CHAPTER-I

INTRODUCTION

"Those diseases and infections which are naturally transmitted between vertebrate's animals and men are called zoonotic disease" (WHO, 2010). It involves a large range of animals that are consumed by humans or associated with them. Among them *Taenia solium* is one of the important neglected parasitic zoonoses disease. *T. solium* is a pork tapeworm which causes intestinal taeniasis and cysticercosis in human and pigs. Cysticercosis is a disease caused by larval stage of the *T. solium* (*Cysticercus cellulose*). The illness are caused by development of characteristic cysts (cysticerci) in different body parts which most often affect the central nervous system (neurocysticercosis), skeletal muscle, eye and skin. Many individuals with cysticercosis never experience any symptoms (asymptomatic) but may be symptomatic in case of central nervous system (CNS) involvement.

Historically the disease has been recognized since 2000 BC by Egyptians. But later Aristotle (384-322 BC) described the infection of pork with tapeworm at ancient Greeks time. The disease was also recognized by Muslim physicians and is thought to be the reason for Islamic dietary prohibition of eating pork. Later in 1850, Germans investigators described the life cycle of *T. solium* (Soulsby, 1982).

Cysticercosis is endemic to many parts of the developing world including Latin America, Sub-Saharan Africa, East Asia, Eastern Europe (Somers *et al.*, 2006; Rajshekhar *et al.*, 2003; Zoli *et al.*, 2003; Garcia *et al*., 2000; White, 2000). Worldwide, more than 4 million people harbor the pork tapeworm and 50 million people are infected with cystic stage. In Asian countries like Vietnam, India, Nepal, China, Korea and Bali (Indonesia) sero-prevalence studies has indicated high rates of exposure to the parasite ranging from 0.02 to 12.6% (Rajshekhar *et al.*, 2003). The incidence of cysticercosis has also increased in the United States due to increased immigration from developing countries and it is estimated that about 1000 new cases of cysticercosis are diagnosed annually. Neurocysticercosis is one of leading cause of late onset-seizure worldwide. An estimated 50,000 people die from cysticercosis each year because of CNS or cardiac complication (WHO, 2010). In California, USA, from the year 1989 to 2000, there was 3.9 per million death associated with human neurocysticercosis (Sorvillo *et al.*, 2004). In Nepal, neurocysticercosis patients has

been found at an overall rate of 2.34 per 1000 OPD visits and rate of neurocysticercosis per epileptic admission episodes ranged between 13.3 -31.7% (Joshi *et al.,* 2004).

Human are host for *T. solium* and they carry tapeworm in their intestine, often without symptoms. The tapeworm eggs are periodically shed in the feces by human reservoir and typically pigs ingest the eggs in contaminated food or water. The pigs subsequently become infected and develop cysticerci in their body tissue. When human eat infected raw or undercooked pork, the life cycle of the tapeworm is completed and the life cycle continues. Human cysticercosis, however develops after human ingest *T. solium* eggs. The eggs are typically spread via food, water or surface contaminated with infected feces and the mode of transmission is usually fecal-oral route. Often times, the eggs may be spread from the hands of infected food handlers who do not clean their hands or from foods fertilized/ irrigated with water containing infected human feces (Scantz *et al.,* 1992). It is also possible for individuals to be infected by autoinfection who carry the tapeworm themselves. The symptoms of cysticercosis may develop from several months to several years after the initial infection. The symptoms depend on the location and number of cysticerci though many individuals with cysticercosis will never develop any symptoms at all. The majority of patients with cysticercosis who present to a health provider have CNS involvement or neurocysticercosis (NCC). Cysticercosis is common in the developing countries due to persistence of poor sanitary condition, free ranging of pigs with easy access to human feces, due to availability of infected pork meat in market and consumption of undercooked meat.

Various studies up to date indicate the endemicity of cysticercosis in Nepal. In the present study, it has aimed to determine the seroprevalence of cysticercosis by collecting the blood samples from clinically diagnosed patients. The pigs being the contributing factor for transmission of cysticercosis, the serum samples of slaughtered pigs were also studied. To verify endemicity and to estimate apparent prevalence of porcine and human cysticercosis different techniques like lingual examination (tongue palpation) in live pigs, Enzyme linked immunosorbent assay (ELISA), which detect parasite antigen (B158/B60 Ag-ELISA and HP10 Ag-ELISA) and enzyme immunotransfer blot (EITB) assay which detects anti parasite antibody are available. In this research, Ag-ELISA has been used to determine the active infection in the study area.

CHAPTER-II

OBJECTIVES

2.1 General Objective

To determine the sero-prevalence of *T. solium* cysticercosis in pigs and human from blood samples.

2.2 Specific Objectives

1. To determine the sero-prevalence of *T. solium* cysticercosis in pigs and human based on Ag-ELISA.
2. To get better understanding of the local epidemiology and to access the public health risk.

CHAPTER-III

LITERATURE REVIEW

3.1 Introduction - Cysticercosis

Cysticercosis is disease caused by larval stage of the tapeworm *Taenia solium* (*Cysticercus cellulose*) which is emerging as a major public health problem of global dimension, especially in the developing countries. The name "*cysticercus*" was given by Laennec, a word derived from the Greek word "Kystic" meaning bladder and "Kercos" signifying tail. It is an ancient parasitic disease rooted in human population of developing countries where pigs are reared as the food source. During the past decade, many surveys have indicated the disease to be a serious emerging problem in many countries of Central and South America, Africa (e.g. Cameroon, Tanzania, Zambia, Madagascar, and South Africa), South Asia (e.g. Nepal and India) and Southeast Asia and the Pacific Region (Somers *et al*., 2006; Rajshekhar *et al*., 2003; Zoli *et al*., 2003; Garcia *et al*., 2000; White, 2000).

Life cycle of *T. solium* was 1st described by Van Beneden (1854). He demonstrated the larval stage (*Cysticercus cellulosae)* in the muscle of the pig after feeding it with eggs from human feces. Kuchenmeister (1855) demonstrated adult tapeworm in the intestine of human (Parija, 2004). Incidence of *T. solium* coincides with the raising of pigs and especially in the areas where human feces are used as fertilizers. Man is the only definitive host of *T. solium* and becomes carrier of an adult tapeworm after the consumption of raw or insufficiently cooked pork meat. The infection is more common in low socio-economic and poor sanitary area.

Worldwide, more than 4 million people harbor the pork tapeworm and 50 million people are infected with cystic stage an estimated 50,000 people die from cysticercosis each year because of CNS or cardiac complication (WHO, 2010). International Task Force for Disease Eradication (ITFDE) had announced *T. solium* cysticercosis among six diseases to be eradicated from the world (Schantz *et al*., 1992). Joshi (1991) was first to observe *Taenia* cysts in pig meat for the first time in Nepal. Various studies up to date have indicated the endemicity of cysticercosis in different part and neurocysticercosis as one of the leading causes of acquired epilepsy.

Trade and industry losses due to condemnation or cheap value of infected pig carcasses and costs involved in diagnosis, treatment and disability of patients with NCC are the major economic burden to be faced by nation of taeniasis and cysticercosis (Carabin *et al*., 2009 and Ngowi *et al.,* 2007).

3.2 Classification of *Taenia solium*

Systematic position of *T. solium* (Kotpal, 2003)

Phylum - Platyhelminthes
Class - Cestode
Sub class - Eucestoda
Order - Taenioidea
Family - Taenidae
Genus - *Taenia*
Species - *solium*

3.2.1 Morphology of *Taenia solium*

Adult Tapeworm:

The adult tapeworm is 3-5m or up to 8m in length (Soulsby, 1982). The body is flattened like ribbon or tape with narrow anteriorly and gradually boarders towards the posterior end. The two flat surfaces represent the dorsal and ventral surfaces. The dorsal surface is closer to testes and the ventral surface is nearer to the female reproductive organs. The body is divided into 3 different parts as scolex, neck and strobila.

Scolex:

The scolex represents anterior end of a body. It is knob-like bi-radically symmetrical and measures approximately 1mm across. Scolex is typically provided with hook lets, hence known as armed tapeworm. It has four suckers and is armed with a conspicuous rosetellum. The latter consists of 2 rows of small and large hooks (130-180 mm long), alternating with each other (Parija, 2004).

Neck:

The neck is short about 5mm -10 mm in length (Soulsby, 1982).

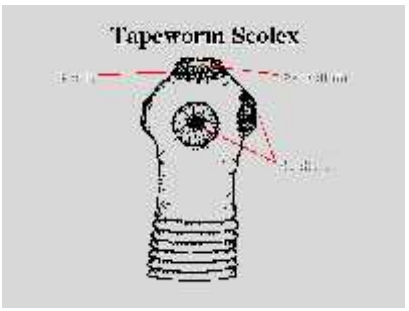
Strobila:

The strobila or body consists of 800-1000 segments or proglottids. Proglottids are a unit part of the body enclosing a complete set of genitalia. According to degree of development, the strobila includes 3 kinds of proglottids: 1) immature; 2) mature; 3) gravid.

The immature proglottids comprise of about 200 anterior proglottids just behind the neck. They are youngest, sexually immature and devoid of reproductive organs. They are short broader than long and rectangular in outline.

The mature proglottids are about 450 mature proglottids forming the middle part of strobila. They are large and square in outline. The anterior part about 100-150 proglottids contain only male reproductive organs. The posterior 250 mature proglottids develop male and female reproductive organs making them hermaphrodite. Each mature proglottids, on one side bears a tiny protuberance, the genital papilla at the tip of which is suited the common genital pore. These pores in the successive proglottids are situated alternately on the right and left sides. Mature proglottids are a complete reproductive unit and produces eggs which are fertilized by it's own sperms (self-fertilization) or by those of other mature proglottids (cross fertilization).

The ripe or gravid proglottids are the oldest and the last 150-350 proglottids up to the posterior of the body. It is longer than broader outline. The male and female reproductive organs have generated exceptionally the highly branched uterus full of fertilized eggs. They are usually 10-12 mm long by 5-6 mm wide and the uterus is fewer than *T. saginata* (15-30 lateral branches). The gravid proglottids each contains about 30000-40000 eggs. The gravid segments after separation passed passively in a chain of 5-6 segments along the feces. Usually only one adult worm is present in intestine of man. It lives for about 25 years (Kotpal, 2003).



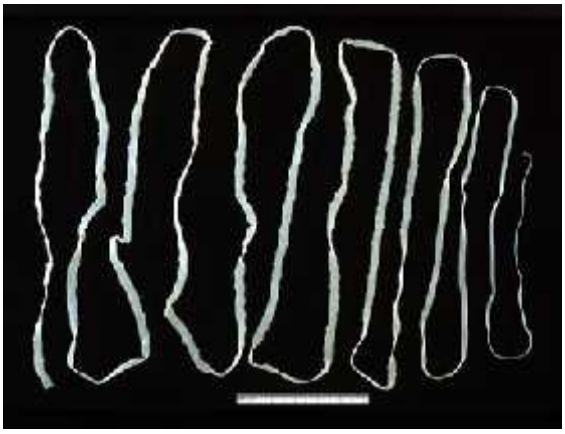


Fig 1: Scolex

Fig 2: Adult *T. solium*

(Source: www.human-healths.com/taenia-solium)

Larvae:

The larvae of *T. solium* are known as *Cysticercus cellulosae*. The cyst is small, oval and fluid filled milky white bladder-like structure. It measures 3mm -15mm in diameter and has a translucent wall through which a single dense white body containing the invaginated scolex can be seen. It is found in muscles or subcutaneous tissues of their intermediate host (generally, pigs as well as human) (Parija, 2004).

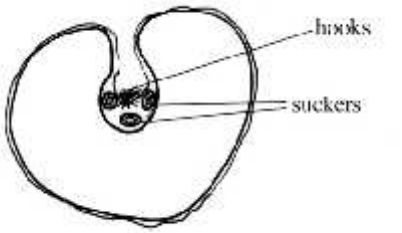


Fig 3: Larvae of *T. solium*

(Source: http://www.wikispot.info/2011/06/cysticercus-cellulosae-cysticercosis.html)

Egg:

Eggs are brown, spherical in form and are characterized by a thick, dark, radially striated shell (embryophore) containing hexacanth (6- hooked embryo oncosphere). The eggs are 26-34 µm in diameter. This is infective to pigs as well as to humans (Parija, 2004).

3.2.2 Life cycle of *Taenia solium*

T. solium has a complex two- host life cycle. Human beings are the only definitive host and harbor the adult tapeworm (taeniasis), whereas both people and pigs can act as intermediate hosts and harbor the larvae or cysticerci. The definitive host, man acquires taeniasis by ingestion of inadequately or improperly cooked pork infected with cysticercerci. In the intestine, cysticerci are digested free of muscles. Then the protoscolex evaginates and attaches itself to the intestinal wall by the help of its suckers and hooks. The adult worm develops from it by forming segments which arise from the caudal end of the scolex. The segments gradually enlarge and mature as they are separated from the scolex by newly formed segments. They subsequently develop into sexually mature adult worms within a period of 62 days to 72 days resulting taeniasis. In a sexually mature adult worm, the eggs are fertilized within the segments and about 2 months after infection, gravid proglottids begin to detach from the distal end and are excreated in the feces. The excreted fertilized eggs can survive for days to months in the enviroment. The pig, which is the usual intermediate host, gets infected by ingesting of food contaminated with eggs or proglottids shed from the human feces. Once the eggs (embryophores) are in the stomach or small intestine of the pig, they hatch out of the eggs, liberating the hexacanth embryo known oncosphere. Oncospheres have an enzymatic system that is activated by the acids and enzymes of the stomach and they attach to the intestinal mucosa by its hooks. Within 24 hours to72 hours, the larva penetrates the mucosa of the stomach or small intestine from these sites and the larvae are carried by high blood flow circulation to the muscle, eyes and the central nervous system (CNS) or other tissues. At these sites, the larvae lose their hooklets, enlarge and develop into cysticercus in 9 to 14 weeks and are comprised of a fluid-filled bladder and an invaginated scolex. Occasionally, humans may become an intermediate host and acquire cysticercosis by ano-oral contamination or by ingesting food contaminated with *T. solium* eggs. Alternatively, those with the adult tapeworm may become auto infected by reverse peristalsis as the eggs or proglottids regurgitates into the stomach and is thus stimulated to hatch. Just as in the pig, the hexacanth embryo penetrates the intestinal mucosa and is hematogenously transported to the muscles, subcutaneous tissues, eyes or CNS of human beings. Cysticercosis, therefore, is not acquired by eating infected pork as is commonly

thought, but usually from *T. solium* eggs passed by human beings carrying the adult tapeworm (Parija, 2004; Kotpal, 2003).

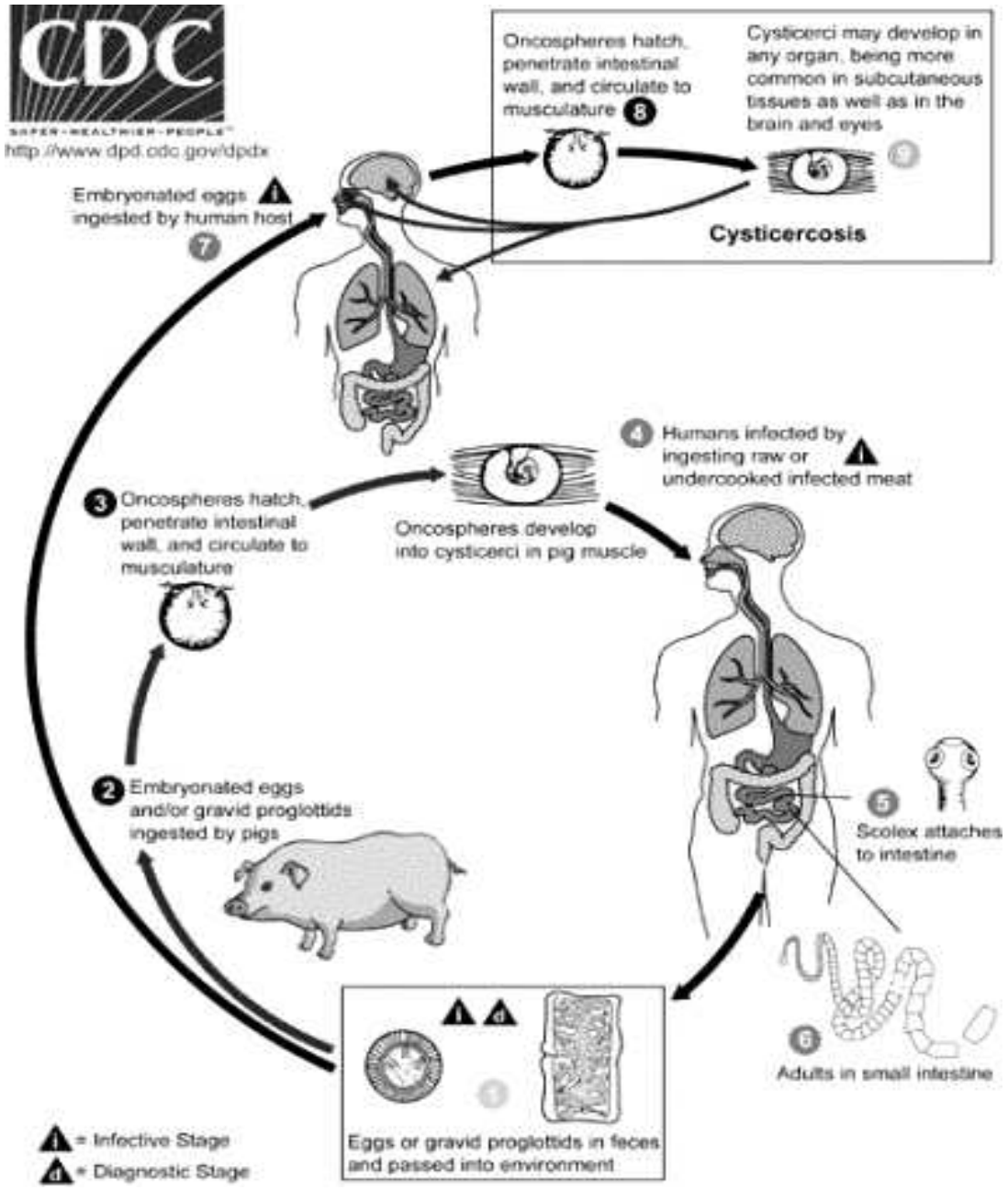


Fig 3: Life cycle of *Taenia solium* with resulting cysticercosis

(Source: http://www.dpd.cdc.gov/dpdx/html/imagelibrary/Cysticercosis)

3.2.3 Clinical Manifestations

The two distinct entities caused by the adult worm and cyst of *T. solium* are:-

- Intestinal taeniasis
- cysticercosis

In Humans

Intestinal taeniasis

Taeniasis occurs only in the human host, after ingestion of undercooked pork infected with cysticerci. Intestinal taeniasis is mostly asymptomatic. The adult tapeworm causes only mild inflammation at the implantation site, without substantial damage to the intestine or none at all. Abdominal pain, distension, diarrhea, and nausea have been attributed to tapeworm infestation and most patients seem to be free of symptoms. Due to which carriers of *T. solium* will neither look for medical care nor notice the tapeworm segments in their stools. Thus identification of *T. solium* infections is important because of the risk of cysticercosis in the carrier or the immediate environment (Garcia *et al*., 2003).

Human cysticercosis

Human beings acquire cysticercosis through fecal-oral contamination with *T. solium* eggs from tapeworm carriers or from contaminated environment. Thus, vegetarians and other people who do not eat pork and have not travelled to an endemic places can acquire cysticercosis from housekeepers or food handlers that have recent emigrants from endemic countries or eating raw vegetable grown in field using sewage mixed water (Scantz *et al*., 1992). Water, wind, flies, and other indirect means of infection also play little part in transmission (Martinez *et al*., 2000). It is potentially a dangerous disease with variable clinical manifestations depending on the sites in which cysticerci are found. The tissues affected by cysticercosis are subcutaneous layers, brain, muscle, heart, liver, lungs, peritoneum, lymph node and tongue.

Different type of cysticercosis found in humans

Muscular and subcutaneous cysticercosis:

It is extra neural cysticercosis (i.e. outside the central nervous system), it causes no major symptoms. Subcutaneous cysticercosis presents as small, movable, painless nodules that are most commonly noticed in the arms or chest. After a few months or

even years, the nodules become swollen, tender, inflamed and then they gradually disappear. Muscular cysticercosis is a casual finding, when radiography is done for an unrelated reason appearing as dot-shaped or ellipsoidal calcifications following the muscle bundles in the thighs or arms. Biopsy or fine-needle aspiration cytology (FNAC) of a subcutaneous nodule may provide rapid, safe, cheaper and reliable tool for diagnosis of cysticercosis infection (Amatya *et al.,* 1999).

Ocular cysticercosis:

Although, ophthalmic cysticercosis is much less common than neurocysticercosis (occurring in 1–3% of all infections), *T. solium* is the most common intra orbital parasite (Rahalkar *et al.,* 2000). Most cysts are found in the vitreous, sub retinal space and conjunctiva. The condition may present as iritis, uveitis, and palpebral conjunctivitis. Intraocular cysts are most frequently found floating freely in the vitreous humour or in the sub retinal space. The cysts in sub conjuctival or sub retinal sites may present as slowly growing nodules, confusing with tumors. Occasionally, sub retinal eye cysts may lead to blindness due to detachment of retina.

Neurocysticercosis:

Neurocysticercosis (NCC) is considered to be the most common cysticercosis of the central nervous system (CNS). NCC is considered to be the main cause of late-onset epilepsy in endemic areas. Seizures/epilepsy is widely reported to be the most common symptom of NCC, occurring in 70–90% of patients (Prasad *et al.*, 2008). While in approximately 60% of the cases, there is an obstruction of the cerebrospinal fluid (CSF) circulation, resulting in hydrocephalus and intracranial hypertension. The lesions in the brain and the consequent onset of symptoms in NCC are mainly determined by (i) the number of lesions (single or multiple cysticerci) (ii) the location of CNS lesions (subarachnoid, intracerebral, intraventricular, intramedullary) (iii) the type of cysticercus (*C. cellulosae, C. racemosus*) (iv) the stage of development and involution of the parasite (vesicular, necrotic, nodular, calcified) and (v) the intensity of the host immune/inflammatory response (Prasad *et al.*, 2008; Takayanagui *et al*., 2006; Garcia *et al*., 2003).

Localization of parasites in the central nervous system

1. Parenchymal:

a. Brain

2. Extraparenchymal

a. Ventricular
b. Subarachnoid
c. Spinal cord

The cysticerci within the brain parenchyma are called parenchymal cysticercosis. Brain parenchymal cysticerci are usually small cysts, single or multiple that tend to lodge in areas of high vascular supply. When cysticerci lodge within the ventricular system, life-threatening acute intracranial hypertension secondary to hydrocephalus may develop.

Extraparenchymal disease is caused by location of cysts in cerebrospinal fluid (CSF) of the ventricles, cisterns, and subarachnoid space or in the spinal cord. Convulsions and/or seizures, intracranial hypertension and psychiatric disturbances are the three important manifestations of the CNS. These may occur separately or combined.

The cysticerci locate most frequently in the meninges, cerebral cortex, ventricles, and less frequently in the parenchyma. The symptoms generally appear several years after the infection, when the death of the larva causes inflammatory reactions. Sometimes, the granulomatous lesions are found in the brain. The cysts may be single 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. Cysticerci invasion of the brain induces inflammatory reaction to meninges, encephalon and vascular regions, resulting in meningo-encephalitis and vasculitis. When these happen, the brain can swell. The pressure caused by swelling is what causes most of the symptoms of neurocysticercosis. Seizures and headaches are the most common symptoms. Death can occur suddenly in the case of epilepsy-like symptoms in heavy infections (White, 2000).

The clinical manifestations of cysticercosis are determined by a strong inflammatory reaction that seems to occur only during and after the death of the parasite. While the

cysticercus generates significant immune responses, the inflammation around viable cysticerci is quite moderate. It is now known that the live cysticercus produces taeniaestatin and paramyosin, which inhibit complement at sites distant from the parasite and may inhibit the proliferation of lymphocytes and macrophages (White, 1997). These actions probably limit the inflammatory reaction while the parasite is alive. The histological features of the immune response in swine complemented by immune histochemical analysis showed the presence of null-γδ-T cells, mononuclear cells and eosinophils, and the co-localization of MHC-II with B-lymphocytes and monocytes / macrophages within the granulomas surrounding the parasites. There was up-regulation of the adhesion CD_4 cells resembling monocytes / macrophages, eosinophils from the central nervous system that may be important for the recruitment of inflammatory cells to the site of the lesion (Londono *et al.*, 2002).

Different clinical symptoms of Neurocysticercosis

Epilepsy / seizures

Epilepsy refers to a generalized state in which a person has recurrent seizures due to chronic, underlying process but a seizure is a paroxysmal event due to abnormal, excessive, hyper synchronous discharges from an aggregate of CNS neurons (Piya *et al.*, 2005). They are caused by the presence of cysticerci in the parenchyma of the brain. Seizures are widely reported to be the most common symptom, occurring in 70–90% of patients, and NCC is considered to be the main cause of late-onset epilepsy in endemic areas (Garcia *et al.*, 2003; Pal *et al*., 2000). Dead or degenerating cysticerci appear to be more frequently associated with epilepsy than living cysts (Prado-Jean *et al.*, 2007)

Meningitis

These patients present with signs and symptoms of increased intracranial pressure associated with mental disturbances, diminution of visual acuity, and generalized seizure.

Intracranial hypertension:

This is caused by obstruction of CSF by the cysts which are found in the ventricles of the brain. Headache, nausea, vomiting, vertigo and altered mental status are the presenting symptoms.

Complications

Intracranial herniations, stroke and status epilepticus are the important complications.

In Animals

Cysticercosis does not usually manifest itself clinically. In some cases, infected swine may experience hypersensitivity of the snout, paralysis of the tongue, produce fever; stiffness of muscles and epileptiform convulsions, but in general, the normal economic life span of the pig is too short for symptoms to become apparent. This occurs soon after ingestion and the animal recovers spontaneously. Cysticercosis infected pigs are usually asymptomatic except in heavy infection which may result in inflammatory response in CNS and muscle (myositis), muscular stiffness, myocarditis and possible loss of condition. Death may occur as a result of degenerative myocarditis. Cysticerci of *T. solium* 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 may damage the parasites, allowing leakage of fluid and perhaps disrupting the parasite's ability to evade the immune response (Fan *et al.,* 2001).

3.3 Epidemiology

3.3.1 Global epidemiology of cysticercosis

Cysticercosis and taeniasis is an ancient parasitic disease rooted in human population of developing countries where pigs are reared as the food source. Since pigs are intermediate hosts of the parasite, completion of the life cycle occurs in the regions where human-pigs interface is very close. Some of the relevant literature reviews related to this are given below.

Gonzalez *et al.,* (1994), determined the prevalence of *T. solium* in sentinel piglets in a disease-endemic zone in Peru using the EITB assay. During the study, out of 12 non-native pigs, 33% (4/12) non-native pigs, acquired new infection and out of 28 native pigs tested, 64% (18/28) acquired the infection. This shows that sero-diagnosis of sentinel piglets can also be taken as a practical method for detection of *T. solium* eggs in the environment.

Porcine cysticercosis in Asian countries varies from 0.02 to 32.5%. In China, the infection is highly variable ranging from 0.84 to 15% (Rajshekhar *et al.*, 2003). The prevalence of porcine cysticercosis in South Africa was found to be 11.9% for lingual examination (tongue palpation) in live pigs, 54.8% for B158/B60 Ag-ELISA, 40.6% for HP10 Ag-ELISA and 33.3% for enzyme immunotransfer Blot (EITB) assay (Krecek *et al*., 2007).

In Latin America, the prevalence of porcine cysticercosis on tongue palpation was reported to be 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).

Prasad *et al.,* (2002) reported 38% taeniasis among 72 family members of a pig farming community and 26% cysticercosis among 50 slaughtered pigs at Utter Pradesh, India. Porcine cysticercosis has been reported to be 8.6% in some region of Nigeria and 11% in the village pigs of western province of Cameroon (Zoli *et al*., 2003).

Krecek (2007) reported the prevalence of porcine cysticercosis to be 11.9% for lingual examination, 54.8% for B158/B60 Ag-ELISA, 40.6% for HP10 Ag-ELISA and 33.3% for EITB in areas of South Africa.

Sikasunge, (2007) also reported the prevalence of *Taenia solium* porcine cysticercosis in free-range pigs in selected districts of Zambia by tongue examination and by detection of circulating antigen (Ag-ELISA) were found to be 10.8% and 23.3% respectively

Lescano *et al., (*2007) estimated that the pig owned by a tapeworm carrier had 4 times higher sero-incidence compared with other pigs and within 50 meter swine sero-prevalence appeared unaffected if the owners harbored. Alcobedes *et al*., (2010) reported the prevalence of porcine cysticercosis in Venezuela to be 65.4% for the Ab-ELISA and 42.3% for the HP10 Ag-ELISA. In west and Central African countries, annual losses of about 25 million Euros have to be beared by the nation due to porcine cysticercosis (Zoli *et al*., 2003).

Human cysticercosis, an infection caused by larvae of *Taenia solium* remains a major public health problem in lower-income, underdeveloped countries. It develops following accidental ingestion of eggs of the pork tapeworm in contaminated food or by fecal-oral route (Somers *et al.,* 2006).The incidence of cysticercosis in vegetarians and other people who do not eat pork have also been found to be exposed to *T.* s*olium* commonly from tapeworm infected food prepares and the consumption of the raw vegetables as salad that is grown in field where human faces are used as manure (Schantz *et al*., 1992).

The parasite commonly infects the central nervous system, causing neurocysticercosis (NCC). Seizures are widely reported to be the most common symptom, occurring in 70–90% of patients, while NCC is considered to be the main cause of late-onset epilepsy and neurological morbidity in endemic areas of the world (Takayanagui *et al*., 2006).

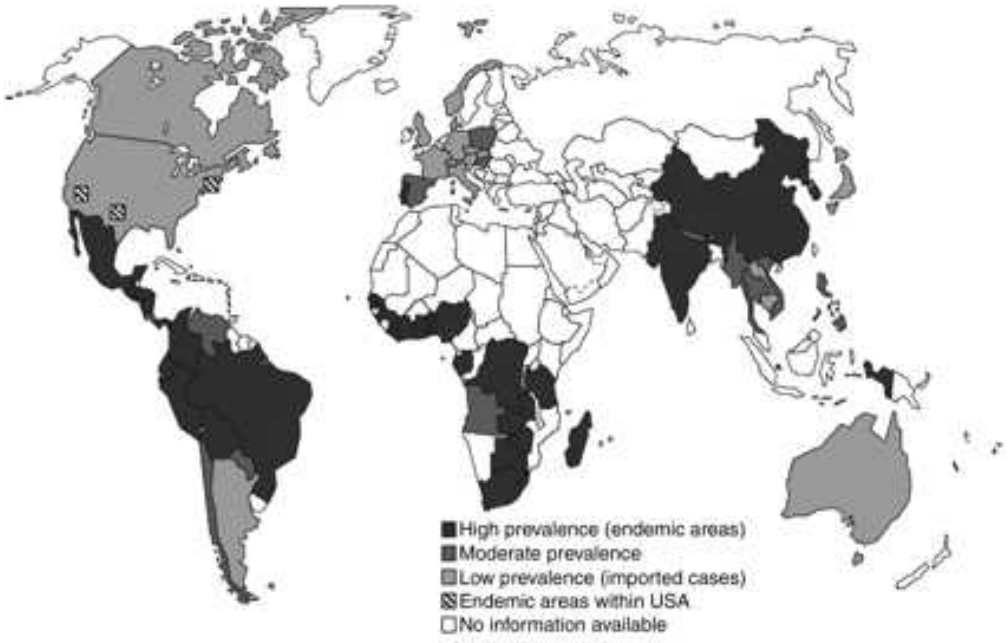


Fig 5: Global distribution of *Taenia solium* cysticercosis/taeniasis (Dorny, 2010)

Cysticercosis has contributed to causation of active epilepsy (AE) in up to 50% Indian patients presenting with partial seizures (Rajshekhar *et al*., 2003). In Utter Pradesh (India), 9.7% members of that community has been reported to have seizures due to neurocysticercosis (Prasad *et al*., 2002). In Latin America, an estimated 75 million persons has been living in endemic areas of cysticercosis and ~400,000 have

symptomatic disease. In Peru, average prevalence rates in areas of endemicity were 6% – 10% and 23,512 – 39,186 of symptomatic NCC cases **(**Bern *et al.*, 1999).

Mittal *et al.,* (2000) performed an ELISA test on the 1881 sera of patient with epilepsy for detection of *T. solium* antibodies. The prevalence of cysticercus antibodies was found to be 10.4 % (n=196).

Erhart *et al*., (2002) performed Ag-ELISA test for evaluation in a village in Northern Viet Nam among which 12 people (5.7%) gave a positive ELISA result among which 5 of them had a history of late onset epilepsy. Subcutaneous nodules were found in 2 of these 5 epileptic patients. Living cysticerci were demonstrated in the brains of 4 of the 5 epileptic patients and in the fifth only one calcified cyst was seen.

Garcia *et al.,* (2003) assessed the prevalence of human taeniasis/cysticercosis and porcine cysticercosis in an endemic area of the Peruvian highlands. The porcine sero-prevalence ranged from 42-75% and human sero-prevalence by village ranged from 7.1-26.9% (mean, 13.9%). Sero-prevalence was higher among individuals with a history of seizures but not in those reporting a history of headache or intestinal taeniasis and prevalence of taeniasis ranged from 0-6-7%.

Martinez-Maya *et al.,* (2003) studied the frequency of *Taenia solium* carriers and its relationship with human cysticercosis in a Mexican locality. Out of 403 fecal samples 5 (1.2%) were positive for *Taenia sp* coproantigen by ELISA. The adult cestode was recovered in only two people. 3.26% (3/92) serum samples were positive for anti-cysticercus antibodies.

Nguekam *et al*., (2003) has reported the sero-epidemiology of human cysticercosis in 4993 individuals from three rural communities of West Cameroon (Bafou, Bamendou and Fonakekeu) by using Ag-ELISA was to be 0.4%, 1.0% and 3.0% respectively. NCC was present in 59.1% of the tested sero- positive persons.

DeGiorgio *et al.*, (2005) have reported the sero-prevalence of *T. solium* cysticercosis and taeniasis by serum immunoblot to be 1.8% and1.1% respectively in the rural area of Southern California, USA. The sero-prevalence of *T. solium* taeniasis was highest in the migrant farm worker community and hand washing frequency was correlated with *T. solium* taeniasis sero-positivity.

Somes *et al.,* (2006) reported the sero-prevalence of cysticercosis was 1.6% and the taeniasis prevalence was 4.5% in the Northeast region of Brazil.

Rodriguez-Hidalgo *et al*., (2006) studied the cysticercosis/taeniasis situation among 1059 inhabitants in an endemic community in the southern Andes of Ecuador. Circulating antigen was detected in 2.25% of the human population by Ag-ELISA, whereas intestinal taeniasis was detected in 1.46% by the formalin-ether technique. In addition, 43% (43/100) and 74 % (74/100) of humans and pigs were positive against *T. solium* cysticerci by enzyme linked immuno electrotransfer blot (EITB) respectively.

Surso *et al*., (2006) reported taeniasis/cysticercosis from several provinces of Indonesia. The highest level endemicity of taeniasis/cysticercosis has been found in Papua and reported that 5 of 58 local people (8.6%) harbored the adult tapeworm *Taenia solium*, whereas 44 of 96 people (45.8%), 50 of 71 pigs (70.4%) and 7 of 64 local dogs (10.9%) were sero-positive for *T. solium* cysticercosis.

Wandra *et al.,* (2006) carried out an epidemiological study in three districts of Bali, Indonesia. Out of total 398 residents, 252 stool samples were examined for of taeniid eggs, copro antigens or copro-DNA for identification of taeniid species, and 311 serum samples were available for detection of antibodies against *Taenia solium* cysticercosis. Taeniasis prevalence was highly variable among three villages (1.1-27.5%), and only osne case of cysticercosis due to *T. solium* infection was detected. All expelled tapeworms were confirmed to be *Taenia saginata* by mtDNA analysis.

Prado-Jean *et al*., (2007) revealed 58.7% and 38.3% positivity in epilepsy cases by using circulating antibodies (Ab) and antigens (Ag) respectively. The risk of epilepsy was high in cases with a positive Ag-ELISA, although less important than in cases with positivity for Ab-ELISA.

Prasad *et al*., (2008) estimate the prevalence of *T. solium* taeniasis in rural pig farming community of North India was 18.6% (172/924). Identification of *T. solium* was confirmed by morphological features of segment and species-specific DNA detection from segments and stool. The factors associated with taeniasis were found to be age above 15 years, history of passage of *Taenia* segments in stool, undercooked pork consumption and poor hygienic habits.

Carabin *et al.,* (2009) estimated the prevalence of *Taenia solium* cysticercosis antigens in 763 residents of three villages in Burkina Faso (i.e. Batondo, Pabre, Nyonyogo) of West Africa to be 10.3%, 1.4% and 0.0%.

Foyaca-Sibat *et al.,* (2009) determined the sero-prevalence of NCC in 296 epileptic outpatients from an area of South Africa and the sero-prevalence of antibodies and antigens to the larval stage of *T. solium* were 32.6% and 7.9% respectively. The proportion of persons with epilepsy attending St. Elizabeth clinic with CT-confirmed NCC was 37%. But in many countries like Japan, South Korea as well as from Central Europe *T. solium* has been eradicated (Pawlowski, 2006).

Anantaphruti *et al., (*2010) carried out a molecular and serological survey on taeniasis and cysticercosis in Central Thailand. Out of 667 stool samples examined 4 (0.6%) were egg-positive for *Taenia* sp. Out of total 159 residents, 9 (5.66%) cases were seropositive for Ag ELISA and they were also confirmed by immunoblot using recombinant chimeric antigen. This all the above reviews shows that the nation is facing a lot of economic burden in trade and industry, due to condemnation or cheap value of infected pig carcasses and costs involved in diagnosis, treatment and disability of patients with NCC.

3.3.2 Epidemiology in context of Nepal

In Nepal many community based surveys have not been conducted yet to represent prevalence of cysticercosis / taeniasis in porcine and human in different part of Nepal. Some of the relevant literature reviews related to this are given below.

Joshi (1991) was first to observe the *Taenia* cysts in slaughtered pig meat in Kangeswari, Kathmandu. In 2005, Bista determined the prevalence of human cysticercosis to be ranges from 0.002- 0.1% in general population of Kathmandu (Bista, 2005).

Joshi *et al*., (2003) reported 14% (34/250) of pigs positive for cysticercosis on post mortem surveys of pigs at slaughter establishments in Kathmandu and Dharan municipality. Ante mortem detection of *T. solium* infection of pigs in Syangja district community indicated 32% (136/419) of pigs positive by lingual examination while

24% (48/201) was serologically positive by Enzyme-linked Immunoelectro Transfer Blot (EITB).

Joshi *et al*., (2004) reported that taeniasis is directly co-related with the environmental condition, the sanitary condition and animal husbandry system of the community. The high prevalence was observed in the ethnic groups i.e. Magar, Sarkies, Damai and Bhote of Syangja districts with 50, 28, 10 and 30% respectively. Human cysticercosis cases reviewed on the basis of the hospital-based data was reported to be 62, 4 and 11 in Patan hospital, Bir hospital and Kanti children hospital respectively.

Joshi *et al*., (2008) has reported the prevalence of *Taenia solium* porcine cysticercosis in Kathmandu and Chitwan valley by lingual and carcass to be 0.63%and 0.94% respectively. Similarly Devleesschauwer (2009) has determined the apparent prevalence of porcine cysticercosis to be 18.21% (73 /401) in the different places of Kathmandu Valley (i.e. Talchhikhel, Kathmandu, Sinamangal) using an antigen-ELISA.

Karna *et al*., (2009) determined the prevalence rate of cysticercosis by lingual examination, carcass examination and ELISA was found out to be 0.05, 0.055 and 35.5% respectively. The collected cysts were confirmed as *Taenia solium* cyst by the histopathology and microscopic examination.

Shakya (2010) has reported the prevalence of *Taenia solium* cysticercosis in pigs in Kirtipur municipality to be 4% (6/150) by lingual examination and Ale (2010) has reported the total prevalence of porcine cysticercosis to 29.3% (87/297) in Kathmandu valley.

Different hospital records and survey has also been conducted to determine the prevalence of cysticercosis in human in which NCC is considered to be the common parasitic infection of the central nervous system and the main cause of the late-onset epilepsy. A retrospective study of 724 epileptic cases from 2000 to 2005 conducted by Piya *et al.*, (2005) in the Kathmandu Model Hospital reported the prevalence of NCC to be 61% out of the total epileptic cases.

Joshi *et al.,* (2001) reported that out of 2533 operated patients only 4 cysticercosis cases were recorded in the registers of Bir Hospital during the period 1995-1997. Out

of 26156 patients operated in Kanti Children Hospital during the period 1993-1997, 11 were found to be positive for NCC. Similarly at neurosurgical OPD at Om Hospital, Sharma (2006) has reported 24 cases of neurocysticercosis out of 54 cases of seizure disorder. Similarly, Pant (2006) has reported 61% of all seizure cases as neurocysticercosis by EITB at Kathmandu Model Hospital.

Chaudhary (2006) has reported 7.18% cases of out of 543 seizure disorder patients NCC in Patan hospital. Agrawal (2006) has reported 66 cases of neurocysticercosis (NCC) at Neurology service T.U Teaching Hospital. Out of which, 63.6% (42/66) showed single ring enhancing lesion and 36.3% (24/66) showed multiple ring enhancing lesions.

Even subcutaneous cysticercosis and ocular cysticercosis had been reported in different hospital of Nepal and has histopatologically diagnosed *T. solium* to be the main causative agent. Amatya *et al*., (1999) studied on 23402 biopsy specimens collected from approx 60 hospitals and clinics scattered throughout Nepal. Among which 62 cases of cysticercosis were diagnosed. Out of these 62 patients, 51 (82%) presented with skin nodules, 6 (10%) with cystic lesions in oral mucosa and 5 (8%) with breast lumps.

Adhikari *et al*., (2007) studied the value of Fine Needle Aspiration Cytology (FNAC) in the diagnosis of subcutaneous cysticercosis among 1334 cases in Tribhuvan University Teaching Hospital. Cysticercosis was diagnosed in 10 (0.7%) cases. Out of 10 cases of cysticercosis, the lesion were located in cheek in 3 (30.0%) cases, neck in 2 (20.0%) cases, forearm in 2 (20.0%) cases, chest wall in 1 (10.0%) case, right arm in 1 (10.0%) case and abdominal wall in 1 (10.0%) case. Even a case of a live cyst in the Anterior chamber (AC) which is rarer than in other type of cysticercosis have been reported by Shariq *et al*., (2007). The cyst was removed intact through limbal incision by viscous expression technique. Histopathology confirmed *Cysticercosis cellulosae* as the infecting agent.

Piryani *et al*., (2007) had reported the diagnostic criteria for neurocysticercosis and the outcome of treated cases at Nepalgunj Medical College (NGMC), Teaching Hospital Kohalpur. 15 neurocysticercosis cases among 14118 patients admitted in Medical Wards with seizure, was diagnosed and managed. The diagnosis was based

on clinical presentation, CT scan findings and high index of suspicion. The use of albendazole, steroids and anticonvulsant drugs; controlled about 93% of neurocysticercosis patients.

Basu *et al*., (2007) reviewed the hospital records among which 124 pediatric impatients were diagnosed of NCC in Manipal college of Medical Sciences and Manipal teaching Hospital, Pokhara. All patients were treated with 28 days course of albendazole, anti-edema drugs and anticonvulsants. Partial seizure was the most prominent presentation. Ninety-eight patients had complete clinical response and 87 of them had complete disappearance of lesion in CT scan at the end of 1 year. Recurrence of seizure was the only residual Symptom found in six (4.8%) patients, all of them having calcified lesions in CT scan.

Karna *et al*., (2009) reported that NCC patients were found at an overall rate of 9.8% (179/1839) epilepsy patients from the survey of 5 hospitals of Kathmandu valley viz. TUTH, Bir, Patan, Norvic and Nepal Medical College.

Ale *et al.,* (2010) has reported the prevalence rate of 13.63% for neurocysticercosis cases from Institute of Medicine (IOM), Maharajgung.

3.4 Diagnostic Approach of Taeniasis/ Cysticercosis

In today's world, taeniasis and cysticercosis is increasing day by day due to change in food habits, people travelling abroad from endemic area and due to lack of sanitation. The diagnosis of teaniasis and cysticercosis is based on the identification of adult worm or proglottids or taeniid eggs in the feces, clinical manifestations, imaging examination, serology and / or histopathology (Yamasaki, 2007a and 2007b). The various methodologies applied for the diagnosis of cysticercosis and taeniasis is briefly described below:

Intestinal Taeniasis:

Taeniasis in human can be examined by:-

i) fecal examination

ii) Serology.

Fecal examination:

It is made by demonstration of eggs and proglottids of *T. solium* in the feces. The eggs or embryophores of *T. solium* are thick, striated, brownish shell can be detected by flotation method of fecal examination (Rodriguez-canul *et al*., 1999). The visual examination and microscopy can diagnose *T. solium* based on morphological characteristics. For the diagnosis of infection with *T. solium*, two problems can be seen as the poor sensitivity of stool microscopy, and the morphological similarity between the eggs of *T. solium* and *T. saginata*. The eggs are morphologically differentiated from other species of *Taenia* by examination of the gravid proglottids occasionally found in the feces. The presence of few lateral branches in uterus on each side, the presence of vaginal sphincter and the absence of an accessory lobe of ovary, are the diagnostic features of the mature proglottids of *T. solium.* Demonstration of the scolex armed with rostellum is the most reliable feature to identify *T. solium.*

Serology

Taenasis in human can also be recognized by detection of taenia copro-antigen in feces using antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA), but the test does not differentiate species (Garcia *et al.*, 2003). Coproantigen detection ELISA has sensitivity of about 95% and sensitivity greater than 99% and is effective tool for epidemiology studies (Garcia *et al.*, 2003). Whereas molecular techniques like DNA probes and polymerase chain reactions (PCR) by using *Taenia* material in feces can differentiate human *Taenia spp.*

Cysticercosis:

The diagnosis of cysticercosis in pigs may pose difficult due to short life before they are slaughtered and absence of clear symptoms so it may require several testing methods like lingual examination (palpation of tongues) and meat inspection. Lingual examination can be applied for the detection of cysticerci during an anti-mortem examination where as meat inspection can be applied mostly in pigs during post mortem examination. Joshi *et al.*, (2008) has also suggested lingual examination method for detection of porcine cysticercosis to be easy, inexpensive and could be utilized as a surveillance tool in developing countries like Nepal where technical

resources and technological capacity are very limited. This method has also been used in endemic areas to reject the pigs that are recognized having *T. solium* larvae under the tongue (Ngowi *et al.*, 2007). This method can be applied mostly in endemic areas for cysticercosis. The slaughter workers in Nepal can easily recognize the cysticerci in the meat and it is named as “Chamle” (resemble as rice grain) or “Pidke” in their local terms. But sensitivity of meat inspection is very low (Joshi *et al*., 2008). Sero-diagnosis of sentinel piglets can be taken as a practical method to detect *T. solium* eggs in the environment. Furthermore, it permits indirect assessment of human risk, which may be useful for monitoring the efficacy of intervention programs (Gonzalez *et al*., 1994). But in human’s different methods can be applied to detect the cysticercosis.

a. Imaging method

This includes the radiological / neuro-imaging examination such as computerized tomography (CT) and magnetic resonance (MRI) of the skull and extremities which show the presence of small calcified areas. This method is used to detect the exact locations and viability of *T. solium* metacestodes (Garcia and Del Brutto, 2003). CT scan and MRI are for the diagnosis of Neurocysticercosis in humans. Imaging techniques and biopsy of the tissues containing parasites provide direct diagnosis of cysticercosis whereas Ag-ELISA, Ab-ELISA, and EITB provide indirect diagnosis of human cysticercosis. When the cysts starts to degenerate, the vesicular fluid becomes opaque and dense, and the edges of the cyst become irregular and shrink and the non-viable cysts appear as whitish, round, calcified nodule and isodense with the surrounding parenchyma in the brain and most common location is in the cerebral hemispheres, mainly at the junction of grey and white matter (Garcia *et al.*, 2003). CT scan are usually used for detection, while MRI are used for cases in which axial skeleton is involved. Calcified cysts are also detected by radiography (White, 2000). X- Rays can also be used for the detection of cysts in the muscles (OIE, 2004). But the diagnosis of neurocysticercosis can however be made with a marked accuracy by combining clinical signs and history with X-ray, CT or MRI, serological tests and laboratory examination. Neuroimaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have been recognized as the gold standard for diagnosing neurocysticercosis

b. Serodiagnosis

Serological tools may be applied on human and pigs in epidemiological studies of cysticercosis and for diagnosis of NCC in human. Two most commonly used tests are: Enzyme linked immunosorbent assay (ELISA) and Enzyme-linked Immuno electro transfer blot (EITB) (Joshi *et al*., 2001). The ELISA is a useful method for demonstration of antibodies /antigens in serum as well as in the CSF but detection of antibodies in the CSF provides better reliability.

i. Antibody- detection method:

Infection with *T. solium* results in a specific antibody response. These antibodies can be detected in serum, in cerebrospinal fluid (CSF) in the case of NCC or even in tear specimens in the case of ophthalmic cysticercosis. Several techniques have been described to detect antibodies to *T. solium* infections in humans and in pigs such as radioimmunoassay, hemagglutination, the complement fixation test, dipstick assay, latex agglutination, enzyme-linked immunosorbent assay (ELISA) and immunoblot techniques (Dorny *et al.,* 2003). Initially, antigens used in antibody-detection assays were cyst fluid, excretion–secretion (ES) products or crude homogenates from cysticerci from either *T. solium* or the related parasites. However, unpurified antigens have moderate sensitivities and relatively poor specificities. Improved protein-purification techniques and research on antigenic properties of cyst fluid and surface proteins has led to the development of better serological tools (Ito *et al*., 2003).

The most specific test so far is the Enzyme-linked immune electro transfer blot (EITB) with an initially reported specificity of 100% and sensitivity of 98% when tested in humans (Dorny *et al*., 2003). This immunoblot uses an enriched fraction of glycoproteins obtained by purifying a raw cysticercus extract by lentil lectin-purified chromatography (LLGP). Reaction with any one of seven specific bands is regarded as diagnostic for cysticercosis. However, the sensitivity of this assay drops dramatically in cases with single cysts in the brain (Deckers *et al.,* 2010).

Although different antibody detection tests have been developed, these assays are less suitable for the intended purpose because detection of *T. solium* specific antibodies in serum only indicates exposure to the parasite and not necessarily established infection, resulting in a transient antibody response (Garcia, 2000). Furthermore,

antibodies may persist long after the parasite has been eliminated by immune mechanisms and/or antiparasitic therapy (Dorny *et al*., 2003). Detection of anti-parasite antibodies in a population in an endemic village does not necessarily reflect the true prevalence, leading to misdiagnosis of a proportion of neurological cases (Bern *et al.*, 1999). It can also lead to superfluous use of antiparasitic therapy in a patient where the parasites are not viable (Garcia *et al.*, 2000).

ii. Antigen detection method:

Considering the drawbacks of antibody detection in clinical settings, antigen detection can provide a valuable alternative in that it reflects the presence of viable parasites. In this respect, antigen detection can also provide a tool for serological monitoring of antiparasitic therapy; antigen levels drop rapidly after successful anthelminthic treatment (Deckers *et al.*, 2010). Several assays have been developed to detect parasite antigens in serum, CSF or urine using either polyclonal or monoclonal antibodies (mAbs). Two mAb-based tests (B158/B60 Ag-ELISA and HP10Ag-ELISA) have been validated and are used routinely for the detection of parasite antigens.

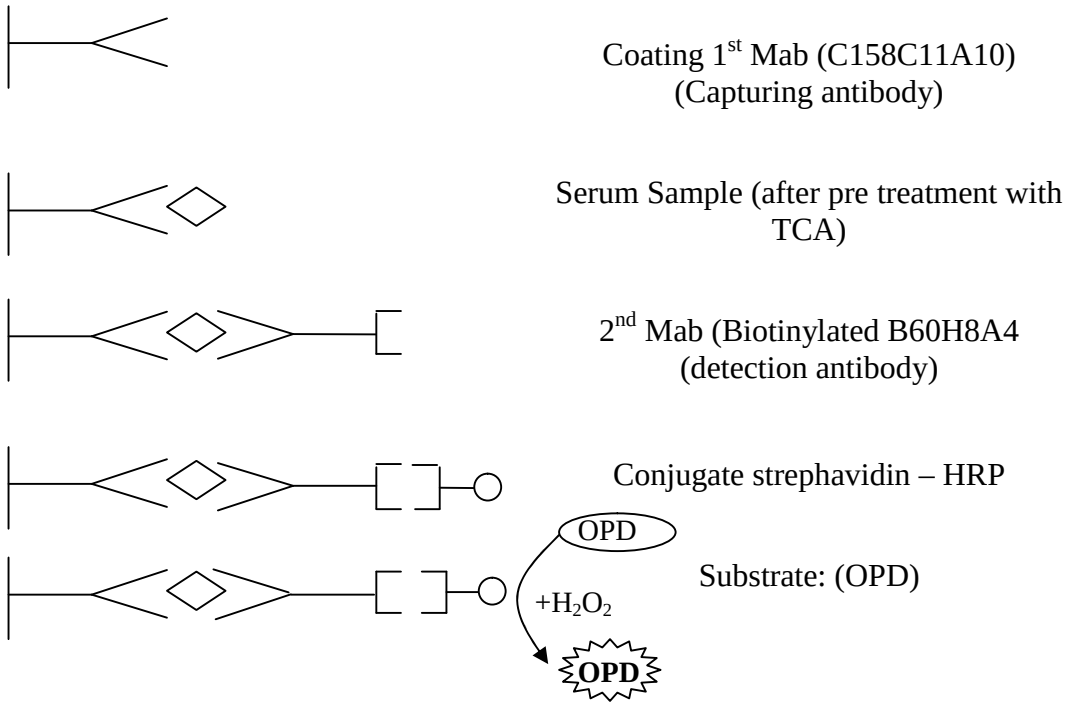


Fig. 6: The general principle of sandwich ELISA

The sensitivity of antigen detecting ELISA is reported to be 92% for viable cyst (Deckers *et al.,* 2010). Antibody detection assays only reflect exposure to the parasite, whereas antigen detection assays indicate the presence of living parasites. Ideally, a combination of both tests is best for sero-epidemiological studies and for supporting diagnosis of NCC by neuro-imaging techniques.

c) Histopatological diagnosis:

The specific diagnosis of NCC is also made by demonstrating cysticerci in the biopsy tissue obtained from the brain during post-mortem. A biopsy or excision of the nodule and histological examination of the cysticerci helps diagnosis of skeletal cysticercosis. Biopsy taking the specimens of subcutaneous or brain tissues reveals typical findings of *T. solium* as scolex with hooks. FNAC (Fine Needle Aspiration Cytology) is one of the rapid tools for pre-operative diagnosis of subcutaneous cysticercosis and it may even obviate the need for open biopsy.

According to Yamasaki (2007a), histopathology provides definitive evidences for the diagnosis of cestodiasis despite the difficulty to specify the causative parasites due to the degeneration and/or calcification of the lesions or artifacts in preparation. He also applied polymerase chain reaction based mitochondrial DNA analysis using formalin-fixed paraffin-embedded sections (FPES) which was very useful for definitive diagnosis. When infection with *T. solium* is not confirmed by histopathological examination, molecular diagnosis will be more useful for definitive diagnosis (Yamasaki *et al.*, 2005). Yamasaki (2007b) reported that since taeniid cestodes are difficult to differentiate because of morphological similarity, BESS T-base analysis and Multiplex PCR are very useful and informative for the species identification.

3.5 Treatment

Neurocysticercosis is associated with substantial morbidity and mortality. Until 1978, the only treatments available were surgery for cyst excision or ventricular shunts or steroids to decrease inflammation. Human cysticercosis treatment includes larvicidal drugs such as albendazole or praziquantal for the viable cysts, corticosteroids to decrease inflammation, anti-seizure medications and surgical interventions (Yancy *et*

al., 2005). In the case of neurocysticercosis, albendazole is the treatment of choice, although praziquantel may also be useful (Garcia, 2003). They are treated with albendazole at a dosage of 15 mg/kg/day for 2 weeks and praziquantel at a dosage of 10 mg/kg/day for 2 weeks additionally. The currently accepted schemes are either 8 days of albendazole treatment (15mg/kg daily) with simultaneous administration of steroids, or 15 days of Praziquantel (50mg/kg daily) (Sotelo *et al.*, 1990). Steriods, dexamethasone or prednisone are helpful in odema or intracranial hypertension. Shorter schemes of 1 day of praziquantel or 3 days of albendazole seem effective for patients with only one lesion, but not for those with many cysts (Pittellal *et al.*, 2001; Corona *et al.*, 1996). Panel of experts agreed on the management of patients with moderate infections and viable cysts (antiparasitic treatment, with steroids); calcified cysticercosis (no antiparasitic treatment); ventricular cysticercosis (neuroendoscopic removal, when available); subarachnoid cysts, including giant cysts or racemose cysticercosis (antiparasitic treatment with steroids) hydrocephalus with no visible cysts on neuroimaging (ventricular shunt, no antiparasitic treatment) and ophthalmic cysticercosis (surgical resection of cysts) (Garcia, 2000). Albendazole therapy is not preferred for NCC though it is cysticidal, it is because cysts are usually in dead condition when patients visit for treatment and it is of no use if cysts are dead already.

For the treatment of taeniasis, two available drugs are Niclosamide and Praziquantel but Niclosamide is the choice of drug as it is not absorbed from the intestinal lumen. With praziquantel, a small risk that asymptomatic viable brain cysts are affected by the drug in serum, causing headache and seizures (Flisser *et al.*, 1993). Praziquantel at the dose rate of 5–10 mg/kg body weight can be given (Sarti *et al*., 2000). A single dose of praziquantel or niclosamide (2 gm) is sufficient to expel adult worms of *T. solium* (Garcia *et al.*, 2003; Allan *et al*., 1997).

Cysticerci may lodge anywhere in the body of the pig, most commonly in the muscle and subcutaneous fat. Although some pigs have massive infections, porcine cysticercosis is rarely associated with symptoms of any kind. Most pigs are killed before the age of 9 months, which is too short a time for the cysts to reach the degenerative stage that is associated with symptoms in human beings. An inexpensive treatment for porcine cysticercosis might increase the value of pigs and block transmission of infection. Porcine cysticercosis treatment with a single dose of 30 mg/kg bodyweight of Oxfendazole (OFZ) is a simple, highly effective (100%),

inexpensive, and potentially sustainable method of treatment that clears all viable cysts in 3 months (Gonzalez *et al.*, 2001). Albendazole sulphoxide at the dose rate of 15mg/kg body weight subcutaneous injection once per day for 8 days was 100% effective against muscular cysticercosis (Peniche-Cardena *et al.*, 2002). Partial protection has been achieved by vaccination of healthy pigs with *T. solium* oncospheral antigens (Verastegui *et al*., 2002).

3.6 Prevention and Control

T. solium is transmitted mainly in rural and polluted areas where pigs have access to untreated human sewage or feces and infected pork is widely available. More developed countries have eradicated cysticercosis by improving sanitation and controlling domestic pig raising. Eradication of *Taenia solium* has been achieved in Europe and North America (Garcia *et al.*, 2003). Only the tapeworm carriers and the infected pigs are important in terms of transmission. Individuals with Neurocysticercosis are a health concern; but unless they also carry an intestinal tapeworm, they do not pose a public-health risk. Transmission of the infection from animals to pigs can be blocked if the sale and consumption of infected pork is avoided.

Closed husbandry practices without contact with human feces can control the porcine cysticercosis. Even if meat are infected, the proper meat processing like freezing (at -20 ^{o}C for 3 days) or thoroughly cooking of meat at 45 - 50°C can kill cysticerci. But the larva can survive in large piece of meat if the centre is inadequately cooked. According to OIE guidelines for meat inspection, the infected carcasses or meats containing live cysts can be inactivated by cooking or heating at 60°C for 15 to 20 minutes and prevention can be done through proper meat inspection at the slaughterhouse. Deep-freezing at -20°C for 4 days can destroy larva (OIE, 2004). Cysticercosis can be overcome by effective health education, human mass treatment against taeniasis or elimination of taeniasis by chemotherapy namely niclosamide and praziquantel, improved sanitation, pig vaccination, treatment of porcine cysticercosis as well as different health education campaigns are the possible measures for prevention and control of *T. solium* and cysticercosis (Sarti and Rajshekhar, 2003). Since little socioeconomic development in endemic areas is expected in the near future, intervention measures for control and eradication are urgently needed.

CHAPTER-IV

MATERIAL AND METHODS

4.1 Study Area

This study was conducted in Kathmandu valley. The Kathmandu valley consists of three districts as Kathmandu, Lalitpur and Bhaktapur, which share its boundaries with Kavrepalanchok district on east, Dhading on west, Nuwakot and Sindhupalchok districts on the north and Makawanpur on the south. This valley is located at an altitude of 1300 m from the sea level and extends about 25 km east to west and 20 km from north to south. Population estimates at Kathmandu for 2010 were about 989,273. The municipal area is 50.67 square kilometers (19.56 square meters) and the population density is 19,500 per km². The temperature in summer (May, June, July) ranges from 19.5ºC to 28.1ºC and in winter (October, January, February) ranges from 3ºC to 19.3ºC (*www*.wikipedia.org/wiki/Kathmandu**).**

The present study was conducted in National Zoonoses and Food Hygiene Research Center (NZFHRC) in collaboration with Central Department of Microbiology. The study was conducted from November 2010 to May 2011. The human blood samples admitted at T.U teaching hospital and Neuro hospital of Biratnagar were taken under the investigation. The blood samples of pigs were collected from different slaughter house at the time of slaughtering.

4.2 The slaughter slabs

In our study for pig samples, two slaughtered slabs were selected based on size (number of pigs slaughtered per day), breed and accessibility. A total of 200 pigs are slaughtered every day in Kathmandu valley (TLDP, 2003). But due to the fact that all are slaughtered at the same time, early in the morning, blood sample from an average 5-6 slaughtered and examined pigs were taken daily. So samples were taken from 2 slabs only: slaughter slab 1 (Teku slaughter center, Bishnumati River) and slaughter slab 2 (Sinamangal slaughter center).

4.2.1 Slaughter slab 1 (Teku slaughter center, Bishnumati River)

It is situated at Bishnumati River at Teku in Kathmandu district. They usually bring pigs from Kavrepalanchowk and neighboring breeding areas. Mostly cross, black and white were slaughtered here. On an average, 4-5 pigs were slaughtered daily.

4.2.2 Slaughter slab 2 (Sinamangal slaughter center)

This slaughter slab is one of the oldest and located at Tilganga, Kathmandu. They slaughtered different breeds of pigs brought from different parts of Kathmandu valley, eastern part of Nepal and India. About 4-5 pigs are slaughtered per day but on the occasion about 10-12 pigs were slaughtered. They usually sell meat to different meat shop of Kathmandu and to owner's of different hotels.

4.3 Sampling Procedure

4.3.1 Specimen Collection and Transport

In case of the pigs, blood samples were collected in numbered sterile plastic tubes at the moment of slaughtering, from posterior venacava and vena porta after removal of the liver. For each sampled pig, the sex, breed, age and origin was recorded and immediately transported to the lab of NZFHRC.

The human blood samples were collected in clean sterile leak-proof, dry containers and labeled properly. Then blood tubes were centrifuged for 15 minutes at 12000 g. When the serum has separated, it is pipette out in a sterile eppendorf tubes and frozen at -20°C until analysis. The data collection form is given in appendix (I, II).

4.3.2 Procedures for Circulating Antigen Detecting ELISA (Ag-ELISA)

For this study, the sandwich Ag-ELISA as described by Dorny *et al., (*2000) and adapated by Dorny *et al.,* (2004) was used. The test uses the IgG type monoclonal antibodies developed for diagnosis of *T. saginata* cysticercosis (Dorny *et al.,* 2000), but cross reaction between antigens produced by *T. solium* and *T. saginata* meatacestodes makes it possible to use for diagnosis of *T. solium* cysticercosis as well (Brandt *et al*., 1992).

First of all serum samples were first pretreated with trichloroacetic acid (TCA). The main aim of pretreatment is to break down immune complexes to obtain free circulating antigen. For pretreatment, 150µl of each sample was mixed with 150 µl of TCA followed by vortexing. These mixtures were incubated for 20 minutes at room temperature and then centrifuged for 10 minute at 12000 g. 100 µl of supernatant was then neutralized by adding a same volume of carbonate/ bicarbonate neutralizing buffer in eppendrofs tube (pH 10). The ELISA test was performed in polystyrene 96-well microplates. All unknown serum samples and 2 positive control samples were tested in 2 well (duplicate). Only 1 well is used for the eight negative control samples. Then polystyrene ELISA plates were coated with a monoclonal Ab (B158C11A10) and the remaining free places were blocked with 1% heat inactivated newborn calf serum in PBS with 0.05% Tween 20 (0.05% PBS-Tw20). Then, trichloroacetic acid pre-treated serum samples were added, after which a second biotinylated detecting monoclonal Ab (B60H8A4) was also added in order to bind the conjugate, streptovidine-labelled horse radish peroxidase (HRP). In the next step, chromogen/substrate solution (OPD Ortho-Phenylenediamine) for HRP was added, together with H_2O_2. The substrate step was incubated in the dark for 15 min. Plates were washed in between the steps with 0.05% PBS-Tween 20. The plates were read after stopping the reaction with H_2SO_4, by using an ELISA reader using 2 filters (492 nm for maximal absorption and 655 nm as background filter).

All positive and unknown samples are done in duplicate. The OD of each sample was displayed as the difference between the maximum absorption and the measured background. If 2 wells containing the same sample give roughly the same OD then the average OD for every sample is calculated as:

$$\text{Average OD} = [\text{OD}_{\text{well 1}} + \text{OD}_{\text{well 2}}] / 2$$

The OD of each serum sample was compared with the mean of negative reference serum samples (n ¼ 8) at a probability level of $P < 0.001$ to determine the result using a modified Student's t test (Sokal & Rohlf 1981). The ELISA values were expressed as a ratio by dividing the OD of the test sample by the OD of the cut-off value.

$$\text{Ratio} = \text{average OD} / \text{cut off}$$

An ELISA ratio >1 was considered positive with a probability of 99.9%. Preliminary results showed that this method has a sensitivity of 94.4% and a specificity of 100% (Erhart *et al.,* 2002).

4.3.3 Statistical Analysis

The data collected in the tables during the study period were analyzed by using Microsoft Excel-2007 and SPSS 16. The statistical parameter like prevalence in the total sample and P value was calculated. The prevalence rate of *T. solium* cysticercosis in slaughter pigs was calculated.

4.3.4 Quality Control

Strict quality control was maintained to obtain reliable results. Strict aseptic condition was maintained throughout the procedure. The internal control of each test was done by a conjugate control (CC), a substrate control (SC) and two positive controls. The CC detects false positive reactions due to a specific conjugate binding. The SC checks if the OPD can change color due to a specific circumstances. The positive controls are used to ascertain positive samples can be detected. The results were interpreted based on the sensitivity and specificity of this test. Using statistical techniques, the sensitivity was estimated at 76.3 to 86.7% and the specificity at 84.1 to 98.9% (Dorny *et al.,* 2004; Krecek *et al.,* 2007). False positive results can however occur due to cross-reaction with cysts of *Taenia hydatigena* and *Taenia saginata asiatica* (Ito and Craig, 2003). Since no data is available on the prevalence of these two parasites in Nepal, the high specificity found in previous studies may possibly not apply here.

CHAPTER-V

RESULTS

A total 340 serum samples were collected including 198 blood samples of pigs and 142 human blood samples from T.U. Teaching hospital and Neuro hospital of Biratnagar during the study period of November 2010 to May 2011. Out of 142 human serum samples, the total prevalence of cysticercosis in human by Ag-ELISA was found to be 10.6% (n=15) (Table 5.1.1). Out of 198 blood samples from pigs, 88.4% (n=175) were from male pigs and 11.6% (n=23) were from female pigs. Out of total 198 samples, 42 were positive for *T. solium* metacestode circulating antigens and the total prevalence of porcine cysticercosis was found to be 21.21% (Table 5.2.1).

5.1 Sero-prevalence of cysticercosis in human

Out of total 142 samples, 16.90% (n=24) were collected from Neuro hospital, Biratnagar and the remaining 83.10% (n=118) were collected from Teaching hospital, Maharajgunj who have been suspected for neurocysticercosis. Out of total 142 epileptic patients, 15 cases were positive for *T. solium* metacestode circulating antigens and the total prevalence of neurocysticercosis was found to be 10.56%.

Table 5.1.1: General prevalence of cysticercosis

STUDY SITE	TOTAL		RESULT		PREVALENCE
	No.	%	POSITIVE	%	
NEURO HOSPITAL	24	16.90%	5	33.33%	10.56%
IOM, KATHMANDU	118	83.10%	10	66.66%	
TOTAL	142	100%	15	100%	

Out of total 142 samples, 59.15 % (n=84) were from male patients and 40.85 % (n=58) were from female patients. The positivity rate of cysticercosis was 80% (n=12) and 20% (n=3) in male and female respectively. But there was no significant association between cysticercosis infection and gender of patients (χ^2_{cal} =2.12).

Table 5.1.2: Distribution of cysticercosis cases in human by gender

PARAMETER	TOTAL		RESULT		P value
SEX	No.	%	POSITIVE	%	
MALE	84	59.15%	12	80%	P > 0.05
FEMALE	58	40.85%	3	20%	
TOTAL	142	100%	15	100%	

Out of total 15 positive cases, high positivity rate i.e. 40% (n=6) was found in the age group 30-40 followed by 20% (n=3) in the age group 20-30 and 13.33% (n=2) in different age groups 10-20, 40-50 and 50-60. No significant association was found between infection and different age group of the patients (P>0.05).

Table 5.1.3: Distribution of cysticercosis in human in different age-groups

AGE GROUPS (YEARS)	TOTAL		RESULT	
	No.	%	POSITIVE	%
0-10	20	14%	0	0%
10-20	23	16%	2	13.33%
20-30	24	17%	3	20%
30-40	32	23%	6	40%
40-50	17	12%	2	13.33%
50-60	11	8%	2	13.33%
60-70	11	8%	0	0%
70-80	4	4%	0	0%
Total	142		15	

5.2 Sero-prevalence of cysticercosis in pigs

A total 198 blood samples were collected from the pigs i.e. 49.5% (n=98) from Sinamangal slaughter center and 50.5% (n=100) from Teku slaughter center. Out of 198 blood samples tested, 42 were found to be positive for the *T. solium* metacestode circulating antigens and the total prevalence of porcine cysticercosis was 21.21%. Mostly white pigs, pakhribas black, cross pigs and hurrah were slaughtered in these slaughter houses.

Table 5.2.1: Distribution of cysticercosis positive pigs in different slaughter slab

PARAMETER	SAMPLE PROCESSED		RESULT		PREVALENC
SLAUGHTER PLACE	No.	%	POSITIVE	%	
SINAMANGAL	98	49.5%	26	61.90%	21.21%
TEKU	100	50.5%	16	38.09%	
TOTAL	198	100%	42	100%	

Usually high number of male pigs (n=175) was slaughter than female pigs (n=23). About 88.4% (n=175) blood samples were collected from male pigs and 11.6% (n=23) blood samples were collected from female pigs. Out of 42 positive cases, 88.09% (n=37) were positive for male pigs and 11.9% (n=5) were positive for female pigs. But no significant difference was found between the infection and sex of pigs (χ^2_{cal} =0.04).

Table 5.2.2: Distribution of cysticercosis positive pigs by sex

PARAMETER	SAMPLE PROCESSED		RESULT		
SEX	No.	%	POSITIVE	%	P VALUE
MALE	175	88.4%	37	88.09%	P>0.05
FEMALE	23	11.6%	5	11.90%	
TOTAL	198	100%	42	100%	

Out of 198 pigs, 28.3% (n=56) were black pakhribas, 34.8% (n=69) were hurrah, 14.6% (n=29) were cross breed and 22.2 % (n=44) were white breed. Among different

types of breed, the highest positivity for *T. solium* metacestode circulating antigens was found to be in black pakhribas followed by hurrah (31%), cross breed (16.6%) and white breed (14.3%) respectively. But no statistical difference was found between the cysticercosis infection and different breeds of pigs (χ^2_{cal} =3.70).

Table 5.2.3: Distribution of cysticercosis positive pigs in different breed

PARAMETER	SAMPLE PROCESSED		RESULT		P VALUE
BREED	No.	%	POSITIVE	%	
BLACK	56	28.3%	16	38.1%	
HURRAH	69	34.8%	13	31%	
CROSS	29	14.6%	7	16.6%	P> 0.05
WHITE	44	22.2%	6	14.3%	
TOTAL	198	100%	42	100%	

More than half i.e. 61.1% of the pigs were brought from different breeding areas of Kathmandu valley, some were brought from the neighbouring districts (Kavre) and remaining was brought from eastern part of Nepal i.e. Sunsari and neighbouring country (India). The high positivity of cysticercosis is found in pigs brought from different part of Kathmandu valley (59.52%) followed by pigs imported from India (21.42%), Sunsari (9.52%) and Kavre (9.52%). No significant difference was found between the origin of pigs (χ^2_{cal} =5.09).

Table 5.2.4: Distribution of cysticercosis positive pigs by its origin

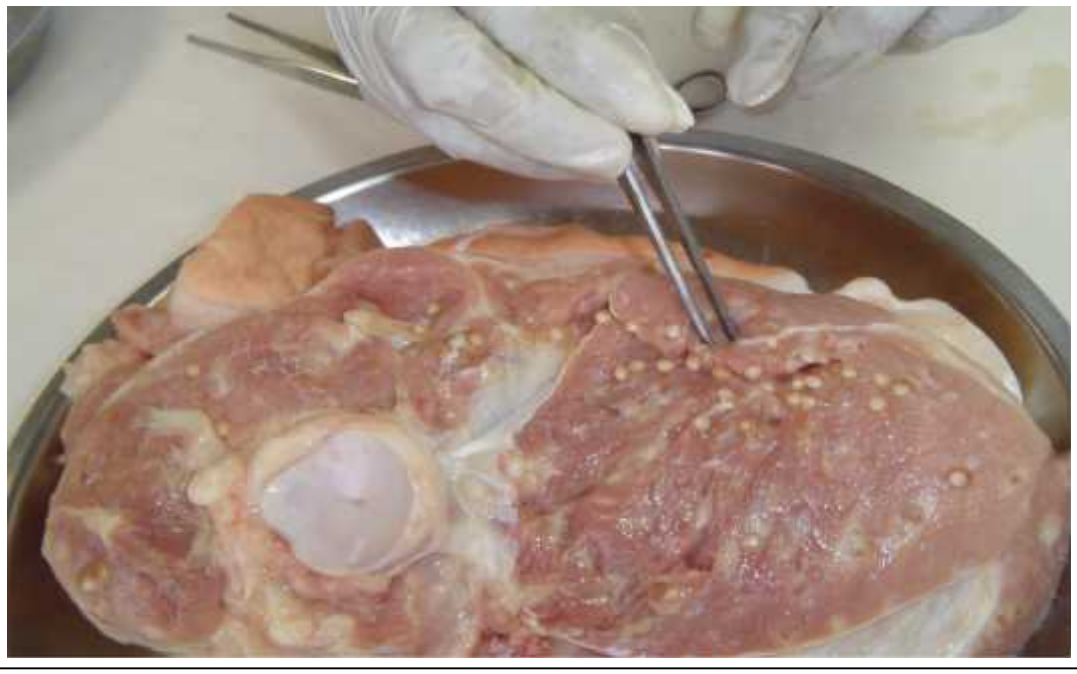
PARAMETER	SAMPLE PROCESSED		RESULT		
PIG'S ORIGIN	No.	%	POSITIVE	%	P VALUE
KATHMANDU	121	61.1%	25	59.52%	
INDIA	41	20.75%	9	21.42%	P>0.05
SUNSARI	28	14.1%	4	9.52%	
KAVRE	8	4%	4	9.52%	
TOTAL	198	100%	42	100%	

LIST OF PHOTOGRAPHS

Photograph 1: Slaughter house of Tilganga with hurrah bree

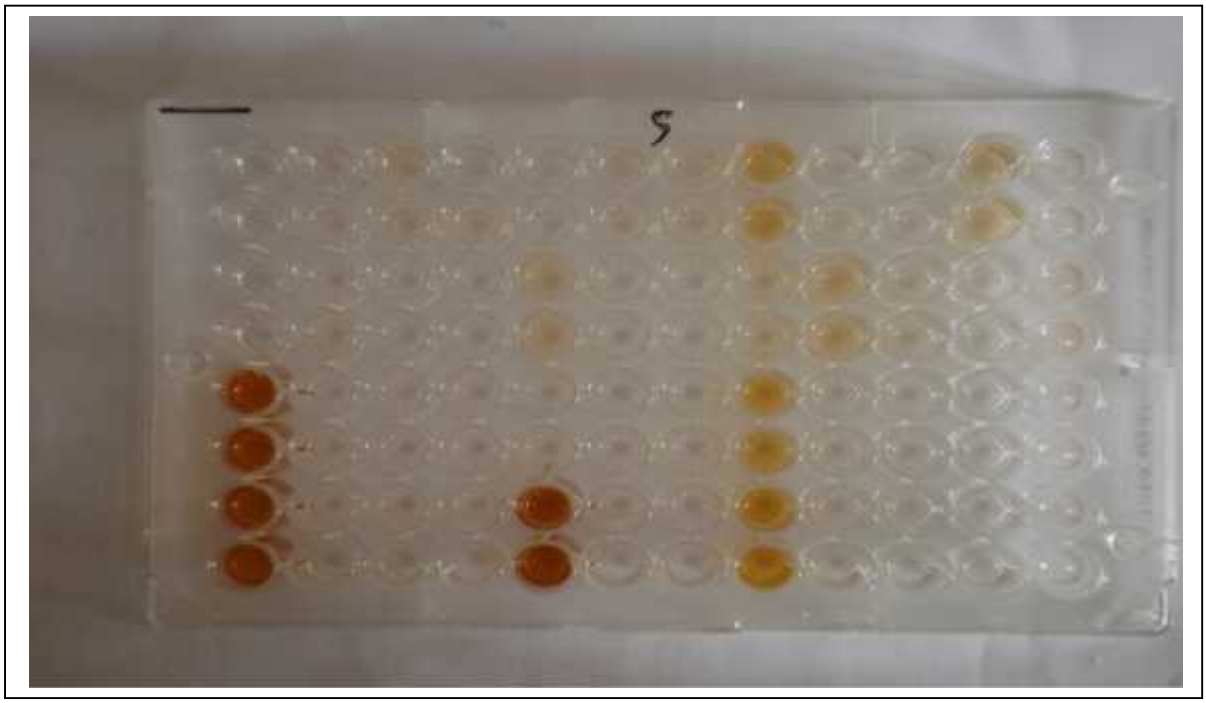
Photograph 2: Breeding areas in bank of Bishnumati River

Photograph 3: Pigs feeding on the sewage waste at Bishnumati River



Photograph 4: Meat with the larva of *T. solium* which was kept for selling in the shop

Photograph 5: Labeling of the samples before processing



Photograph 6: ELISA plate after performing ELISA test, showing positive by color change

Photograph 7: Elisa reader

CHAPTER –VI

DISCUSSION

6.1 Discussion

Nepal is an agricultural country with poor economy. Agriculture contributes 38.15% of the nations GDP and livestock contributing almost 27.66% of Agricultural GDP (MoAC, 2006). Pig farming is increasing in Nepal day by day with the annual population growth rate of 4.55% (CLDP, 2003). Pork contributes about 7.32% of total share in Nepal and other as buff contributes 64.68% and poultry 7.18% (CBS, 2006a). Per capita meat consumption pattern in Nepal is 8.7 kg/per person/year (TLDP, 2003). The pork consumption is highest (38%) in world scenario followed by poultry (30%) and beef (25%) (Bhattarai, 2005). Meat being good source of protein, vitamins, minerals and fat necessary for good health and growth, the consumer demand for pork has dramatically increased these days. In recent years, pig breeding and marketing has been a traditional occupation of some communities of Nepal and pork constitutes as part of their food as well. It has also become one of the good sources of income among many farmers. Pork meat is also rich in thiamine (B_1) which helps to maintain the circulatory and nervous systems actively and also aids the body in storing and releasing energy. Many studies up to date has shown that as pig farming is increasing day by day in Nepal, the incidence of *T. solium* cysticercosis infection in both pigs and human population is being endemic. In Nepal, yearly 2.73 per 1000 inhabitants' life are lost through NCC-associated epilepsy. *T. solium* cysticercosis is responsible for 1% of disease burden of all diseases in Nepal (Dorny, 2010).

Using a cross-sectional study, the prevalence of cysticercosis in pigs and humans were determined. A total 340 blood samples were processed for detection of *T. solium* metacestode circulating antigens by using Ag–ELISA. Out of 340 blood samples, 198 blood samples were collected from pigs during the time of slaughtering and 142 blood samples were collected from human who has been suspected for neurocysticercosis in T.U. Teaching hospital and Neuro hospital of Biratnagar. Out of total 142 epileptic patients, 15 cases were found to be positive for *T. solium* metacestode circulating antigens. The total prevalence of neurocysticercosis was found to be 10.56%. Similar result has been reported by many previous studies conducted in different places of

Nepal. Ale (2010) reported the prevalence of neurocysticercosis to be 13.63% in the patients visiting Institute of Medicine (IOM), Maharajgung. Priyani *et al.*, (2007) reported 13.3% (15/112) neurocysticercosis cases with seizure, at Nepalgunj Medical College (NGMC). Similar findings was reported by Pandey (2007) i.e. 13.34% of total epileptic admission Episodes in TUTH. During 2002-2008 neurocysticercosis (NCC) patients were found to be 9.7% (179/1839 epilepsy cases) from data survey of 5 hospitals (Norvic, TUTH, Patan, Bir and NMC) (Karna, 2009).

But in 2006, high rate of NCC has been reported in different research conducted in different hospitals of Kathmandu. Panta, (2006) has diagnosed 61% NCC cases by EITB at Model Hospital. Similarly Neupane (2006) has diagnosed, 23% out of total 200 seizures disorders cases as NCC by CT scan and MRI in Shree Birendra Hospital, Chhauni. In the same year, Sharma (2006) has reported 43.64% out of 55 cases of seizure disorder patient as NCC based on CT scan at OPD section of OM Hospital. All these research shows that NCC is prevalent in Nepal since many years. But as compared to previous research, our study shows decrease in NCC cases, this may be due to increase level of personnel sanitation and hygiene, deworming practice and eating of well cooked pork.

Out of 142 human samples, 59.15% (n=84) blood samples were collected from male patients and 40.85% (n=58) were collected from female patients. Out of 15 positive samples, 80% (n=12) were positive for male patients and 20% (n=3) were positive for female patients showing high positivity rate of cysticercosis in male. On the sex wise distribution, Karna (2009) has also reported similar results with high positivity prevalence in male (66.5%) than in females (33.5%). Pandey (2007) has also reported the high positivity in male (58%) than in females (42%). Similarly the findings of Sapkota (2005) also showed higher prevalence in male i.e. 59% than in females (41%). Therefore high prevalence of infection in male population may be due to high consumption of raw pork or barbecued meat along with drink which leads to taeniasis and autoinfection leads to NCC. But no significance difference was found between cysticercosis infection and the gender of the patients ($P>0.05$).

On the age wise distribution, out of total 15 positive cases, high positivity rate i.e. 40% (n=6) was found in the age group 30-40 followed by 20% (n=3) in the age group 20-30. Similarly 13.33% (n=2) were found positive in different age groups 10-20, 40-

50 and 50-60. Similar results has also been reported by Karna (2009) with the highest positivity of cysticercosis in the age group 15-34 years (37.5%) and 22.35% for >35 years. Sapkota (2005) reported highest (40%) NCC in >35 year followed by 37.5% in 15-34 years and 22.5% in less than 14 years. Younger age group of mostly second and third decades was mostly affected in a study at TU Teaching hospital (Agrawal, 2006). Age group of 21-30 years was mostly affected by NCC at OM hospital (Sharma, 2006). Therefore our study and previous studies shows the high positivity cases of neurocysticercosis are mostly prevalent in productive age group. Since these groups are more involved in numerous daily activities they have greater chances of exposing to the disease distribution sources than other groups. However, low incidence at the low age group also suggested that these groups are less exposed to the agent in less numbers than others. There was no significant association between cysticercosis infection and different age-group of patients ($P>0.05$). During this study, extensive data about patient's socioeconomic condition couldn't be taken which could have further explored the causes of this result in the study.

The sero-diagnosis of piglets can be taken as practical method to detect *T. solium* eggs in the environment as well as the pigs being the contributing factor for transmission of cysticercosis in human population, the sero-prevalence of cysticercosis in pigs was also carried out. Out of 198 blood samples of pigs, 21.21% (n=42) were found to be positive for *T. solium* metacestode circulating antigens by using Ag-ELISA.

Many previous researches conducted in Kathmandu valley and different parts of Nepal show that the porcine cysticercosis is endemic with high risk to human population. Devleesschauwer (2009) has reported the similar findings with the apparent prevalence of porcine cysticercosis to be 18.21% (73/401) in the Kathmandu Valley. Ale (2010) reported prevalence of the porcine cysticercosis to be 29.3% (87/297). Out of which, 29% (29/100) were obtained from lalitpur district, 39% (39/100) from Bhaktapur district and 19.59% (19/97) from Kathmandu district.

Although detection of cysts in pig's carcass during dissection is "the gold standard" for diagnosis, this technique was not used due to lower sensitivity as well as due to different technical constraints. If the pigs are highly infected then only the larva are able to detect in the carcass inspection otherwise it may be detected by chance at low infection level (Krecek *et al*., 2008). By lingual and carcass examination, the lower prevalence has be reported by Joshi *et al*., (2008) in pigs of Kathmandu and Chitwan

valley to be 0.63% and 0.94% respectively. Similarly Shakya (2010) has reported the prevalence of porcine cysticercosis by tongue palpation and caracass inspection in Kirtipur municipality to be 3.33% (6/150).

Out of 198 pig's blood samples, 88.4% (n=175) were collected from male pigs and 11.6% (n=23) were collected from female pigs. Among 42 positive samples, 88.09% (n=37) were positive for male pigs and 11.9% (n=5) were positive for female pigs. The more positivity prevalence of cysticercosis in male pigs was observed because more than eight times male pigs were slaughtered than females (88.4% versus 11.6%) in these areas.

Due to high demand of meat, pigs are mostly brought from different breeding areas of Kathmandu valley, Kavre districts, from the eastern part of Nepal (Sunsari districts) as well as from the border areas and India for slaughtering in different slaughter slabs. More than half i.e. 61.1% of the pigs were brought from different breeding areas of Kathmandu valley. The high positivity of cysticercosis was found in pigs brought from different part of Kathmandu valley (59.52%) followed by pigs imported from India (21.42%), Sunsari (9.52%) and Kavre (9.52%). Mostly hurrah breed (kalo dharane bangur) was brought from the eastern part of Nepal and India and usually other breeds were reared for meat in different parts of breeding areas of valley. Out of 198 pigs, 28.3% (n=56) were black pakhribas, 34.8% (n=69) were hurrah, 14.6% (n=29) were cross breed and 22.2% (n=44) were white breed. Among different types of breed, the highest positivity for *T. solium* metacestode circulating antigens was found to be in black pakhribas (38.1%) followed by hurrah (31%), cross breed (16.6%) and white breed (14.3%) respectively. The breeding areas and the other parameters like slaughtered place, sex and breed of the pigs was found to have no significant association with the cysticercosis infection ($P>0.05$).

Our findings were in agreement with findings of Devleesschauwer (2009). But in the study conducted by Pandey (2007), the breed was regarded as a significant risk factor local hurrah pigs were found to have 7.69 times more risk of infection than improved breeds (P=0.048). In the residential area of the ethnic population, high rate of porcine cysticercosis and taeniasis has been reported due to poor sanitation, lack of education and close relation between pig farming and community.

The pigs slaughtered at the Sinamangal slaughter center were found to have high rate of sero-positivity for cysticercosis than Teku because the pigs slaughtered at Sinamangal were mainly brought from different breeding regions of Kathmandu and from the eastern parts of Nepal especially Sunsari districts. After direct observation on the breeding region of Kathmandu i.e. Jadibuti (the bank of Manohara river) and Kalimati (the bank of Bisnumati river), these areas were found highly polluted. The practices of directly mixing of untreated sewage to the river, semi-intensive husbandry practices and direct access of pigs to human feces were found to be possible cause for porcine cysticercosis. In the Sunsari districts and many eastern parts of Nepal, according to Sharma (2006) majority of the farmers are following scavenging system for pig farming and open defecation practice is highly present. The poor sanitary condition, low socio-economic status and lack of education among farmers were major cause for cysticercosis among pigs and humans.

After conversation with the butchers and shopkeeper, it was found that most of them were not aware of porcine cysticercosis and *Taenia solium*, but frequently the larva of *T. solium* have been observed by them on the meat surface. They have named “Pidke” or “Chamle” to larva on the local terms. The few of them only had the knowledge about tapeworm infection in humans was due to eating infected pork. They were ignorant of the fact that a person with taeniasis can act as a carrier of the infection and could infect themselves and another person. Majority of the butchers were found selling the infected meat in the market to the consumers. So, more risk has been observed to consumers of eating infected meat in the study areas knowing or unknowingly which has played an important role in maintaining the life cycle of cestode in the communities. Pigs are slaughtered here by using sharp rod or hammer without following any meat inspection for different zoonotic diseases.

Kathmandu being the capital city of Nepal, migration rate of population from rural area to this area is very high so migration of tapeworm carrier from different endemic area of the country to the valley and exposure to the disesase distribution is high increasing the risk of cysticercosis to the surrounding, families and themselves. Schantz *et al.,* (1992) has reported the incidence of cysticercosis even in vegetarians from tapeworm infected food prepares and the consumption of the raw vegetables. In Kathmandu, practice of using sewage mixed water sources for irrigation and washing green vegetables before taking to the market by farmers may be other contributing factor for transmission of *Taenia* eggs in humans.

Mainly two monoclonal Ab-based tests (B158/B60 Ag-ELISA and HP10 Ag-ELISA) have been validated and are used routinely for the detection of parasite antigens (Dorny *et al*., 2003). The sensitivity and specificity of this ELISA are generally considered to be high (Poudyel *et al*., 2002; Dorny *et al*., 2004; Krecek *et al*., 2008). The neuro imaging studies, such as computed tomography (CT) and/or magnetic resonance imaging (MRI) are indispensable tools in diagnosis but these procedures are expensive, mainly available in urban areas, and often inaccessible to inhabitants of endemic rural areas. Human cysticercosis frequently occurs without brain involvement and consequently clinical symptoms are often lacking. Ag- ELISA is cabable of detecting infections with only a few cysts, and was under an experimental setting able to detect even a single cyst infection (Nguekam *et al.*, 2003; Dorny *et al*., 2004). The positive antigen detection in pigs/humans can provide a valuable alternative in that it reflects the presence of viable parasites. On the other hand, the test certainly underestimates the prevalence of cysticercosis in a community because all patients harbouring only calcified cysticerci are not detected. Antigen detection can be used as a tool for serological monitoring of anti-parasitic therapy: antigen levels drop rapidly after successful antihelminthic treatment (Garcia *et al.*, 2000; Nguekam *et al*., 2003). Thus, antigen detection in neurocysticercosis cases is a useful tool, which may support clinical diagnosis when neuroimaging studies are not available or are inconclusive (Dorny *et al.*, 2003).

The findings of this study show that porcine cysticercosis is highly endemic in the different parts of Nepal, the human population living at these areas and consuming such infected meat seems to have high risk of getting infected with cysticercosis/taeniasis. Therefore the poor sanitary conditions, unplanned urbanization, free ranging of pigs with easy access to human feces, availability of infected pork meat in market and consumption of undercooked meat are found to be contributing factor for the cause of cysticercosis/taeniasis. Due to which country is facing the major economic burden and losses due to condemnation or cheap value of infected pig carcasses and costs involved in diagnosis, treatment and disability of patients with NCC. This strongly indicates a need for comprehensive program to combat the problems of cysticercosis and taeniasis associated morbidity and mortality in Nepal.

CHAPTER VII

CONCLUSION AND RECOMMENDATION

7.1 Conclusion

This study shows that *T .solium* cysticercosis is endemic and highly prevalent in pigs and human population of Nepal. But still no eradication, preventive measures and control programs has been launched for disease control in Nepal. The evidence of cysticercosis through this study clearly shows that this chronic infection is a potential zoonotic threat. The life cycle of *T. solium* is sustained because pigs have access to infected feces and measly pork available for consumption in the market. So, strict practice of hygienic slaughtering and meat inspection act and regulation should be implemented strictly as soon as possible. Control or eradication of *T. solium* cysticercosis has been achieved to date only in European countries and North America through significant improvements in sanitary conditions and developing functional slaughtered house control systems. But in endemic areas of developing countries control is limited by economic and sanitary conditions. The poor sanitary condition, open field defecation, availability of infected meat in market and use of sewage mixed water source for irrigation and washing vegetables are the possible factors for the causes of cysticercosis in human and pigs. Thus this baseline data could play a guiding role in setting up the control programs as well as provide guidelines to extend the research to different regions of the country, and to pay more attention for identification and quantification of risk factors, for porcine as well as human cysticercosis and taeniasis.

7.2 Recommendation

1. *T. solium* taeniasis/cysticercosis cases should become notifiable to enable investigations at the farm / production level and favour interruption of the parasitic cycle.
2. Cysticercosis - free husbandry should be encouraged.
3. Since the consumption of infected pig meat plays an important role in the transmission of the *T. solium* cycle, proper slaughterhouse and meat inspection act and regulation should be strictly followed for illegally established slaughter houses in Kathmandu valley.
4. Open field defecation should be discouraged and time to time mass human chemotherapy for elimination for taeniasis should be conducted.
5. Different health and hygiene awareness information campaigns by posters, leaflets or by different direct/ indirect means should immediately be conducted from grass-root level to high authorized level to control the taeniasis and cysticercosis**.**
6. Different research programs in different regions of the country and more attention for the identification and quantification of risk factor for porcine cysticercosis as well as human cysticercosis and taeniasis is important. The co-operation or network between different stakeholders, government, NGO's, hospitals, educational institutes and different international and inter-sectoral collaborations are necessary to exchange of knowledge and information.
7. In other to facilitate the interrelation between the antigen of *T. hydatigena* and *T. s. asiatica* in Nepal further research are necessary in molecular levels.
8. The study was conducted in limited measure of sample from limited places. Study for large places is suggested to outline the broad situation.

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APPENDICES

Appendix 1

Data of blood collection of Hospitals patients

Hospital: Date:

S.N	Surname	Sex	Age	Cut off value	OD value	Ratio	Result

Appendix 2

Data of Blood collected and Carcass Examined Pigs

Slaughter House ( )

Pigs brought from:

Average daily slaughter:

S.N.	Sex	Age(months)	Breed	Cut off value	OD value	Ratio	Results
S 1							
S 2							
S 3							
S 4							
S 5							
S 6							
S 7							
S 8							
S 9							
S 10							
S 11							
S 12							
S 13							
S 14							
S 15							
S 16							
S 17							

H-Hurrah, W-white, B- black pakribas, C- Cross

APPENDIX 3

Equipment and materials used during the study

Equipments

Autoclave:	Sternite, Japan
Centrifuge:	Heltich, Japan
Refrigerator:	LG Korea
Deep freeze:	Korea
Hot air oven:	Memmert, Germany
Incubator:	Sakura, Japan
Weighing balance:	Choyo MP, Japan
ELISA reader with 490 filters:	Belgium
Parafilm	VWR
Eppendorf tubes, safe lock (2 ml)	VWR (211-2120)
Immunoplate Maxisorp F96 ELISA plate	VWR (NUNC439454)

REAGENTS	**COMPANY OR SUPPLIER**	**REFERENCE CODE**
Biotin protein labeling Kit:	Roche	1418165
Carbonate-Bicarbonate Buffer tablets	Sima-Aldrich	C3041
H_20_2 30% P.A. ISO 250 ml	VWR	211-2120
Monoclonal antibodies	please contact ITM	
Na_2CO_3 1.06392.1000	VWR	
$NaHCO_3$ 1.06329.1000	VWR	
Newborn Bovine Serum, 100 ml (NBCS)	Invitrogen	16010-167
OPD tablets	DAKO	S2045
PBS tablets	Oxoid	BR0014G
Steptavidin-HRP (Jackson immunoresearch)	Lucron	016-030-0840

TCA 98% 100g	Sigma-Aldrich	11,611- 4
H_2SO_4 0.5 Mol/l	VWR	
1.09981.0001		
Tween 20	VWR	
8.22184.0500		

DIFFERENT MATERIALS USED:

Rile disposable plastic pipettes (25 ml)

Automatic pipettes (0.5 µl-10µl, 50µl-200µl, 100µl-1000µl) + tips

Magnetic stirrer

Aluminum wrapping foil

Eppendorf tubes (1.5 ml + rack)

Disposable syringe

Measuring flasks and beakers

Falcon tubes

All of these equipments were brought from the local suppliers.

Appendix 4

A. Composition and preparation of buffer solutions

Phosphate buffered Saline (PBS)

The PBS buffer is prepared by using tablets.1 tablet in 100 ml of distilled water, yields 100 ml PBS buffer, pH 7.3

Trichloroacetic acid (TCA)

The solution used for the “pretreatment” of the serum samples is a 5 % (w/v) solution in RO-DI water.

Washing buffer

The washing buffer consists of PBS with 0.05% (v/v) Tween 20. 10 PBS tablets are mixed with 1 liter of Distilled water and 0.5 ml (500µl) Tween 20 is mixed well.

Blocking buffer

The Blocking buffer consists of washing buffer + 1 % (v/v) of Newborn Calf Serum (NBCS). 0.5 ml (500 µl) of NBCS is mixed with 49.5 ml of washing buffer.

Coating Buffer

The coating buffer is prepared by using powder-filled capsules. 1 tablet in 100 ml of RO-DI water yields a 0.05 M carbonate/bicarbonate buffer, pH 9.6

Alternative:

Stock solution A: Na_2CO3 (0.06M) =0.159g/25ml

Stock solution B: NaHCO3 (0.06M) = 0.504g/100ml

10 ml A+ 50 ml B+175 ml RO-DI water.

A and B are carefully added until pH 9.6 is reached.

Then volume is adjusted to 250 ml with RO-DI water.

Based on:

Na_2CO_3: 0.06mol/l= 0.006 mol/100ml= 0.0006 mol/10 ml

$NaHCO_3$:0.06 mol/l= 0.006 mol/100ml= 0.003 mol/50 ml

Finally we have 0.0036 mol/250ml= 0.0144M

Calculation of the pH:

pH = pKa + log (salt)/(acid)

Salt: Na_2CO_3 concentration of 0.0006 mol in 250 ml, hence 0.0024 M

Acid: $NaHCO_3$ concentration of 0.003 mol in 250 ml, hence 0.012 M

pH= 10.25 + log 0.0024 / 0.012

pH= 9.55

By adding a little of solution A or B, the desired pH of 9.6 can be obtained.

Coating buffer should be stored at +4 ºC and can be kept for a maximum of 3 months.

Neutralisation buffer

(Carbonate/Bicarbonate Buffer, 0.156 M, pH 10)

Stoke solution A: Na_2CO_3 (0.61 M) = 6.466 g/100 ml

Stock solution B: $NaHCO_3$ (0.61 M) = 5.124 g/100ml

72 ml A+ 55 ml B +300 ml RO-DI water.

Then pH to 10 is adjusted by adding either A or B.

Once the pH is set, the volume is adjusted to 500 ml with RO-DI water.

Based on:

Na_2CO_3: 0.61 mol/l= 0.061 mol/100ml = 0.044 mol/72 ml

$NaHCO_3$: 0.61 mol/l= 0.061 mol/100ml = 0.034 mol/55ml

Finally we have 0.078 mol/500 ml=0.156 M

Calculation of the pH:

pH = pKa + log (salt/acid)

Salt: Na_2CO_3 concentration of 0.044 mol in 500 ml, hence 0.068 M

Acid: $NaHCO_3$ concentration of 0.034 mol in 500 ml, hence 0.068 M

pH= 10.25 + log 0.088/0.068

pH = 10.36

By adding a little of solution A or B, the desired pH of 10 can obtained. Neutralization buffer should be stored at + 4 ºC and can be kept for a maximum of 3 months.

Substrate (OPD)

In 12 ml distilled water 2 tablets OPD tablets is mixed well and 5µl H_2O_2 is mixed well. But H_20_2 is added to the mixture of OPD solution just before adding putting in the plate.

Streptovidin

In 10 ml blocking buffer, 2µl of streptavidin is mixed.

B. Monoclonal antibodies used

The monoclonal antibodies are developed produced and labeled by Prince Leopold Institute of Tropical Medicine, Department of Animal Health, Antwerp (Antwerp), Belgium.

1. **The Capturing antibody**

   The capturing antibody (B158C11A10) is used. In 1 ml coating buffer 3ul capturing Ab is mixed.

2. **The detecting antibody:**

   The detecting antibody (B60H8A4) is labeled to biotin. In 10 ml blocking buffer 25 µl of detecting antibody is mixed.

C. Controls

The plate layout is as shown in Table 9.Note that +1, +2 and X1 –X40 are 2wells/sample, while -1 to -8 is 1 well/sample.

Table 9: Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	SC	-1	X1	X5	X9	X13	X17	X21	X25	X29	X33	X37
B	SC	-2	X1	X5	X9	X13	X17	X21	X25	X29	X33	X37
C	CC	-3	X2	X6	X10	X14	X18	X22	X26	X30	X34	X38
D	CC	-4	X2	X6	X10	X14	X18	X22	X26	X30	X34	X38
E	+1	-5	X3	X7	X11	X15	X19	X23	X27	X31	X35	X39
F	+1	-6	X3	X7	X11	X15	X19	X23	X27	X31	X35	X39
G	+2	-7	X4	X8	X12	X16	X20	X24	X28	X32	X36	X40
H	+2	-8	X4	X8	X12	X16	X20	X24	X28	X32	X36	X40

Internal quality control:

Substrate and conjugate controls

There is a conjugate and substrate control build in each plate (CC and SS respectively).Non-specific reactions between the plate, coating/blocking and conjugate are interpreted by conjugate control. The quality of the substrate (e.g. by influence of light) can be traced by substrate control. Both controls need to be Negative (below cut off value).

Table 10: SC and CC controls

ELISA step	SC	CC
Capturing antibody	Only coating buffer	Capturing antibody
Blocking	Blocking buffer	Blocking buffer
Samples	Blocking buffer	Blocking buffer
Detecting antibody	Blocking buffer	Detecting antibody
Streptavidin	Blocking buffer	Streptavidin
Substrate	Substrate	Substrate

Table 10: shows the contents of SC and CC controls for each step of the ELISA assay.