or horses. It occurs in temperate regions of Europe and Asia (Murrel *et al.*, 2000). *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 for non-encapsulating species in some cases). *Trichinella murrelli* is found in wildlife and occasionally horses, humans and domestic pigs. It is a North American species. *Trichinella nelsoni* has been reported in

wildlife in Africa. It has greater resistance to high temperature as compared with other species of *Trichinella* (Pozio and La Rosa, 2000).

Two species of *Trichinella* do not form a capsule in muscle. They are *Trichinella pseudospiralis* and *Trichinella papuae*. *Trichinella pseudospiralis* is cosmopolitan in distribution and has been recovered from raptorial birds, wild carnivores, rats and marsupials in Asia, North America and the Australian subcontinent (Pozio, 2000). *Trichinella papuae* has only been reported from Papua New Guinea to date. In addition to the species described, several other isolates are recognized. As molecular techniques are refined and further comparative studies are performed on *Trichinella* isolates, further taxonomic resolution is proposed for this genus (Murrell *et al.*, 2000). A new *Trichinella* species that infects both reptiles and mammals called *Trichinella zimbabwensis* was discovered in Zimbabwe. This species is non-encapsulated (Pozio *et al.*, 2002). All *Trichinella* species are considered infectious for human.

2.3.2 Life cycle

This nematode has no stages outside a host. The adult worms are found attached to or buried in the mucosa of the duodenum. After mating, the males die. Females (viviparous) produce living young larvae (approximately 1,500 per female over a period of two months depending upon the amount of worm burden and the immunological response of the host self cure phenomenon) and then die. Larvae enter the lymphatics and mesenteric veins and are found throughout the arterial circulation between the 7^{th} and 25^{th} day after infection via the hepato-portal system through the liver, heart and lungs (Straw *et al.*, 1999). From three weeks on, they are transported to striated muscles, penetrate individual fibers, and cysts are formed around the juveniles. Within cysts, juveniles remain viable for many years, up to 25 years in man and 11 years in pigs because they trigger the formation of a nurse cell around the muscle. When viable encysted juveniles are ingested, they are digested from the cysts and pass to the duodenum where they mature.

2.3.3 Pathogenicity and clinic

The pathogenicity of all the different species of *Trichinella* has not yet been totally explored in animals and humans. For the most common, *T. spiralis,* the following clinical signs have been found.

Trichinellosis is rarely detected clinically in animals. Trichinellosis can range from asymptomatic to fatal, depending on the infective dose and natural resistance of different host species. In humans, in the week following ingestion of infected meat, a patient may show the intestinal phase of pathogenicity, the nausea, vomiting, diarrhoea and abdominal discomfort due to intra-intestinal activities of the adult worms (Urquhart *et al.*, 1996). In the parasitemic phase, the worms get circulated in almost every organ of the body. In the muscle or rheumatoid phase, there is sudden onset of muscle soreness and pain. In case of *Trichinella* infection, the symptoms like fever, edema of the upper eyelid and urticarial rash can be seen in 2 to 8 weeks after ingestion. Eye pain, photophobia, thirst, profuse sweating, chills and weakness may also occur. Recurring high fever (as high as 104°F) usually stops after 1 to 6 weeks. In most severe infections cardiac and neurological complications like blindness and heart attack may occur and sometimes result in sudden death during 3 to 6 weeks of infection. Laboratory tests will show a rapid increase in eosinophils level in blood (Soulsby, 1982).

2.3.4 Epidemiology

Trichinellosis is recognized in more than 100 animal species in areas with different geographical and ecological characteristics (Pozio, 2000). Independent sylvatic and domestic zoonosis cycles of infection occur. The sylvatic cycle involves wild carnivores such as foxes, jackels, wild boars, black bears, bush pigs, walrus etc. And these animals maintain the transmission (Soulsby, 1982). Humans may become infected following the ingestion of game meat. The synanthropic zoonosis cycle occurs primarily in pigs and rats; occasionally cats, dogs and humans may become infected. The anaerobic metabolism of larvae in nurse cells allows their survival in

extremely decayed meat. Encapsulated larvae in the decomposing carcass function similarly to the larvae of other nematodes, suggesting that the natural cycle of *Trichinella* includes a free-living stage when the parasite is no longer protected by the homeothermy of the host (Pozio, 2000). Outbreaks of trichinellosis have been associated with horses. Horses are susceptible to infection and may be infected if processed horse feed contains remnants of infected meat. Pigs, dogs, cats, horses, rats and many wild animals such as, bears, wolves, wild boars, foxes and arctic marine mammals can serve as reservoirs for *Trichinella* (Urquahart *et al.*, 1996). Trichinosis occurs worldwide and affects peoples of all ages. The global prevalence of the disease is difficult to evaluate but as many as 11 million people might be infected (Dupouy-Camet, 2000). Depending on local customs regarding eating pork or undercooked meat the incidence of disease is variable. The disease is particularly worrisome in the Balkans, Russia, the Baltic republics, in some parts of China and Argentina (Dupouy-Camet, 2000).

Many reports on *Trichinella* are found from China. In the report compiled by Mingyuan and Pascal (2002), the first outbreak of trichinellosis in China was reported in Tibet in 1964. After that until 2002, a total of 25,161 human cases had been reported and out of them 240 were death cases. Epidemiological surveys have shown that 94.3% of human trichinellosis outbreaks nationwide were caused by the consumption of raw or under-cooked pork. The prevalence of porcine trichinellosis was recorded at 4% in China. The Nanyang province showed a prevalence of 32.2% while in Beijing it was 7.3%, in 1997. Dog trichinellosis varied from 9.82% to 44.8% in different provinces of China while the rat trichinellosis was from 1.98–15.06% (Wang and Cui, 2001). The method of diagnosis was microscopy in all cases. Seroepidemiological surveys by ELISA using E/S antigen in human populations of ten Chinese provinces showed a prevalence of trichinellosis to be 5.5% (Wang and Cui, 2001).

The first case of trichinellosis in man from India was discovered incidentally during drainage of psoas abscess (Mohan *et al.*, 2002). In a study conducted in Hongkong, the sera of 18 Gurkha/Nepali patients with clinical manifestations of acute

trichinosis were tested for *T. spiralis* antibodies by IgE, IgM and IgG-ELISA, IgG radioimmunoassay (RIA) and the indirect haemagglutination test (IHA). Ninety four percent of patients were positive for IHA, IgG-RIA and IgG-ELISA. In muscle biopsies, results were positive for only in 56% of patients (Au *et al.*, 1983). The serological study by EITB showed two positive cases of trichinellosis out of 425 sera of local pigs of Nepal (Joshi *et al.*, 2004b) although in ELISA by synthetic beta-tyvelose an unexpectedly high prevalence was noted.

2.3.5 Mode of transmission of *Trichinella spiralis*

Trichinella spiralis, the agent of the domestic type of trichinellosis, has a typical life cycle pattern in pigs, rats and humans. Transmission occurs by ingestion of raw or undercooked meat containing *Trichinella* larvae. Pork and pork products are the most likely sources but beef products, which may become inadvertently adulterated with raw pork during processing, may also be a source. There is no person-to-person spread of trichinosis (Urquhart *et al.*, 1996). The usual incubation period is 8 to 15 days. If a large number of larvae is ingested, symptoms may occur more rapidly. Animal hosts may remain infective for months, and meat from these animals remains infective until sufficient cooking, freezing or irradiation kills the larvae. Disease in pigs is perpetuated by swill feeding, eating infected rodent carcasses, tail-biting, infestation by faeces from freshly infected animals or feeding of non sterilized human food residuals (Urquhart *et al.*, 1996). Farm management practices play an important role in the outbreak of trichinellosis in pig farms (Gamble and Bush, 1999; Gamble *et al.*, 1999). Transplancental transmission of larvae occurs in mice and humans, but not in pigs (Bowmann, 1999).

2.3.6 Diagnosis

Diagnostic tests for *Trichinella* infection fall into two categories: direct detection of first-stage larvae encysted or free in striated muscle tissues, and indirect detection of parasites by tests for specific antibodies (OIE, 2000).

2.3.6.1 Serodiagnostic methods

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

The sensitivity of serological methods is equal to or better than direct methods. In light or moderate infections in pigs, a serological response is often not detected before 3 weeks or longer after muscle larvae become infective (Gamble, 1996). The time of seroconversion is correlated to infection dose (Nöckler *et al*., 2000). In old infections (sows), the antibody level decreases 4-5 months after infection. In such cases, a false-negative serological result might be obtained. In serology, the enzyme linked immunosorbent assay (ELISA) is the best method for the ante-mortem detection of *Trichinella* infection (OIE, 2000). Infection level as low as one larva per 100 grams of tissue has been detected (Gamble *et al.*, 1983). The specificity of ELISA for *Trichinella* infection is directly linked to the type and quality of the antigen employed in the test. Excretory-secretory (E/S) antigens, which are metabolic products collected by in-vitro culture of muscle larvae provide the most specific and economical source. There are no known cross-reactions using these antigens. The use of E/S antigens in ELISA is recommended for surveillance programs (Straw *et al.*, 1999). E/S antigens in ELISA can be used to detect the antibody level of other species of *Trichinella* too. In case of somatic antigens, such as crude worm extracts, cross-reactions with antigens of other nematodes may cause false-positive results (Gamble *et al.*, 1983).

A synthetic glycan antigen has been developed for use in ELISA. Recently, it has been reported that a synthetic glycan antigen, beta-tyvelose appeared to be less sensitive than the E/S antigens and beta-tyvelose antigen may not be suitable for screening of trichinellosis in pig herds (Moller *et al.*, 2005). However, due to failure

to detect infected pigs during both the early and the very late stages of infection, ELISA can not be used to replace the direct method. Therefore, to confirm the seropositive case, 100g or more muscle tissue has to be tested by digestion method (OIE, 2000).

2.3.6.2 Identification of the agent

Two general methods, compression or digestion of muscle tissue, are used for the direct detection of *Trichinella* infection. *Trichinella* larvae are seen under a microscope/trichinoscope. The predilection sites in pigs are in descending order of the diaphragm (crus), tongue, masseter and abdominal muscles, although this partially depends on the degree of infection and types of pig rearing (indoor/outdoor) (OIE, 2000). The direct method will identify the infected pigs 17 days after exposure but remains effective if larvae are viable. The selection of an appropriate method for direct inspection for *Trichinella* infection depends on the facilities available and the number of samples to be tested.

a) Trichinoscopy (compression) method

Twenty-eight small pieces of muscle of about 2mm × 10mm (size of an oat grain), with a total weight of about 0.5g, should be taken from the prescribed predilection sites. The muscle pieces are compressed between two glass plates until they become translucent, than examined individually for *Trichinella* larvae, using a trichinoscope or a conventional stereo-microscope (15–40 × magnification) (OIE, 2000). The specialized microscope the trichinoscope has an estimated efficiency of detecting as few as three to five larvae/gram of tissue. It has the disadvantage of requiring considerable time and labour for the inspection of multiple samples from each carcass. It is also very difficult to detect the larvae of *T. pseudospiralis*, which are free in muscle cells, with this method.

b) Digestion method

This method involves the digestion of individual or pooled muscle tissue samples, followed by selective screening, filtration or sedimentation procedures. Finally these samples are examined microscopically for the presence of larvae. Stirring or homogenizing the digest mixture may mechanically assist digestion methods. The magnetic stirrer method is a very popular method (ECC, 1984). Digestion methods have an efficiency of approximately three larvae/gram of tissue examined when the tissue sample size is one gram. As a confirmatory test, the digestion of up to 100g of tissue will ensure an accurate diagnosis. The digestion method is applicable in the slaughterhouses where a large number of pigs are slaughtered for pool sampling. This method is cost effective for the pooled samples and even more sensitive than the direct microscopy. In the digestion technique, individual samples of 100g may be taken from one animal, or multiple samples may be collected from a number of animals to make a 100g pool. The sizes of the samples that make up the pool determine the sensitivity of the method (OIE, 2000).

2.3.6.3 Polymerase Chain Reaction (PCR)

For species differentiations of *Trichinella,* PCR is applied. Species identification will be a valuable tool in the study of parasitic epidemiology and then the assessment of risk factors. It has been claimed that a single PCR test for simple differentiation of all currently recognized genotypes of *Trichinella* is made possible (Appleyard *et al*., 1999).

2.3.7 Prevention and therapy

The prevention of trichinellosis by proper meat inspection is a classic example of successful veterinary public health measures (Urquhart, 1996). In countries where domestic pig infections are virtually non-existent, monitoring of *Trichinella* infection in wildlife could also contribute to understanding the infection pressure from nature to livestock. *Trichinella*-free pig farming is a feasible option for controlling this

zoonosis, even in endemic areas. All animals with access to the environment, or animals that are fed with potentially *Trichinella*-infected feed (swill, carcasses) will always constitute a public health threat, and must be inspected individually at slaughter (swine, horses, wild boars). If no control system exists, for whatever reason, the public should be educated not to consume improperly cooked meat.

Cooking meat products to an internal temperature of at least 65.6°C to 77°C can kill the *Trichinella* larvae. Freezing pork less than 6 inches thick for 20 days at -15°C or three days at -20°C kills larval worms (Straw *et al*., 1999). It is recommended to cook wild game meat thoroughly. Freezing wild game meats, unlike freezing pork products, even for long periods of time, may not effectively kill all worms. Cook all meat fed to pigs or other wild animals. Do not allow hogs to eat uncooked carcasses of other animals, including rats, which may be infected with trichinellosis. Clean meat grinders thoroughly while preparing ground meats. Curing (salting), drying, smoking, or microwaving meat does not consistently kill infective worms.

Pigs infected with *Trichinella* are not treated with anthelmentics as the treatments are costly and results are unsatisfactory. Treatment for a person with trichinellosis is also not yet totally satisfactory; it involves administration of the drug thiabendazole or mebendazole (Soulsby, 1982). The patient is usually given analgesics and corticosteroids to reduce inflammation and ease the pain.

2.3.8 Carcass judgment

According to Herenda *et al.* (2000), a carcass infected with *Trichinella* should be condemned. This attempt is very essential in the prevention and control of trichinellosis.

3. MATERIALS AND METHODS

3.1 Farming systems and breeds of pigs

There are three pig production systems in Nepal. These are indoor, mixed and outdoor. In the indoor system, improved pigs are reared, although some indigenous pigs may be reared in this way. Although these pigs are protected from external inclement conditions they are nevertheless prone to disease associated with poor sanitation and other adverse indoor keeping conditions for example ventilation, crowding etc. In the mixed (indoor/outdoor) system, both indigenous and improved (crossbred) pigs are reared outdoors during the day and kept indoors during the night (Dhaubhadel, 1992). Under this system the opportunity for eating contaminated feeds and acquiring infections like parasitic infestation and other disease problems is very high. Few native pigs, e.g. Hurra and Chwanche are reared in the free ranging system (out door system) (Joshi and Shaha, 2003).

Nepal has three pig breeds identified as indigenous in the country. They are Bampudke, Chwanche and Hurra (Joshi and Shaha, 2003). Bampudke pigs are found in the lower hills while the Chwanche are found in the middle mountainous regions and Hurra in the Terai. Over the years, government institutions and non-governmental agencies have imported some exotic breeds like the Hampshire, Landrace, Tamworth, Saddleback and Fauyen, with a view to upgrading native ones. The Pakhribas Agricultural Centre, a government institute, focuses on improving the black pig called the Pakhribas Cross. But, these Pakhribas Cross breeds are popular in the country with the common name of “Dharane” pigs.

3.2 Study area

Nepal has four climatic seasons (a) spring: March-May (b) summer: June-August (c) autumn: September-November (d) winter: December-February. The Kathmandu Valley, which is bowl-like in topography, stretches for approximately 25 km from east to west and about 20km from north to south. This valley lies at a height of approximately 1300 meters. The heights of the surrounding mountains range from 1500 meters to 2800 meters. The temperatures of Kathmandu Valley during winter and spring are in the range of 1.9ºC to 20.7ºC and 10ºC to 28ºC, respectively. The mean rainfall in winter is 20mm while in spring it is 55mm. There are three districts in Kathmandu Valley namely, Kathmandu Lalitpur/Patan and Bhaktapur. Towards the south-west of the valley there is the Dhading District; towards the north-east and eastern part the Nuwakot and Kavrepalanchowk districts are situated, respectively. The map of Nepal showing the study area and the places of origin of slaughter pigs is presented in Figure 1.

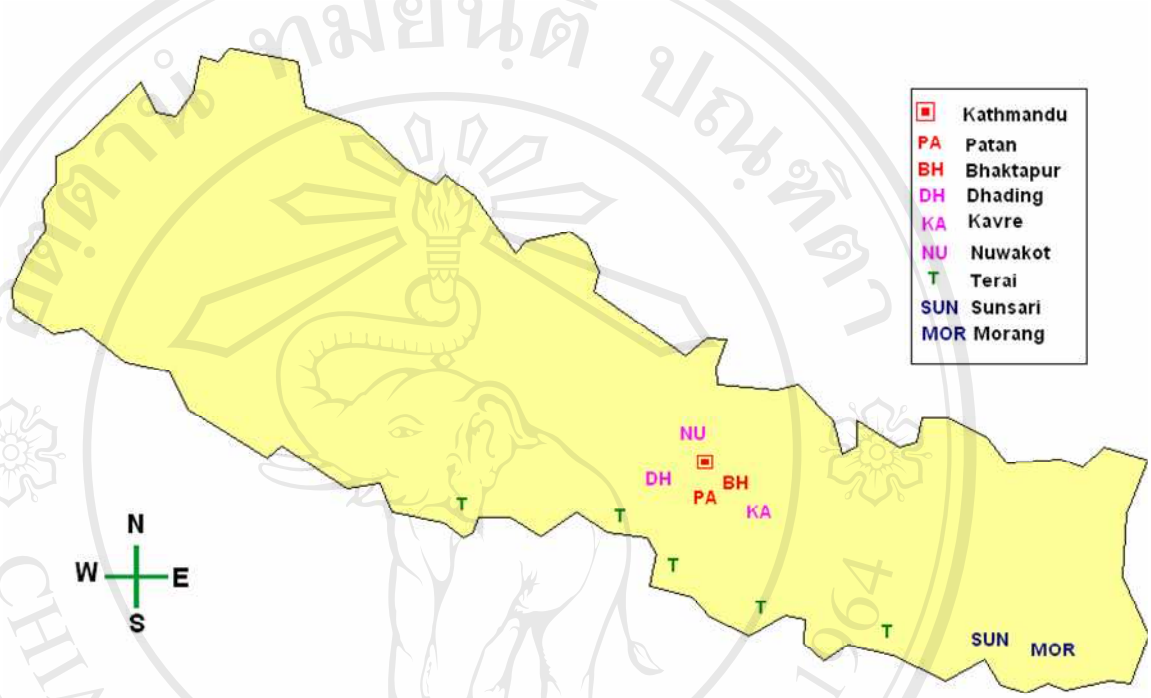

Figure 1; Map of Nepal showing the study area and the places of origin of slaughter pigs.

3.3 Slaughter Slabs

According to the Third Livestock Development Project (TLDP, 2002), a total of 50 pigs are slaughtered every day in Kathmandu Valley. There is no pig slaughterhouse in the valley. Pigs are slaughtered at slaughter-slabs. The slabs were numbered as Slab-1, Slab-2, Slab-3, and Slab-4. Slab-1 is located in the Patan District. Slab-2 and Slab-3 are located in the Kathmandu District whereas, Slab-4 in the Bhaktapur District. Among these slabs, Slab-1 also called “Nippon”, is the registered slaughter slab for pig slaughtering under the Department of Livestock Services Ministry of Agriculture and Co-operatives. All other slabs are unregistered. Slaughter pigs in these slabs come from different areas of Kathmandu Valley, in addition to those, from eastern Nepal, Terai and adjoining districts of the valley i.e Dhading, Kavrepalanchowk and Nuwakot. Dharane pigs are brought from eastern Nepal and Hurra from the Terai. Most pigs coming from adjoining areas and Kathmandu Valley are of the improved breeds.

a) Slab-1

“Nippon”, Slab-1 is the modern one. A meat shop is attached to this slab. About 8-10 pigs are slaughtered per day. The main importance of this slaughter slab is slaughtering of only male Dharane pigs. Nippon has selective clients for the meat sale, who prefer the meat from male Dharane pigs. These clients are of Rai and Limbu communities, being migrated from eastern part of Nepal.

b) Slab-2

This slab is situated near the Bishnumati River. The slab started slaughtering pigs about 20 years ago. In addition to slaughtering improved breeds of pigs, Hurra pigs are also slaughtered here. The Hurra breed looks quite similar to wild boar and hence, in the market, the meat of Hurra is as popular as “wild boar meat”. About 10 pigs are slaughtered per day.

c) Slab-3

This is the oldest slaughter slab, situated at the centre of Kathmandu Valley, but has not been registered yet. Pigs slaughtered here come from the Kathmandu Valley and the adjoining areas. They are mostly white coloured pigs. Although there is no meat shop attached to the slab nevertheless the offal is sold at the slab itself. About 7-10 pigs are slaughtered per day.

d) Slab-4

This slaughter slab is situated in the Bhaktapur District. Here, all types of pigs are slaughtered. The capacity of this slab is 8 to 10 pigs per day. Pigs come from Kathmandu Valley and nearby areas only. There is no meat shop at the slaughter slab.

3.4 Sampling procedure and biological sample collection

3.4.1 Sample size determination

The total pig population in the country is about 0.9 million (CBS, 2002). Since there was no reliable information on prevalence of porcine cysticercosis and trichinellosis, the sample size was calculated on the basis of an expected conservative prevalence of 50%, 95% CI, 5% error (Win Episcope 2.0) and for that, 385 carcass examinations and serum collections were sufficient for the prevalence survey for cysticercosis and trichinellosis. The study population was the slaughtered pigs at the 4 slabs.

3.4.2 Collection of biological samples

3.4.2.1 Sampling schedule

This study was a cross sectional study design, in which the individual pig was the sampling unit. Sampling methods consisted of convenient sampling of the slaughter slabs and random sampling of slaughter pigs at the time of slaughter. The visitation weeks for slaughter slabs were chosen randomly. The samples were collected from November 2004 to April 2005. A total of 504 carcasses were examined for cysticercois with an equal number of samples distribution at each slab (126) since the over all number of pigs slaughtered in each slab remained in the similar range. In total, 400 serum samples were collected and each slab accounted for 100 sera. The total visitation days to the slaughter slabs for meat inspection were 84 days and for serum collection 67 days. Sample collection was done 4 days a week.

3.4.2.2 Collection of cysts and blood samples

The method of meat inspection was based on the OIE guidelines for meat inspection of cysticercosis (OIE, 2004). Meat inspection was done by visual inspection of the carcass, its cut surfaces and the organs within it to look for the cysticerci. The external and internal masseters and the pterygoid muscles, were each examined and incisions were made. The freed tongue was examined visually and palpated. Pericardium and heart were examined visually. The heart was incised once lengthwise through the left ventricle and inter-ventricular septum, thus exposing the interior and cut surfaces for examination. The muscles of the diaphragm, after removal of the peritoneum, were examined visually and incised. The esophagus was examined visually.

In the examination process, if a cyst was found at the inspection sites, the whole carcass was then further inspected in detail taking permission from the butcher. The muscles examined were external masseter, internal masseter, psoas muscle, tricep brachii, forelimb muscle, hind limb muscle and abdominal muscle. Cysts were

counted and recorded according to the scoring method. The scoring method consisted of if no cyst = 0, cyst 1 to 5 = 1, and more than 5 = 2. The samples from each part were collected in a small polythene bag separately and were kept frozen at -25°C until the final morphological examination was done.

The traditional method of pig slaughter does not include the bleeding step. Scalding follows right after stunning and then the evisceration. Thus, the blood was collected from the heart during the evisceration. The blood was immediately transported to Central Veterinary Laboratory (CVL), Tripureshwor, Kathmandu and centrifuged to separate the serum. The serum samples were stored at -25°C at the same laboratory.

3.5 Microscopic examination of cysts

Frozen cysts were thawed in the beginning. The cysts then were soaked in the 10% hydrochloric acid (HCL) for about two minutes to dissolve the outer layer. With the help of a tiny needle the invaginated scolex was separated and examined under microscope at 400× (ocular 10×, objectives 40×) magnification. Confirmatory diagnosis of morphological examination was conducted at the Parasitological Laboratory Regional Center for Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai, University, Thailand.

3.6 Indirect, non-competitive Enzyme Linked Immunosorbent Essay (ELISA) for *Trichinella*

ELISA test was conducted at Regional Center for Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand. The method was the standard method described by the National Reference Laboratory for Trichinellosis, Federal Institute for Risk Assessment (BfR) Berlin, Germany. The detail protocol developed by the BfR for the indirect non-competitive ELISA for *Trichinella* is as follows.

The ELISA-Kit consisted of

a) Microtitre plates coated with *Trichinella* antigen (excretory-secretory antigen of *T. spiralis* larvae); 50µl *Trichinella* E/S antigen per well, storage at 4-8ºC
b) *Trichinella*-positive control serum (1ml, lyophilized), storage at –20ºC
c) *Trichinella*-negative control serum (1ml, lyophilized), storage at –20ºC

Additional buffers and reagents:

a) Phosphate Buffer Saline (PBS buffer) (not included, to be prepared according to protocol)
b) Anti-pig IgG-peroxidase conjugate pre-diluted 1:10, (1ml), storage at –20ºC (SIGMA, product No. A5670)
c) ABTS buffer, dry matter from Boehringer, storage at 4-8ºC
d) Tablets chromogen ABTS, storage at 4-8ºC

Preparation of ELISA reagents

1) PBS-Tween 20 (pH = 7.2-7.4): PBS-T was prepared by mixing following reagents.

KH_2PO_4	0.4g
$Na_2HPO_4 \times 12\ H_2O$	5.8g
NaCl	16.0g
KCl	0.4g
Tween 20	1.0 ml
Aqua dest	2000ml

2) Preparation of ABTS buffer (pH 3.4-3.6): Take 1.67g of citric phosphate buffer and add 100 ml of aqua dest. Mix two tablets of chromogen ABTS (100gm) to 100 ml of ABTS buffer. The resulting solution is the substrate in ABTS buffer.

Test procedure for ELISA

a) Wash the microtiter plate 1 × with aqua dest and 3 × with PBS-T (each for 3 min).
b) Dilute the test and control sera in PBS-T (1:100) and put into the wells (volume 50µl).

Example of 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 = Field sample

c) Incubate for 30 min at 37ºC and wash as under b.
d) Add 50µl anti-pig IgG peroxidase-conjugate (pre-diluted 1:10) at final dilution of 1:1200 in PBS-T to all wells.
e) Incubate for 30 min at 37ºC. Wash under b and finally wash again 1 × with aqua dest.
f) Add 50µl of freshly prepared ABTS prepared under 2 (substrate indicator) to all wells.

g) Read the plate for extinction value/optical density value (OD) of the samples at 405nm if the positive control serum has an OD of 1.300-1.400. To reach this OD value, incubate at room temperature for about 20-30 minutes.

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

$NE = OD\text{-}OD_{neg}$

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%.

$$\text{ELISA-index (\%)} = \frac{mNE_{sample}}{mNE_{pos}} \times 100\%$$

d) Evaluation of test results:

"*Trichinella*-negative" (-) ELISA-index (%) < 8

"*Trichinella*-questionable" (?) 8 ≤ ELISA-index (%) < 14

"*Trichinella*-positive" (+) ELISA-index (%) > 14

3.6.1 Titration procedure

The positive and doubtful samples were confirmed by titration according to procedure described in Nöckler *et al.* (1995).

The ELISA plate is washed with PBS buffer and blot dry, in the first 4 wells of the first column 10µl of negative control (diluted in PBS 1: 100) is added and in the

remaining 4 wells the positive control is added with the same dilution and amount. The positive and doubtful samples found in the screening test are diluted as 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280 in PBS. Dilution steps are made as follows.

In the first row of ELISA plate except the first well, mix 10µl of sample and 90µl of PBS and in wells of the remaining rows except the first put 50µl of PBS in each. Now, mix sample and PBS thoroughly in the first row and draw out 50µl from there and put into the second row, mix well in the second row and again draw 50µl from there and put to the 3rd row. In the same way go up to the last row and from the last row discard 50µl solution. Thus, required serial dilutions can be obtained. Now, incubate the plate for half an hour at 37ºC, take it out of the incubator and wash by buffer three times and blot dry. Add the conjugate and incubate again for half an hour at 37ºC. Wash the plate by PBS once and three times with distilled water. Finally, add ABTS and read.

Criteria for evaluation of titration results

The sample is positive if ELISA index is eighty percent and above in 1:10 dilution. The border line titer is defined as the titration step at which the sample still shows the positive OD value. The minimum border line titer should be 1:80 for positive samples. If the ELISA index at 1:10 dilution is less than 40% the sample is a negative sample. An ELISA index of 40% to 80% at 1:10 dilution is described as a doubtful sample (Nöckler *et al.*, 1995).

3.7 Questionnaire survey for neurocysticercosis

Questionnaires (reproduced in the Annex 1) were administered to the hospitals along with the authorization letter from the Public Health Department, Kathmandu Metropolitan City (KMC), and the Directorate of Animal Health, Department of Livestock Services (DLS), Ministry of Agricultures and Co-operatives, Nepal. Table 2, shows the details about the hospitals of Kathmandu Valley and those surveyed for neurocysticercosis (NCC). In total, 8 hospitals of the valley were surveyed. The concerned specialists and medical recorders of the respective hospitals answered the questionnaires after reviewing the hospital records of 2000 to 2004. The survey was conducted once a week from November 2004 to April 2005.

Table 2; Different hospitals surveyed in Kathmandu Valley for neurocysticercosis, Nov. 2004 to April 2005.

	Types of hospitals		
Description	**National**	**Private**	**Community**
Total hospitals	13	30	9
Hospitals with NCC data	6	NA*	0
Hospitals surveyed	3	5	0

NA* Information not available

The admissions in the hospital do not represent the number of in-patients, as a person may have more than one admission within the year. Therefore, the results are presented in terms of NCC patients per 1,000 admission episodes and NCC patients per 1,000 admission episodes and out patients' department (OPD) visits.

3.8 Data management and analysis

Data entry and statistical analysis were carried out using the Microsoft Excel (MS-Excel) Statistical Software (Version 2002) and NCSS (Version 1997). A significant level (alpha) equal to 5% ($p = 0.05$) was used at which difference between values were considered statistically significant.

Prevalence and sero-prevalence were estimated as the number of infected and/or exposed individuals from the total analyzed. In case of trichinellosis, the ELISA indices were calculated by using the formula described in the protocol of ELISA from BfR. The prevalence and the confidence intervals were calculated by using the method described by Collett (1999). Kruskal Wallis test was used to find the significant difference in the prevalences among slaughter slabs, areas of origin and farming systems for cysticercosis as well as trichinellosis. Means and standard deviations (SD) as well as ranges for the small and large rostellar hooks were calculated. In hospital survey data, the percentages of NCC cases in terms of epileptic admission episodes were calculated. Demographic characteristics of NCC cases were calculated in percentage.

4 RESULTS

4.1 Sources and some characteristics of slaughter pigs

Slaughter pigs in Kathmandu Valley were from 9 different areas in Nepal. These areas are three districts of Kathmandu Valley namely Kathmandu, Patan and Bhaktapur, and three adjoining districts. The latter are Dhading, Kavre and Nuwakot. Other sources of those pigs were the Morang and Sunsari districts of eastern Nepal and the Terai region. The ages of those pigs ranged from 4 months to 42 months with the most prevalent age being 7 months. A total of the sampled carcasses for meat inspection, 69% (349/504) were male pigs and 31% (155/504) females. In serum collection, the distribution by sex was 68% (273/400) males and 32% (127/400) females. Slaughter pigs were comprised of six breeds. They were Dharane, Landrace, Yorkshire, Hurra, Local Cross and Hampshire.

4.2 Prevalence of porcine cysticercosis

Out of 504 carcasses examined during this study only 5 carcasses were positive for cysticercosis, giving the prevalence of 0.99% (95% CI: 0.32-2.29).

Table 3 shows the distribution of cysticercosis positive carcasses by farming systems, areas of origin and slaughter slabs, in Kathmandu Valley. About seventy four percent (372/504) of sampled carcasses were from the indoor managed pigs. None of them was positive for cysticercosis in post mortem inspection. Twenty five percent (127/504) of the sampled carcasses were from mixed farmed pigs. Four were positive among them giving the prevalence of 3.14%. Of the five carcasses examined from free-ranged pigs one was found positive for cysticercosis. Overall there was a significant difference among the farming systems in the prevalence of cysticercosis (Kruskal Wallis, H = 28.08, df = 2, p = 0.001).

Table 3; Distribution of cysticercosis positive carcasses by farming systems, areas of origin and slaughter slabs, in Kathmandu Valley, Nepal, Nov. 2004 to April 2005.

	Total carcasses	**% Positive**	**95 % CI**
Farming systems			
Indoor	372	0	
Mixed	127	3.14 (4)	0.86-7.86
Outdoor	5	20 (1)	0.50-71.64
Areas			
KTM Valley	243	0.41 (1)	0.01-2.27
Eastern Nepal	130	1.53 (2)	0.18-5.44
Terai	66	1.51 (1)	0.03-8.15
Adjoin districts	65	1.53 (1)	0.03-8.27
Slabs			
Slab-1	126	0.79 (1)	0.02-4.34
Slab-2	126	1.58 (2)	0.19-5.61
Slab-3	126	0.79 (1)	0.02-4.34
Slab-4	126	0.79 (1)	0.02-4.34

( ) = Number of positive samples

KTM = Kathmandu

About 48.2% (243/504) of examined carcasses were of the pigs from Kathmandu Valley alone. Of those, only one was positive, giving the prevalence of 0.41%. Twenty six percent (130/504) of the examined carcasses were from the pigs of eastern Nepal among them two were positive. Thus, the prevalence of slaughter pigs of eastern Nepal was 1.53%. Carcass examination of the pigs of Terai and adjoining regions of Kathmandu Valley accounted for 13% (66/504) and 13% (65/504) respectively. Only one carcass was positive for cysticercosis in each of these areas with the prevalence of 1.51% and 1.53%, respectively. The Kruskal Wallis test showed no significant difference in the prevalences among these areas ($H = 1.60$, $df = 3$, $p = 0.65$).

An equal number of carcasses was examined in each slab. The prevalence in slab 2 was 1.58 % whereas the prevalence in each of the other slabs was 0.79%. There was no significant difference in the prevalences among the slaughter slabs (Kruskal Wallis, H = 0.60, df = 3, p = 0.85).

4.3 Microscopic examination of cysts

The cysts collected during meat inspection were examined microscopically for the rostellar hooks. One cyst from each sample of muscle, tongue and heart was examined. There were two types of rostellar hooks. Those were small and large rostellar hooks. All were found to have sickle-shaped structure. The sizes of the small hooks ranged from 91µm to 108µm with a mean value of 99µm and standard deviation (SD) of 6µm. The sizes of the large hooks ranged from 132µm to 156µm with a mean of 143µm and SD 6µm (Table 4). The morphological characteristics (size and shape) of the rostellar hooks were comparable to those of cysticercus cellulosae (Soulsby, 1982).

Table 4; Results of the microscopic examination of cysts for rostellar hooks.

Types of hooks	Mean (µm)	Standard Deviation (µm)	Range (µm)
Small (15)*	99	6	91-108
Large (15) *	143	6	132-156

( )* = Number of hooks examined

4.4 Predilection sites

Heart, diaphragm and other muscles were found heavily infected in all positive carcasses. The esophagus and tongue were also found to be moderately to heavily infected. Among other muscles, the external masseter, internal masseter, psoas muscle, tricep brachii, forelimb muscle, hind limb muscle and abdominal muscle were examined and all of them had score 2 in the scoring system. Table 5 shows the results

of the record of cysts in positive samples. Heart, diaphram and muscle showed 2 scores in all positive carcasses whereas esophagus and tongue each had three 1 score and two 2 scores. No cyst was found in liver, lung and kidney.

Table 5; Cysts recorded in different organs and muscles in positive carcasses (n*=5).

Organs	**Score 0 = no cyst**	**Score 1 = 1-5 cysts**	**Score 2 > 5cysts**
Heart			5
Oesophagus		3	2
Diaphram			5
Muscle			5
Tongue		3	2

n* = Total number of positive carcasses

4.5 Seroprevalence of trichinellosis

Of the total 400 serum samples tested by ELISA, four were positive and one was doubtful. Table 6 shows OD values and ELISA indices of positive and doubtful samples in AB-ELISA. Sample 78, slab-4 showed the highest (0.59) OD value. But the ELISA index was the highest (39.30) in the sample number 73, slab-4. The sample number 87, slab-3 showed the OD value of 0.21 and ELISA index of 9%. In the re-screening stage this sample showed the ELISA index of 8.46%, which is lower than 14%. Therefore, the sample was confirmed doubtful.

Table 6; OD values and ELISA indices of positive and doubtful samples for AB-ELISA for *Trichinella*.

Slabs	Samples	OD Value	ELISA Index %	Result
Slab-2	47	0.46	18.66	+
Slab-3	65	0.31	16.57	+
Slab-3	87	0.21	9.00	?
Slab-4	73	0.59	39.30	+
Slab-4	78	0.48	29.76	+

? = Doubtful

4.5.1 Titration results

Table 7 shows the titration results of the positive and doubtful samples in screening test. All of them showed the border line titer of 1:80 and above. The highest border line titer was 1:540 from sample number 47, slab- 2.

Table 7; Titration results of the positive and doubtful serum samples in slaughter pigs in screening test.

Slabs	Samples	Border titer	Result
Slab-2	47	1:540	+
Slab-3	65	1:80	+
Slab-3	87	1:80	+
Slab-4	73	1:160	+
Slab-4	78	1:80	+

Out of 400 serum samples examined, 4 samples were positive for *Trichinella* antibodies giving the seroprevalence of trichinellosis 1% (95% CI: 0.27 - 2.54).

Table 8 shows the distribution of serum samples found positive for *Trichinella* antibodies by farming systems, areas of origin and slaughter slabs. Sera from indoor managed pigs were 71.2% (385/400), and from mixed farmed pigs 28.2% (113/400).

All 4 positive sera were from indoor managed pigs giving the prevalence of 1.4%. There was no significant difference in the seroprevalences of trichinellosis among farming systems (Kruskal Wallis, H = 1.62, df = 2, p = 0.44).

Table 8; Distribution of serum samples found positive for *Trichinella* antibodies by farming systems, areas of origin and slaughter slabs, in Kathmandu Valley, Nepal, Nov. 2004 to April 2005.

	Total serum samples	**% Positive**	**95% CI**
Farming systems			
Indoor	285	1.4 (4)	0.38-3.55
Mixed	113	0 (1) *	
Outdoor	2	0	
Areas			
KTM Valley	193	1.55 (3)	0.32-4.47
Eastern Nepal	104	0 (1) *	
Terai	54	0	
Adjoin districts	49	2 (1)	0.05-10.85
Slabs			
Slab- 1	100	0	
Slab- 2	100	1 (1)	0.02-5.44
Slab- 3	100	1 (1) (1)*	0.02-5.44
Slab- 4	100	2 (2)	0.24-7.03
Total	400	1 (4)	0.27- 2.54

( ) = Number of positive samples

( )* = Number of doubtful samples

About 48.2% (193/400) of sampled sera were of the pigs of Kathmandu Valley. Sera of pigs of eastern Nepal accounted for 26% (104/400), and those of Terai and adjoining areas of Kathmandu Valley were 13.5% (54/400) and 12.2% (49/400), respectively. *Trichinella* positive samples were found in Kathmandu Valley and adjoining areas giving the seroprevalences of 1.55% (3/193) and 2% (1/49),

respectively. There was no significant difference in the seroprevalences of trichinellosis among these areas (Kruskal Wallis, H = 2.72, df = 3, p = 0.43).

An equal number of serum samples was tested for ELISA from each slaughter slab. The seroprevalence of sampled pigs at slab 4 was 2% and at slab-2 and slab-3 it was 1% in each. The doubtful sample was from slab-3. There was no significant difference in the seroprevalences of trichinellosis among slaughter slabs (Kruskal Wallis, H = 2.01, df = 3, p = 0.56).

4.6 Results of hospital survey of NCC

The questionnaire survey revealed that all hospitals used either MRI or CT scan or both for the diagnosis of NCC. Overall, the answers for the suspected cause of NCC were pork, green salad, dirty food habit and poor personal hygiene. Annex 2 shows the distribution of total patients, epileptic patients and NCC patients in all 8 hospitals for the period of 2000 to 2004. The data of Bir Hospital (Bir), Tribhuwan University Teaching Hospital (TUTH), Patan Mission Hospital (Patan), Norvic Escorts International Hospital (Norvic), Kathmandu Medical College Teaching Hospital (KMC) and Nepal Medical College Teaching Hospital (NMC) were from admission episodes only. Data from B&B Hospital and Om Hospital were the combined data of admission episodes and out patients' department (OPD) visits. The data from Norvic Hospital and B&B Hospital were of a three years period (2002-2004) only.

a) Occurrence of NCC cases among total admission episodes and total epileptic admission episodes

Table 9 shows the distribution of NCC cases in terms of total admission episodes and total epileptic admission episodes. NCC per 1,000 admission episodes was the highest (4.56) in Norvic Hospital and the lowest (0.16) in KMC Hospital. Similarly the percentage of NCC in terms of epileptic admission episodes was the highest (30.5)

in Patan Hospital and the lowest (6.74) in KMC. During 2000-2004, NCC patients were found at the overall rate of 1.02 per 1,000 admission episodes in 6 hospitals. In the same hospitals, the occurrence of NCC was 18.7% of epileptic admission episodes.

Table 9; NCC cases in six hospitals of the Kathmandu Valley, during 2000-2004.

Patients	**Hospitals**						
	Bir	**TUTH**	**Patan**	**Norvic***	**KMC**	**NMC**	**Total**
Total admission episodes	47,707	80,420	88,200	4,166	36,632	28,726	28,5851
Total epileptic admission episodes	92	471	641	120	89	159	1,572
Total NCC cases	21	40	195	19	6	13	294
NCC per 1000 admission episodes	0.44	0.49	2.21	4.56	0.16	0.45	1.02
NCC per epileptic admission episodes (%)	22.8	8.5	30.5	15.5	6.74	8.17	18.7

*The data were available for the period of three years only (from 2002 to 2004).

NCC = Neurocysticercosis

TUTH = Tribhuwan University Teaching Hospital

Norv = Norvic Escorts International Hospital

KMC = Kathmandu Medical College Teaching Hospital

NMC = Nepal Medical College Teaching Hospital

The individual NCC patients' list in the data from 4 hospitals namely TUTH, Norvic, KMC and NMC showed that the patients were from all over Nepal. The patients from the Kathmandu Valley accounted for 32% (n = 78) in those data.

b) Occurrence of NCC cases among total admission episodes and OPD visits and total epileptic admission episodes and OPD visits

During 2002-2004, 1.3 NCC cases were found among 1,000 admission episodes and OPD visits at the B&B hospital (Table 10). For a longer duration (2000-2004), NCC cases at the Om hospital were at the rate of 1.5 per 1,000 admission episodes and OPD visits. NCC cases accounted for 42.5% and 43.5% of all admission episodes and OPD visits of epileptic patients at the B&B hospital and OM hospital, respectively. Overall, from two hospitals, 1.5 NCC cases were found among 1,000 admission episodes and OPD visits in those two hospitals. NCC cases accounted for 43.2% of all admission episodes and OPD visits of epileptic patients in those data.

Table 10; NCC cases at B&B hospital and OM hospital during 2000-2004.

Patients	**Hospitals**		
	B&B*	**OM**	**Total**
Total admission episodes and OPD visits	145,009	349,306	494,315
Total epileptic admission episodes and OPD visits	466	1,251	1,717
Total NCC cases	198	544	742
NCC per 1000 admission episodes and OPD visits	1.50	1.5	1.5
NCC per total epileptic admission episodes and OPD visits (%)	42.5	43.5	43.2

*Data were available for 3 years period only (from 2002 to 2004).

c) Demographic characteristics of NCC patients in the Kathmandu Valley

Table 11 shows the distribution of NCC cases in terms of demographic characteristics. Of the total 294 patients from 6 hospitals, 59% were males whereas 41% were females. The distribution was the highest (40%) in the age group of above 35 years. For the age groups of 0-14 years and 15-34 years, it was 22.5% and 37.5%, respectively. The highest (48%) occurrence was noted in lower socioeconomic status groups whereas the distribution among middle and upper economic status was 35% and 17%, respectively. The occurrence of NCC in vegetarian people was 36%. Distribution by caste was 31% in Brahmins and 69% in non-Brahmins.

Table 11; Demographic characteristics of NCC cases, at time of diagnosis.

Sex (%)		Age (years) (%)			Eco-status (%)			Food Habit (%)		Caste (%)	
♂	♀	0-14	15-35	>35	Uper	Middle	Lowr	Veg	Nonveg	Brah	NonBra
59	41	22.5	37.5	40	17	35	48	36	64	31	69

♂ = Male

♀ = Female

Veg = Vegetarian

Nonveg = Non-vegetarian

Brah = Brahmins

NonBra = Non-Brahmins

Eco-status = Socioeconomic status

Uper = Upper

Lowr = Lower

5. DISCUSSIONS AND CONCLUSIONS

Over the years, government institutions and non-governmental agencies have imported and introduced improved breeds of pigs in different parts of Nepal and possibilities of developing small piggeries to generate cash have increased (Dhaubhadel, 1992). This has eliminated the religious prejudice, which had imposed restrictions of pig farming in high social groups in Nepal. The annual population growth rate (4.55%) of pig is higher than any other food animals (1.93% in buffaloes, -0.82% in sheep, 2.03% in goats) (CLDP, 2003). But on the other hand, pig farming has not yet reached to full commercialization. Pigs are reared with poor nutrition and in minimal housing conditions.

A prevalence survey of porcine cysticercosis and trichinellosis in slaughter pigs in Kathmandu Valley was conducted for the period of Nov. 2004 to April 2005. All together, about 40 pigs were slaughtered per day in 4 slaughter slabs from where the samples were collected. This accounted for 80 % (40/50) of the total of slaughtered pigs in the valley. The sample size in each slab was calculated according to probability proportional sampling. The visitation weeks for the slabs were randomly chosen and the frequency of visits was made consistently (4 days a week). In total, 504 pigs were sampled by random sampling at the time of slaughter during the survey. Thus, the study design was able to represent the prevalence of slaughter pigs in the valley.

There was no control over pig slaughtering due to lack of meat inspection in the country. An attempt was made to sample pigs from all slaughter slabs of the valley. However, due to few pigs with inconsistent slaughtering at other slabs this was not always possible. This was the main limitation of this study.

5.1 Porcine cysticercosis and human neurocysticercosis

The fact that the pork industry is still underdeveloped, and lack of awareness of butchers about porcine cysticercosis, tongue palpation to examine for cysticerci due to cysticercus cellulosae in pre-slaughter pigs is not normally practiced in Nepal. In other cysticercosis endemic areas, tongue palpations have been used to reject the pigs that are recognized to have *T. solium* larvae under the tongue for fear of losing money due to carcass condemnation in meat inspection (Ngowi *et al.*, 2004). In light infections the tongue palpation is not sensitive (Scuitto, 1998). Therefore, this study was aimed at surveying of porcine cysticercosis using meat inspection, to determine the predilection sites for the cysts, and to identify *Taenia* species involved.

Cysts being harbored by the heart, other muscles and diaphragm were more than those located in the tongue and esophagus. Among other muscles, the external masseter, internal masseter, psoas muscle, tricep brachii, forelimb muscle, hind limb muscle and abdominal muscle were examined and all showed high infection. These results are in accordance with Boa *et al.* (2002). However, due to very few positive results, a clear determination of the predilection sites could not be made.

The examination of the rostellum from the scolex of the collected cysts revealed two different sized sickle-shaped hooks, small with the range of 91µm to 108µm and a mean of 99µm and large hooks with the range of 132µm to 156µm and amean of 143µm. The morphological characteristics (size and types) of the rostellar hooks were comparable to those of cysticercus cellulosae (Soulsby, 1982). Thus, it was concluded that all cysts found in the meat inspection were due to *T. solium* infection in pigs, and no cyst of *T. asiatica* was found in this study.

The prevalence of cysticercosis in slaughter pigs in Kathmandu Valley was 0.99% (5/504) in this study. All carcasses of indoor managed pigs which accounted for 74% (372/504) of all samples were negative for cysticercosis. Twenty five percent (127/504) of the sampled carcasses were from mixed farmed pigs. The slaughter prevalence in those mix farmed pig carcasses was 3.14%. Of the five carcasses from

free-ranged pigs one was positive for cysticercosis. Overall, there was a significant difference among the farming systems in the prevalence of cysticercosis. Due to the fact that many pigs were from indoor farming and none of them found positive, the over all prevalence was low in this study. In contrast, Ngowi *et al.* (2004) has reported 6.5% prevalence in indoor raised pigs although free ranged pigs showed 17.8% prevalence.

The ages of the slaughter pigs ranged from 4 months to 42 months with the most prevalent age being 7 months. Of the sampled carcasses 69% (349/504) were male pigs and 31% (155/504) females. About 48.2% (243/504) of the examined carcasses were of pigs from Kathmandu Valley alone and only one was positive for cysticercosis, giving a prevalence of 0.41%. Twenty six percent (130/504) of the examined carcasses were from the pigs of eastern Nepal and only two were positive, giving a prevalence of 1.53%. Carcasses of pigs from Terai and adjoining areas of Kathmandu Valley accounted for 13% (66/504) and 13% (65/504), respectively. Only one carcass was positive for cysticercosis in each of these areas resulting in prevalences of 1.51% and 1.53%, respectively. Thus, these results indicated that *T. solium* was endemic in all the areas of origin of slaughter pigs. However, it can be assumed that pigs presented to Kathmandu Valley are those of improved hygienic farms. In general, pigs of remote areas would never reach the valley. So, prevalence of cysticercosis in slaughter pigs of Kathmandu Valley serves as an indicator of high prevalence in other parts. This could be postulated from Joshi *et al*. (2004a), who reported 32% (136/419) of cysticercosis by tongue palpation of local pigs in rural areas in western Nepal.

Joshi *et al.* (2004a) reported 14.28% (28/196) of porcine cysticercosis in slaughter pigs in Kathmandu Valley in 1997. This significant decrease of prevalence obtained in this study can not be easily explained. However, it can be hypothesized that it could either be due to the change in management of the pig farming, sources of the slaughter pigs, different study designs or different sensitivities in meat inspection by the investigators, or due to improvement of general hygienic conditions in the country during the last 8 years.

Porcine cysticercosis (methods being the meat inspection or tongue palpation) in different countries of Asia has been reported in the range of 0.02% to 32.5% (Rajshekhar *et al.*, 2003). The findings of this study agreed with the findings of southern Vietnam, where the prevalence was recorded 0.9%. Similarly, the results of this study are found to be in accordance with the results presented in some parts of China. In China, porcine cysticercosis is highly variable, ranging from 0.84% to 15% and in some areas as high as 40% (Rajshekhar *et al.*, 2003). However, there is a lack of real prevalence data to be compared with this study in Asia, as the data from most Asian countries are either unreliable or outdated as stated by Rajshekhar *et al*. (2003). Porcine cysticercosis in a pig farming community in India was found to be 26% (Prasad *et al.*, 2002). However, the findings of this study were of slaughtered pigs, which do not represent a single particular community. The prevalence obtained in this study can be compared to that of some West African countries e.g. Burkino Faso (0.6%) and Senegal (1.2%) (Zoli *et al*., 2003).

According to Boa *et al*. (2002), the chance of detecting porcine cysticercosis in meat inspection when the regulations are followed is low. This study followed the OIE guidelines from which regulations are derived in each country. The sensitivity of meat inspection was reported to be very low (OIE, 2004). On the other hand, slaughter slabs had poor facilities for meat inspection as well as inadequate lighting. Due to these limitations, positive cases might have been missed and the prevalence could be even higher than reported in this study. However, since there was no gold standard test developed for porcine cysticercosis, consequent meat inspection was chosen as the appropriate approach for Nepal.

During 2000-2004, neurocysticercosis (NCC) patients were found at an overall rate of 1.02 per 1,000 admission episodes in the combined data of 6 hospitals (Bir, TUTH, Patan, Norvic, KMC, NMC) in the Kathmandu Valley. In the same hospitals, the occurrence of NCC was 18.7% of epileptic admission episodes. Among these hospitals, NCC cases were the highest (4.5 per 1,000 admission episodes) at the Norvic hospital, and the lowest (0.16 per 1,000 admission episodes) at KMC. The

high occurrence of NCC cases at the Norvic hospital could be due to selection bias as patients visited to Norvic Hospital for neurological problems. Norvic hospital specializes in neurology.

Overall, from B&B Hospital and Om Hospital, 1.5 NCC cases were found among 1,000 admission episodes and out patients' department (OPD) visits. NCC cases in terms of admission episodes and OPD visits of epilepsy were 43.2% in those data. From this it was clear that NCC was the major contributory factor in the occurrence of epilepsy in Nepal. Among 121 focal seizure cases, 73% were diagnosed as NCC by EITB at Modal Hospital, Nepal (Dhakal *et al.*, 2005). However, in this study the epileptic patients included all types of epilepsies. Comparatively, in Rajbhandari (2004), of the total 300 epileptic patients in Shree Birendra Military Hospital 47% were diagnosed NCC by CT scan and MRI. In the analysis of 1026 epileptic patients comprising of various types of seizures, 34.6% were diagnosed as NCC in a Hospital in north-west of India (Singh *et al.*, 2005). In the report of Del Bruto *et al.* (2005), one third of epileptic patients were NCC cases. Studies from countries where neurocysticercosis is endemic, had reported that up to 50% of all cases of adult-onset epilepsy were due to neurocysticercosis (Roman *et al*., 2000).

Out of total 23,402 general surgery specimens 0.26% were diagnosed as cysticercosis by histopathology in Patan Hospital during 1993 to1998 (Amatya and Kimula, 1999). A diagnosis of cysticercosis was made in 0.01% (n = 25,033) of pathological specimens examined at Bir Hospital, Kathmandu Nepal from 1995 to 1997 (Joshi *et al.*, 2004a). But, in this study, the survey revealed all NCC patients were diagnosed by imaging techniques (CT scan and MRI).

NCC cases by age groups revealed that 40% were above 35 years followed by 37.5% in 15-34 years old and 22.5% in less than 14 years. In contrast, in a review by Roman *et al.* (2000) age groups found affected in Mexico were as follows. The age of 25-44 years (42%), 45-64 years (20.4%), 15-24 years (16%) and below 15 years (14.5%). According to Ikejima *et al.* (2005), 77% NCC cases were in 18-59 year age group.

Distribution of NCC cases by sex revealed 59% in males and 41% in females in the data of 6 hospitals. In contrast, Amatya and Kimula (1999) reported 61% female and 39% male in the data of Patan Hospital. In this study, 48% of NCC patients were from a lower socioeconomic status. Thirty six percent of the total NCC cases were from vegetarians; elsewhere in India, 95% NCC patients were vegetarians (Rajshekar *et al.*, 2003).

Individual NCC patients' list from 4 hospitals revealed that 32% (25/78) of the NCC patients were from Kathmandu Valley. However, it should be considered that inability to pay for costly diagnosis and treatment of the rural population underestimated the situation of NCC outside the valley. On the other hand, the CT scan and MRI have been used for the quicker and more accurate diagnosis of NCC. They are sometimes limited by the small size of the visualized lesions and atypical images, which are difficult to distinguish from abscesses or neoplasms (Sako *et al.*, 2005).

5.2 Trichinellosis

Four hundred serum samples were collected from the pig carcasses during meat inspection for cysticercosis. Out of those 400 sera, 4 were positive for AB-ELISA of *Trichinella,* giving a seroprevalence of trichinellosis in slaughter pigs in Kathmandu Valley 1%. This finding shows that pigs from Kathmandu Valley and adjoining areas have trichinellosis. This conclusion is further supported by the fact that three positive cases were from Kathmandu Valley whereas one was from adjoining areas. Although the proportion of the sera from indoor managed pigs was 71.2% (285/400), and from mixed farmed pigs was 28.2% (113/400), nevertheless all 4 positive samples were from indoor managed pigs, giving the prevalence of 1.4%.

Comparatively, Joshi *et al.* (2004b) reported a prevalence of 0.47% of trichinellosis by EITB (2 out of 425) in slaughter pigs in Kathmandu Valley.

However, using synthetic beta-tyvelose antigen in ELISA they unexpectedly obtained high positive cases. According to Gamble *et al*. (1983), the use of E/S antigens increases the sensitivity and specificity of the diagnosis of *T. spiralis* in pigs, thus increasing the detection of natural infections, even those with very low parasitic densities. Recently, it has been reported that a synthetic glycan antigen, beta-tyvelose appeared to be less sensitive than the E/S antigens and beta-tyvelose antigen may not be suitable for screening of trichinellosis in pig herds (Moller *et al.*, 2005). The findings of this study can be compared with the findings in some parts of China as the seroprevalence of porcine trichinellosis in China varies from 0.09% to 29.63% (Wang and Cui, 2001).

In this study all the positive sera were from pigs of less than 9 months of age. About 10% (40/400) of the serum samples were from the pigs of over 1.5 years. But, it is worth noting that ELISA fails to detect infected pigs during both the early and the very late stages of infection (OIE, 2000). Thus, it is likely that positive cases might have been missed, and the prevalence could be even higher than reported here. It has been reported that the time of seroconversion is correlated to infection dose and there are no known cross-reactions using E/S antigens in ELISA (Nöckler *et al*., 2000). Therefore, it can be assumed that one doubtful result obtained in this study could be due to old infection or low infection dose of *Trichinella* in the pig.

Management practices of the farms are associated with the occurrence of trichinellosis (Gamble and Bush, 1999; Gamble *et al.*, 1999). Rodents play an important role in the transmission of *Trichinella* in the domestic cycle. Kitchen wastes, which are not heat treated properly, are the risk factors for trichinellosis (Urquhart *et al.*, 1996). In Nepal, pig farming is not yet commercialized and the bio-security measures have not been introduced in pig farms (Dhaubhadel, 1992). Feeding of offal and kitchen waste is very common in pig farming in Nepal (Joshi *et al.*, 2004b). In this study the positive pigs were from indoor farms in which the pigs were confined in the same place under minimum housing systems until they were marketed. Therefore, cannibalism and the access of rodents in the indoor farms could

not be avoided. This could explain why positive cases were recorded in the slaughter pigs.

Direct demonstration of the parasites is required for the proof of the presence of *Trichinella*. Isolation of the parasite for identification of species should be followed by direct demonstration in order to study the epidemiology of trichinellosis in Nepal in the near future.

5.3 Conclusions and recommendations

This study has shown that the zoonotic parasite, *T. solium*, is a real public health threat in Nepal. The serological evidence of trichinellosis in pigs suggested that *Trichinella* exists in Nepal, too.

Whilst studies in the slaughter slabs and hospitals are useful in improving the understanding of taeniosis/cysticercosis situation, well-designed community-based studies for example, cohort or the case-control studies examining the burden of the cysticercosis in pigs, and taeniosis in humans are recommended. Similarly, seizure disorders due to NCC in humans and their ecological determinants are much required.

Animal Slaughtering and Meat Act (1999) mandates the slaughterhouse construction, meat inspection and control. Therefore, there is an urgent need to implement this act in Nepal. The cysticercosis positive carcasses must be deep frozen for a week before sale. Similarly, uncooked *Trichinella* positive carcasses should not be delivered to the market.

Community health education about meat-born diseases should address cysticercosis and trichinellosis, and the knowledge has to be imparted effectively to the workers, butchers, meat sellers and meat consumers. Adequate sewage disposal systems should be implemented at every slaughter slab. The kitchen waste and offal should be sterilized before feeding to pigs.

The serological evidence of trichinellosis has suggested that direct demonstration of the parasites is required by digestion method, which is the gold standard test for trichinellosis, for the proof of the presence of *Trichinella.* The isolation of the parasites for identification of species has to be followed accordingly. Therefore, establishment of cost-effective laboratory facilities for diagnosis of trichinellosis should be brought to the attention of the veterinary and public health authorities in Nepal.

Intensive pig farming could reduce the risk of cysticercosis and trichinellosis in human and animal population. The control strategies for cysticercosis in particular should be to prevent pigs from having access to human faeces. This can be achieved by good toilet facilities for humans and controlling pigs from letting them loose to run freely. In endemic areas, human mass treatment against taeniosis is recommended with anthelmentics at required intervals.

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7. ANNEXES

Annex 1; Questionnaires for hospital survey of human *T. solium* cysticercosis

Date_____

Name of the Hospital_____Location_____

Name of the doctor_____

1. Patients' residence areas

_____

_____

2. Total number of patients in the hospital

a) year 2000_____ b) 2001_____c) 2002_____d) 2003____e) 2004_____

3. How many epileptic cases are found for the period of

a) year 2000_____b) 2001_____c) 2002_____d).2003____e) 2004_____

4. How many confirmed neurocysticercosis cases

a) year 2000_____ b) 2001_____c) 2002_____d)2003____e) 2004_____

5. What is the method of diagnosis used in the Hospital?

a) CT scan____b) ELISA_____ c) MRI_____d) Others

6. Age groups (years) of NCC cases

a) 0-14_____b) 15-34_____c) above35_____

7. Sexes

a) Male____ b) Female____

8. Patients' socioeconomic status

a) Upper____

b) Middle____

c) Lower____

9. Food habit

a) Veg_____b) Nonveg (Pork eating) _____

10. Suspected cause of neurocysticercosis

a) Pork____ b) Salad___c) Other_____

11. List the patients according to the caste.

a) Brahmins____ b) Non- Brahmins_____

12. Other comments

_____

_____

_____

Annex 2; Total admission episodes, total epileptic admission episodes and NCC cases by hospitals and years in the Kathmandu Valley.

	Patients' profile (total, epileptic and NCC) in different hospitals								
Year	**Hospl**	**Bir**	**TUTH**	**Patan**	**Nor**	**KMC**	**NMC**	**B&B***	**Om***
2000	Total	9082	14806	15322		4661	4716		53645
	Epilep	28	103	123		8	24		229
	NCC	1	7	52		1	1		103
2001	Total	9741	15504	16586		5271	5703		55351
	Epilep	25	86	147		12	33		208
	NCC	6	16	38		2	1		98
2002	Total	8646	15900	17976	1213	8512	6504	39428	57201
	Epilep	19	78	108	35	16	42	186	285
	NCC	7	7	64	6	0	5	64	121
2003	Total	10228	16706	19143	1324	8872	6202	48459	81405
	Epilep	13	96	137	38	13	32	174	314
	NCC	4	7	15	9	2	2	56	141
2004	Total	10010	17508	19173	1629	9316	5601	57122	101704
	Epilep	7	108	126	47	28	28	106	314
	NCC	3	3	26	4	1	4	78	141

* Patients included admission episodes and OPD visits

Epilep = Total epileptic patients admission episodes and OPD visits

NCC = Neurocysticercosis

Epilep = Epileptic patients

Hospl = Hospital

TUTH = Tribhuwan University Teaching Hospital

Nor = Norvic Escorts International Hospital

KMC = Kathmandu Medical College Teaching Hospital

NMC = Nepal Medical College Teaching Hospital

B&B = Banskota and Baidhya

CURRICULUM VITAE

Personal background

Name:	Buddhi Sagar Sapkota
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Work Experiences

Teacher at Raktakali English Secondary School, Kathmandu Nepal (1991-1994)
Teaching mathematics and science in high school level

Technical manager at Ratna feed industries private Ltd, Kathmandu Nepal (1999-2001)
Worked as technical consultant for livestock farming, feed formulation, hatchery and breeding management and meat inspection

Chairman of Community Health Promotion and Zoonosis Center, Kathmandu (2000-2003)
Non governmental organization (NGO) working for community health promotion and zoonosis control, I was involved in the joint program of the said NGO and Kathmandu Metropolitan City in rabies control program in Kathmandu Valley

Director of Pet love Vet. Clinic, Kathmandu (2001-2003)
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DECLARATION

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

Name Buddhi Sagar Sapkota

Signature 

Date of submission 20th September 2005