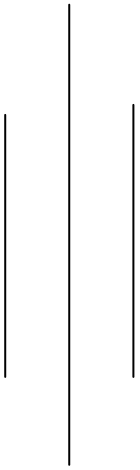
ROLE OF SEROLOGY, NEUROIMAGING AND STOOL EXAMINATION IN DIAGNOSIS OF NEUROCYSTICERCOSIS



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Dr. Kalyan Sapkota

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LIST OF ABBREVIATIONS

EITB: Enzyme linked immunelectro-transfer blot

ELISA: Enzyme linked immune-sorbent Assay

CDC: Centre for Disease Control and Prevention

CECT: Contrast enhanced Computed Tomography

CNS: Central Nervous System

CSF: Cerebrospinal fluid

CT scan: Computed tomography

EEG: Electro encephalogram

ICT: Intra-cranial tension

IgG: Immunoglobulin-G

MRI: Magnetic Resonance Imaging

MTH: Manipal Teaching Hospital

NCC: Neurocysticercosis

NPHL: National Public Health Laboratory

OD: Optical density

OPD: Out Patient Department

WHO: World Health Organization

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CHAPTER-I
INTRODUCTION

Neurocysticercosis (NCC) is the infection of Central nervous system by the larval stage of *Taenia solium* (pork tapeworm). It is the most important parasitic neurologic disease and a common cause of epilepsy in Asia, Africa and Latin America; representing enormous cost for anticonvulsant and medical resources (1). *Taenia solium* causes two different diseases. When the adult cestode infests the human intestine, taeniasis develops; it is generally asymptomatic, but the host becomes a continuous source of taenia eggs, which are expelled every day in the feces, which may then contaminate vegetables and food in areas with poor sanitary conditions. Human cysticercosis occurs by ingestion of faecally contaminated food, water or vegetables containing eggs of *T. solium* (2-4). However, ingestion of infected pork only causes intestinal tapeworm infestation (taeniasis) (5).

This tapeworm is a public health problem in most developing countries where pigs are raised and pork is consumed and where poverty, illiteracy and deficient sanitary infrastructure are common (3). The main objective of this study is to find out the diagnostic significance of Serology and Neuroimaging and to detect intestinal carriers of the tapeworm.

Cysticercosis caused by infection with larval stage of *Taenia solium* has been described as both an ancient disease (Del Brutto & Sotelo 1988) and a modern day plague (Brown & Voge 1985). In endemic countries taeniasis/cysticercosis is extremely common; and neurologically symptomatic individuals, although many represent only the 'tip of the iceberg' (1, 6). In most endemic villages more than 10% of the general populations are seropositive, and the proportion can reach 25% (1, 4). In population-based studies, 10–18% of asymptomatic individuals have CT features that suggest neurocysticercosis, mainly brain calcifications in seronegative individuals (1). WHO estimated that 50 million persons, predominantly from developing

countries, are infected with taeniasis, and 50,000 people die of the disease each year (1).

Cysticercosis of the central nervous system is the most important neurological disease of parasitic origin in humans. It causes serious morbidity and in areas where *T. solium* is endemic, it is known to be a leading cause of epilepsy (3, 5, 7), which has profound social, physical and psychological consequences. NCC is the important cause of chronic epilepsy which places particular demand on the health services.

Diagnostic certainty of NCC is based on combined neuroimaging studies, immunodiagnostic technique, clinical presentation and epidemiological evidence suggestive of Neurocysticercosis (8). The clinical diagnosis is impaired by polymorphism and non-specificity of the symptoms (9, 10). Depending upon the cysticerci in the brain and the reaction they induce within the brain and their site in brain, they produce varieties of symptoms. Most common being seizure, headache, nausea, vomiting and altered mental status. And the lab facilities for serology are lacking in most of the health centers where the disease is endemic. Any study of NCC is limited by the difficulty in clearly establishing the diagnosis. Therefore case definition is limited to CT scan finding and serology. Diagnosis is important for proper evaluation of the patient condition and appropriate treatment as the outcome with early treatment is very good

Neuroimaging technique such as CT scan have attributed to more accurate diagnosis and better understanding of pathophysiology. However, only the presence of cystic lesion showing the scolex is considered pathognomonic (8). CT scan has been claimed to have sensitivity and specificity of 95 % for diagnosing NCC (6).

The detection of specific antibody against *T. solium* cysticercal antigen has been considered important diagnostic element for NCC especially when neuroimaging technique are unavailable or inconclusive. Immunodiagnostic techniques include detection methods for specific antibody and for circulation parasite antigen in serum and cerebrospinal fluid (11). Infection with *T. solium* results in specific antibody

response mainly IgG class. Different techniques have been described to detect antibody to *T. solium*. The most specific test is Enzyme Linked Immunoelectro-Transfer Blot (EITB). However in developing countries ELISA (Enzyme linked Immuno-sorbent Assay) is preferred because of better availability, simplicity and lower cost compared with EITB (12). ELISA measures specific IgG antibody to partially purified antigen from cysticercal fluid, serum sensitivity of 75-87% and specificity of 75% has been reported (12). Analysis of antibody response indicated that the optimum threshold titres for seropositivity were 1:800 for the ELISA. When used with these thresholds, the ELISA gave a sensitivity, specificity, positive and negative predictive values and diagnostic efficacy of 89%, 81%, 79%, 90%, 85%, respectively (13). Serologic test can be very useful for confirmation of neuroimaging finding for differential diagnosis of other cyst forming condition (14, 15).

Taenia carriers are potent source of NCC, endangering everyone coming into contact with them (1). Garcia et al (16) demonstrated that tapeworm carriers are at high risk of developing massive brain infection with viable *T. solium* cysticerci. 25% of the patients with cerebral cyst were found to harbor adult *T. solium* in their intestine or have history of such infection. Detecting ova in children with NCC is worthwhile as a public health measure to prevent further exposure and to implement preventing measure to control faecal-oral transmission. Rates of taeniasis as determined by stool examination for ova have been reported to range between 0.1-6% in endemic countries (4, 17). The frequency of autoinfection in individuals with taeniasis is not known. Dixon and Lipscomb noted that nearly 25% of patients with neurocysticercosis either had harboured or were harbouring a tapeworm. Up to 15% of patients harbour a tapeworm at the time of diagnosis of neurocysticercosis,and the proportion with tapeworms is directly related to the number of cerebral parasites (18).

In context of Nepal, the disease is endemic because of lack of proper sanitary measures, unavailable of clean and safe water; traditional pig-rearing technique, lack of health education, and also the contributing factors are inaccessibility to proper health care, lack of awareness by the medical community and difference in quality and availability of services. Human taeniosis and human and porcine cysticercosis are

reported among the major zoonotic diseases in Nepal (20, 21). The few baseline studies carried out among the different ethnic groups in the country indicate very high prevalences of human taeniosis and porcine cysticercosis (20). In Nepal up to 50% of pigs rearing farmers are infected with *T. solium* tapeworm (12). *T. solium* cysticercosis is a major health hazard in the country (Amatya et al 1999, Shrestha et al 1989). And NCC is the commonest cause of seizure in Nepal. According to study done by Basu et al (21) in western Nepal, 124 cases of NCC were diagnosed in period of 4 years (2000-2003) and 47.6% of these cases were carriers of Taenia species on stool examination.

The clinical presentation diverse, ranging from asymptomatic to severe neurological disease. Clinical symptoms of NCC generally occur as a result of inflammatory reaction around cysticerci and that stage is usually associated with degeneration of the cysticerci. Early diagnosis of NCC by serology may therefore provide opportunities for prevention of clinical symptoms and complication arising thereafter. The epilepsy occurring due to NCC has social and economic consequences. National program has mainly focused on the intestinal parasite, but little is talked about the cysticercosis. It should be addressed with proper anthelminthics therapy, along with health education, environmental sanitation. Although the condition is rampant, very little study has been carried out to find out the prevalence of taeniasis and cysticercosis and their impact upon the health of the individual, and about the knowledge, attitude and practice.

This research will identify the method for diagnosing cysticercosis more effectively. It will also provide information about sub-clinical infection and intestinal carriers of the parasite, thereby indicating the burden of the disease, and awareness for the prevention and control of the disease.

This study primarily focuses on the distribution and prevalence of cysticercosis in Western Nepal. The study evaluates the clinical profile and explores the polymorphism in clinical presentation and the outcome with treatment in pediatric neurocysticercosis. It also determines the effectiveness of serology testing and neuroimaging technique in correctly diagnosing the disease. The stool examination

helps to determine the prevalence of intestinal tapeworm and the burden of disease. Detecting ova in children with NCC is worthwhile as a public health measure to prevent further exposure and to implement preventing measure to control faeco-oral transmission. The infection with intestinal parasite and cysticercosis can be controlled with proper public health measures thereby reducing the morbidity and mortality due to the disease. This research work creates an advocacy for health worker and public health officials, which will surely help in implementing control measures and increasing awareness among general population. This study also suggests new ways to identify and suspect cysticercosis and will recommend to the health officials and researchers in Taenia/cysticercosis control, prevention, management and research.

CHAPTER-II

OBJECTIVES

2.1 General Objectives

To evaluate the diagnostic significance of serology and neuro-imaging technique in neurocysticercosis; and to determine the intestinal carriers of the parasite in neurocysticercosis in pediatric age group.

2.2 Specific Objectives

a) To identify the clinical profile of pediatric neurocysticercosis.

b) To find out the diagnostic tools used to confirm the diagnosis of suspected neurocysticercosis.

c) To determine the radiological findings in suspected neurocysticercosis cases.

d) To analyze the stages and distribution pattern of cyst in neurocysticercosis as seen in CT scan.

e) To identify the sensitivity and specificity of serologic test (Enzyme Linked Immuno-Sorbent Assay-ELISA) and Neuroimaging technique (Computed Tomography (CT) scan) in diagnosing Neurocysticercosis (NCC).

f) To determine the seroprevalence of cysticercosis in hospitalized pediatric population.

g) To screen and identify the intestinal carrier of parasite in neurocysticercosis patients.

h) To evaluate the treatment modalities and outcomes of pediatric neurocysticercosis.

CHAPTER-III

LITERATURE REVIEW

3.1 *Taenia solium*

Cysticercosis is a systemic illness caused by dissemination of the larval form of the pork tapeworm, *Taenia solium*. Encystment of larvae can occur in almost any tissue. Involvement of the central nervous system (CNS), known as neurocysticercosis (NCC), is the most clinically important manifestation of the disease. NCC is caused by the tissue-invading larvae (*Cysticercus Cellulosae*) of the pork-tapeworm; *Taenia solium*. Cysticercosis is considered the most common parasitic disease of the central nervous system (1). This disease is one of the main causes of epileptic seizures in many less developed countries and is also increasingly seen in more developed countries because of immigration from endemic areas (1). Little information is available on the natural evolution of taeniasis or cysticercosis. *Taenia solium* infection and the resulting disease neurocysticercosis are endemic in less developed countries where pigs are raised as a food source (4). It is now increasingly diagnosed in more developed countries owing to immigration of tapeworm carriers from endemic zones.

3.2 Life cycle/Parasitology

Taenia solium is a zoonotic cestode. *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 (4).

Humans are the definitive *T. solium* hosts and can carry an intestinal adult tapeworm (taeniasis), often without symptoms. The adult stage is a 2- to 4-m-long tapeworm that lives in the small intestine of humans. No other final hosts are known for *T. solium* tapeworms in nature. The lifespan of the adult *T. solium* is also unknown; it varies from 20–25 years (4). Intermittent fecal shedding of egg-containing proglottids or free

T. solium eggs ensues, with the intention that the intermediate host (normally pigs) will ingest the excreted eggs in contaminated food or water. The natural intermediate host is the pig, harboring larval cysts anywhere in its body. *T. solium* embryos penetrate the GI mucosa of the pig and are hematogenously disseminated to peripheral tissues with resultant formation of larval cysts (cysticerci). When undercooked pork is consumed, an intestinal tapeworm will again be formed in human, completing the life cycle of the worm.

Human cysticercosis occurs when *T. solium* eggs are ingested via fecal-oral transmission from a tapeworm carrier (3, 4). The human then becomes an accidental intermediate host, with development of cysticerci within organs. After ingestion of Taenia eggs containing infective oncospheres, the parasites become established in the tissues as larval cysts and reach their mature size in about 3 months. The parasite may locate almost anywhere in the body. The infection burden varies from a single lesion to several hundreds, and lesions may range in size from a few millimeters to several centimeters.

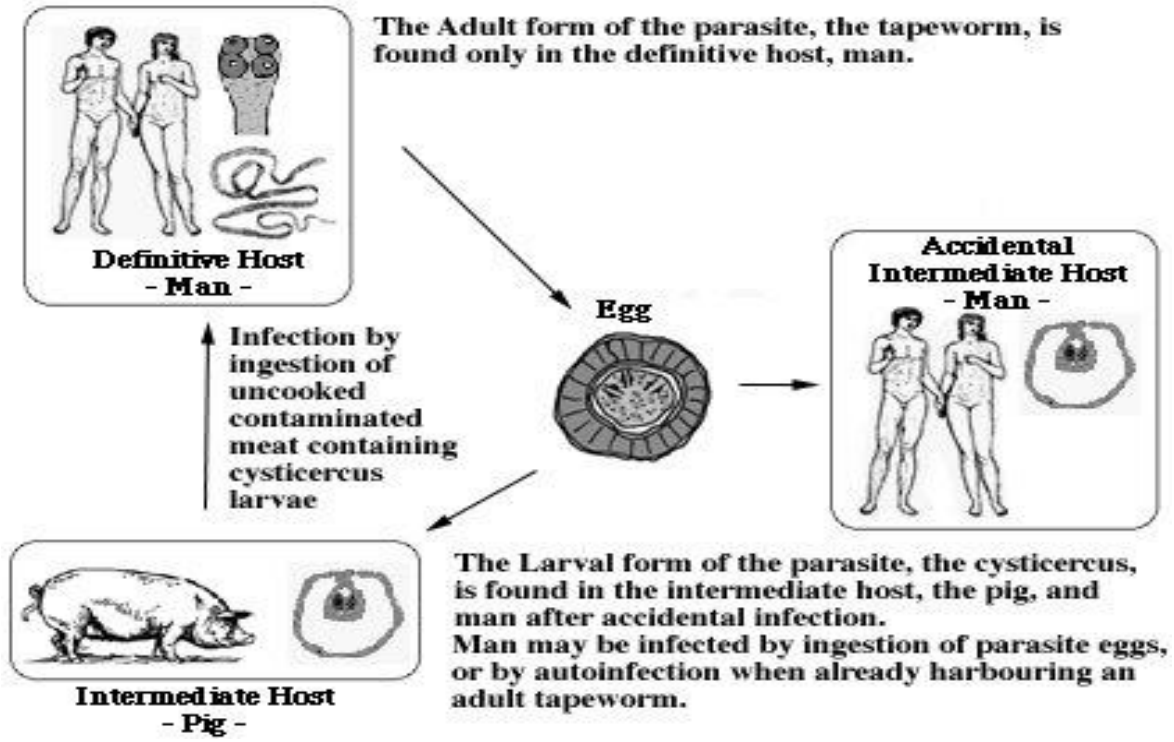

Fig. 3.1: Life cycle of *T. solium*

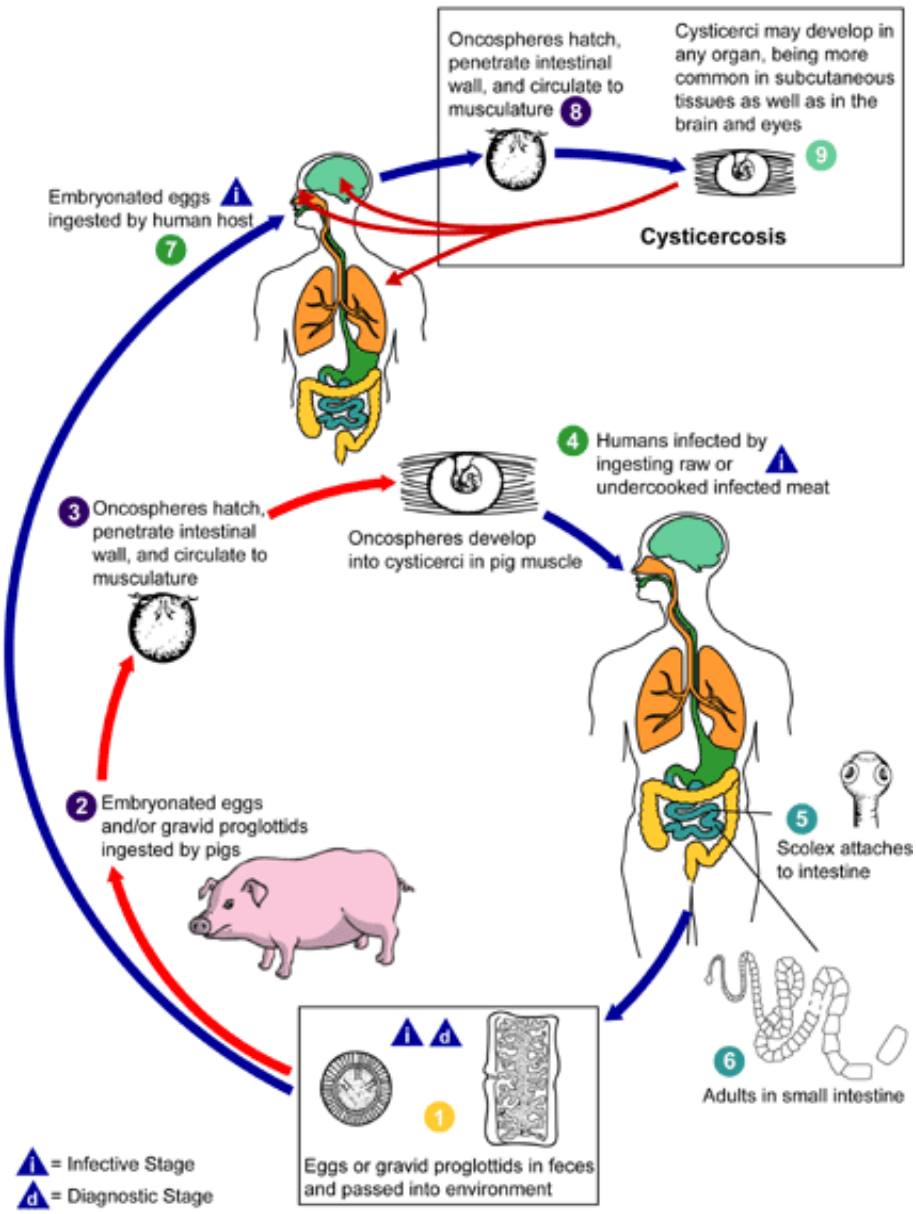

Fig. 3.2: Life cycle of *Taenia solium*

3.2.1 Taeniasis

Taeniasis occurs only in the human host, after ingestion of undercooked pork infected with cysticerci. The larvae evaginate in the small intestine; the head (scolex) attaches to the mucosa and begins forming segments (proglottids).

The frequency of autoinfection in individuals with taeniasis is not known. Dixon and Lipscomb noted that nearly 25% of patients with neurocysticercosis either had harboured or were harbouring a tapeworm. Up to 15% of patients harbour a tapeworm at the time of diagnosis of neurocysticercosis, and the proportion with tapeworms is directly related to the number of cerebral parasites, which strongly suggests autoinfection. In other cases the tapeworm carrier can be found in the patient's household (18)

3.2.2 Human cysticercosis

Cysticercosis is infection with the larval stage of the parasite. Human beings acquire cysticercosis through faecal-oral contamination with *T. solium* eggs from tapeworm carriers (3, 4). Thus, vegetarians and other people who do not eat pork can acquire cysticercosis (4). Water, wind, flies, and other indirect means of infection play little part in transmission.

The invasive oncospheres (embryos) in the eggs are liberated by the action of gastric acid and intestinal fluids and actively cross the bowel wall, enter the bloodstream, and are carried to the muscles and other tissues (6). At small terminal vessels, they establish and encyst as cysticerci, reaching their definitive size of about 1 cm in 2–3 months. Clinical manifestations depend on the affected organ; neurocysticercosis and ophthalmic cysticercosis are associated with substantial morbidity.

Cysticerci may be found in almost any tissue. The most frequently reported locations are skin, skeletal muscle, heart, eye, and CNS. Host inflammatory response to cysticerci depends on the parasite's ability to evade host immunity. Lack of inflammation occurs with both healthy cysticerci and those that have involuted, termed "active" and "inactive" disease, respectively. Inflammation is restricted to currently degenerating cysts whose ability to evade host defenses is faltering. Upon involution, cysts undergo granulomatous change and exhibit calcification. Cysts in various stages of viability can be seen simultaneously in one host.

3.2.3 Neurocysticercosis

The parasite commonly infects the central nervous system, causing neurocysticercosis, a pleiomorphic clinical disorder. After entering the central nervous system, cysticerci are viable and elicit few inflammatory changes in the surrounding tissues. Cysticerci may remain for a long time in this stage, protected by the blood-brain barrier (4, 9) and active immune-evasion mechanisms by the cysticerci. After a variable and unknown time (estimated to be several years) the parasite degenerates with associated immune-mediated inflammation. Cysticerci cause symptoms because of mass effect or by blocking the circulation of cerebrospinal fluid, but most symptoms in neurocysticercosis are the direct result of the inflammatory process that accompanies cyst degeneration. Clinical manifestations are thus related to individual differences in the number, size, and topography of lesions and in the severity of the host's immune response to the parasites (6). Symptoms and signs are varied and non-specific.

3.3 Pathogenesis

Cysticercus is a fluid filled sac which varies in size from 0.5 to 5 cm or more in diameter. It has got a wall composed of three layers; innermost is reticular layer, middle one is cellular layer and outer most is cuticular layer. Scolex is a structure which resembles adult *T. solium* and found in invaginated form inside the cysticercus sac. Scolex can be easily identified in biopsy or necropsy material.

3.3.1 Localization of parasites in the central nervous system

i. Parenchymal Neurocysticercosis
ii. Subarachnoid neurocysticecosis
iii. Ventricular neurocysticercosis
iv. Spinal cord neurocsticercosis

Brain parenchymal cysticerci are usually small cysts, single or multiple, that tend to lodge in areas of high vascular supply. The process of degeneration of parasitic cysts

involves a continual process that has been categorized by Escobar (1983) in four histopathological stages: 1) Vesicular, 2) Colloidal, 3) Nodular-granular and 4) Calcified.

The clinical stages of NCC namely are Active, Transitional and Inactive (27). Vesicular is active form, colloidal and granular nodular represent transitional stage while nodular calcified stage is inactive stage of NCC. CT and MRI findings of Parenchymal neurocsyticercosis depend on stages of development of the parasite within the nervous system:

3.3.2 Clinical staging of Neurocysticercosis

1) Vesicular (living) cysticerci-is a small and rounded low-density areas that are well demarcated from the surrounding brain parenchyma, these cysts lack perilesional edema and has minimal enhancement after contrast medium administration, which is due to little or no host response. At this stage, the scolex usually is identified as an eccentric nodule within the cyst.

2) Colloidal cysticerci-appear as ill defined lesion surrounded by edema. Most of them show a ring pattern of enhancing cyst without a well-defined scolex. This stage represent acute encephalitic phase of neurocysticercosis in which the host's immune system is actively reacting against the parasite.

3) Nodular-granular cysticerci-appear as nodular hyperdense lesion surrounded by edema and demonstrate contrast enhancement.

4) Calcified (dead) cysticerci- normally appear on CT as small hyperdense nodules without perilesional edema which is recognized as nonenhancing punctuate calcification on CT.

3.4 Clinical Manifestation

The clinical manifestations of neurocysticercosis are nonspecific and vary (9), depending on the number, localization, the individual immune response to the parasite, and developmental stage of the *T. solium* cysticerci in the CNS. The usual symptoms and signs are manifestations of three major patho-physiological processes as namely, the mass effect, the inflammatory response evoked by the parasite and the obstruction of the foramina and ventricular system of the brain. These symptoms include headache, hydrocephalus, chronic meningitis, or symptoms due to a space-occupying central nervous system (CNS) lesion (22). Isolated non-neurological manifestations, such as ocular or dermal cysts, account for <5% of cases of symptomatic disease (10). The interval from infection to onset of symptoms is lengthy.

The stage of cysticerci reflects the signs, symptoms and treatment of neurocysticercosis. In the case of **parenchymal neurocysticercosis**, four major stages have been classified: In stage 1, immature cysts appear within 1-4 weeks during which the oncosphere lodges to the brain and finally expands into a cyst. It is mainly asymptomatic, although flu-like illness, rare seizures, rare increased intracranial pressure from massive infestation has been recorded. In stage 2, the cysticerci become mature and viable about 2 months after egg ingestion. The cyst possesses a protoscolex with the cyst bladder and causes no or minimal surrounding inflammation or edema. The cysticerci also down-regulate host cellular immunity. Stage 2 cysts are also asymptomatic, and can persist for more than 10 years.

Stage 3 is typified by colloid or degenerating cysts with thick cystic fluid, thickened capsule, and appear two to 10+ years after the cyst becomes mature. The cyst no longer prevents a host immune response and its antigens leak from the bladder wall. The intense inflammation is provoked around the degenerating cyst. Most patients bearing stage 3 develop clinical signs and symptoms such as seizures, occasional focal neurological signs, headaches, nausea, vomiting, lethargy from increased intracranial pressure and altered mental status. At stage 4, the cyst is calcified. The surrounding

inflammation drops since the dead cyst no longer produces foreign antigens. Common clinical features include persistent non-provoked seizures although most of the patients are asymptomatic.

In a small number of patients, primarily children, parenchymal neurocysticercosis may presents with an encephalitis-like clinical presentation due to an uncontrolled inflammatory response to the numerous cysticerci.

In **meningeal cysticercosis**, cysticerci often do not develop into typical cysts, and become racemose, lacking a scolex and becoming lobes in thin-walled bladders. These cysts increase and slowly leak their antigen into the subarachnoid CSF producing meningitis and can further develop into arachnoiditis, which may lead to obstructive hydrocephalus, cranial nerve involvement, intracranial hypertension, arterial thrombosis and stroke. Cysticerci in the cerebral ventricles frequently cause obstructive hydrocephalus. The hydrocephalus can be caused by obstruction from the cysticercus or from associated ependymitis. Patients usually present with headaches, but may also develop symptoms of elevated intracranial pressure (nausea, vomiting, blurred vision, or dizziness) or altered mental status. Patients may also present with seizures from associated parenchymal cysticerci.

In **intraventricular cysticercosis**, the cysts occur in the lateral, third or fourth ventricles which may be asymptomatic or if they block the flow of CSF, they may cause increased intracranial pressure.

3.5 Epidemiology

This tapeworm is a public health problem in most developing countries where pigs are raised and pork is consumed and where poverty, illiteracy and deficient sanitary infrastructure are common (3, 31). Millions of persons are affected by *T. solium* taeniosis/cysticercosis in Latin America, Asia, and Africa where the disease is a factor in the extremely high prevalence rates of epilepsy.

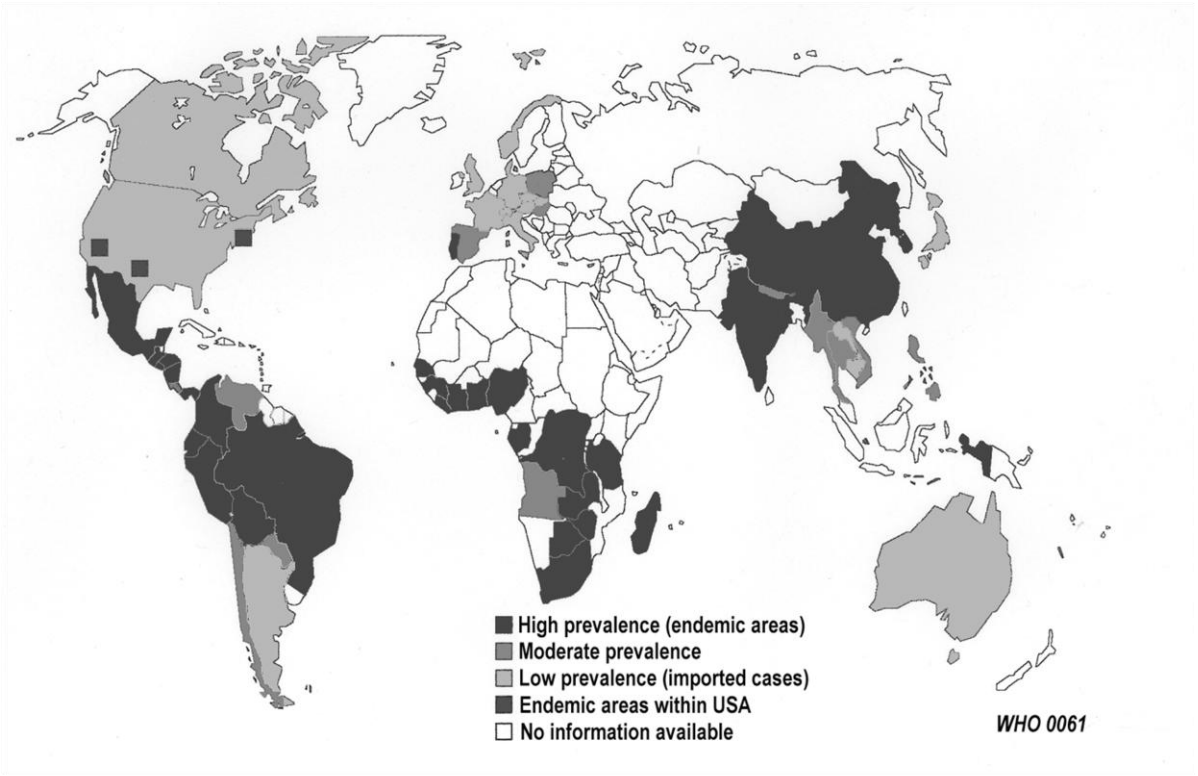

Fig. 3.3: Map showing areas where cysticercosis is endemic. Countries in black represent countries where cysticercosis is endemic; countries in grey represent those where cases have been reported.

According to the Commission on Tropical Diseases of the International League Against Epilepsy, the age-adjusted prevalence of active epilepsy in tropical countries ranges from 10 to 15 per 1000 inhabitants, almost twice the level in western countries (1, 23). Brain imaging has revealed that 50-70% of all patients with NCC present with seizures. NCC is the leading cause of the onset of epileptic seizures in persons aged over 25 years in countries where *T. solium* infection is endemic, and it is also an important cause of seizures in pediatric age groups (1, 24). WHO estimated that approximately 50 million persons, predominantly from developing countries, are infected with taeniasis, and 20 million people are infected with *T. solium* cysticerci and 50,000 people die of the disease each year (1). The disease is endemic in the Andean area of South America, Brazil, China, the Indian subcontinent, Mexico and Central America, Papau New Guinea, South-East Asia, and sub-Saharan Africa (2).

Human and porcine taeniasis/cysticercosis is reported to be among the major zoonotic diseases in Nepal (19, 20). Particular ethnic groups, which could comprise up to 25% of the population of Nepal, are pig farmers and pork consumers with very low hygienic and sanitation practices, and with no control of pig husbandry and slaughtering. Epilepsy cases in Nepal are increasing, with studies showing that up to 7.3 per 1,000 populations may suffer from epilepsy, and almost 50% of the cases are due to neurocysticercosis (25). Joshi *et al* (2001a) showed that the seroprevalence by ELISA and prevalence by lingual palpation was 23.5% (204 pig sera) and 32.5% (419 pig tongues), respectively.

The load of the disease is substantial in Nepal. An astonishingly high rate of taeniasis of 50% was reported from an area in Nepal populated mainly by pig-rearing farmers (17). The disease is one of the main causes of epileptic seizures, and accounts for as high as 50% of patients presenting with partial seizures (17)

3.6 Diagnosis

The consistent diagnostic criteria of the NCC is based in combined neuroimaging studies, immunodiagnostic technique, clinical presentation and exposure history (8). Diagnostic certainty of NCC is based on Computed Tomography (CT) findings, positive serology, clinical and epidemiological evidence suggestive of Neurocysticercosis (8). But the lab facilities are lacking in most of the health centers where the disease is endemic. Any study of NCC is limited by the difficulty in clearly establishing the diagnosis. The only true measure for the diagnosis of NCC is brain biopsy (26), which is clearly impractical. Therefore case definition is limited to CT scan finding and serology. Diagnosis is important for proper evaluation of the patient condition and appropriate treatment as the outcome with early treatment is very good

The diagnosis of neurocysticercosis is difficult because clinical manifestations are nonspecific, most neuroimaging findings are not pathognomonic, and some serologic tests have low sensitivity and specificity.

A set of diagnostic criteria was proposed in 1996 (15) and recently revisited (8), based on objective clinical, imaging, immunological, and epidemiological data; these criteria consist of four categories that are stratified according to their diagnostic strength (Appendix 1). These criteria provide two degrees of diagnostic certainty: definitive diagnosis, in patients who have one absolute criterion or in those who have two major plus one minor and one epidemiologic criteria; and probable diagnosis, in patients who have one major plus two minor criteria, in those who have one Major plus one minor and one epidemiologic criteria, and in those who have three minor plus one epidemiologic criteria. This chart of diagnostic criteria for neurocysticercosis has not yet been tested in hospital-based studies.

3.7 Diagnostic studies

Confirming the diagnosis in neurocysticercosis is always challenging due to wide variation in clinical presentation and limited availability of the tests in areas where the disease is endemic. Commonly used tests include neuroimaging studies, serology, and biopsy in tissue cysticercosis and stool examination to identify the carrier stages of the parasite.

3.7.1 Neuroimaging

Neuroimaging is the first diagnostic tool to be used in any of the suspected cases of neurocysticercosis. It includes either computed tomography (CT) scan or Magnetic Resonance Imaging (MRI). Even soft tissue plain X-ray may show calcification of inactive cysts, which appear as oblong-shaped lesions. But it only detects the calcified stage of the cysts and has low sensitivity and specificity. Neuroimaging studies helps in depicting active, transitional and inactive lesion. It also helps in clinical staging of the cyst in parenchymal neurocysticercosis (32). Neuroimaging technique such as CT scan have attributed to more accurate diagnosis and better understanding of pathophysiology. However, only the presence of cystic lesion showing the scolex is considered pathognomonic (8).

i. Brain CT scan

CT scan is the recommended first imaging study to be done in any suspected neurocysticercosis. It is widely available, less expensive and easy to perform. Both plane CT scan and with contrast administration is done for contrast and non-contrast studies. Non-contrast study may show focal areas of edema, cystic lesion, or calcification. Contrast study may show non-enhancing cystic lesion with or without edema or ring enhancement signifying inflammation surrounding the cyst. CT scan has been claimed to have sensitivity and specificity of 95 % for diagnosing NCC, although CT images are rarely pathognomonic for this disease. Computerized tomography (CT) indicates structural disease but misses brain stem and intraventricular neurocysticercosis lesions and, if not performed with enhancement, misses those that are isodense

ii. MRI of Brain

MRI is recommended as an adjunctive diagnostic tool to CT scan. MRI may show a mural nodule within the cyst representing larval scolex and also may show cysticerci within the ventricular system, which are often missed by CT scan.

3.7.2 Serology

It is the most useful of lab tests. Sensitivity of serology is directly linked to number of parasite lesions and the stage of the lesion. Single lesion and calcification are more likely to be associated with a false-negative assay result. False-positive results may be caused by other parasitic infections. The detection of specific antibody against *T. solium* cysticercal antigen has been considered important diagnostic element for NCC especially when neuroimaging technique are unavailable or inconclusive. Immunodiagnostic techniques include detection methods for specific antibody and for

circulation parasite antigen in serum and cerebrospinal fluid. Infection with *T. solium* results in specific antibody response mainly IgG class. Different techniques have been described to detect antibody to *T. solium*. The most specific test is Enzyme Linked Immunoelectro-Transfer Blot (EITB). However in developing countries ELISA (Enzyme linked Immuno-sorbent Assay) is preferred because of better availability, simplicity and lower cost compared with EITB (12). ELISA measures specific IgG antibody to partially purified antigen from cysticercal fluid, serum sensitivity of 75-87% and specificity of 75% has been reported (12). Analysis of antibody response indicated that the optimum threshold titers for seropositivity were 1:800 for the ELISA. When used with these thresholds, the ELISA gave a sensitivity, specificity, positive and negative predictive values and diagnostic efficacy of 89%, 81%, 79%, 90%, 85%, respectively (13). Serologic test can be very useful for confirmation of neuroimaging finding for differential diagnosis of other cyst forming condition (14, 15).

3.7.3 Stool Examination

Many patients have simultaneous intestinal tapeworm infestation. *Taenia* carriers are potent source of NCC, endangering everyone coming into contact with them (1). Garcia et al (16) demonstrated that tapeworm carriers are at high risk of developing massive brain infection with viable *T. solium* cysticerci. 25% of the patients with cerebral cyst were found to harbor adult *T. solium* in their intestine or have history of such infection. Detecting ova in children with NCC is worthwhile as a public health measure to prevent further exposure and to implement preventing measure to control faecal-oral transmission. Stool examination to detect ova/parasite is done either by simple method or by implementing concentration method to increase the sensitivity.

3.8 Review of previous studies

According to the Commission on Tropical Disease of the International League against Epilepsy, the age adjusted prevalence of active epilepsy in tropical countries range from 10 to 15 per 1000 population, almost twice the level in western countries. Brain imaging has revealed that 50-70% of all patients with NCC presents with seizures (28).

In the Cysticercosis Surveillance Program in Los Angeles Country from 1988-1990, 138 cases were reported which represent annual incidence of 0.6 per 100,000 population. Hispanics, mostly Mexican immigrants had the highest rate (1.6 per 100,000). In 7% of the cases, tapeworm carrier was identified.

Study done in Peru and Mexico (7) demonstrated seroprevalence ranging from 8-12% and confirmed that NCC is the major cause of neurologic morbidity in those countries. According to A. Carpio (9) Immunological assay detect positivity for human cysticercosis in 8-12% of people in some endemic region which indicate the presence of antibody against the parasite but not necessarily active or CNS infection, at such instances, CT/MRI is the reliable tool.

In a series of 991 patients with simple partial seizure in South India 40% of patients were found to have either active NCC, calcification on CT scan consistent with prior cysticercosis or single enhancing lesion (27). In a separate study in Honduras reported in 1999 showed that when a diagnosis of NCC had been made, seizures were the presenting symptoms in 52% of cases.

Serology sensitivity in cases with multiple cysts was 94% and of only 28% was found in cases with single cyst in brain (29). Serological tests are helpful for confirmation of imaging technique for differential diagnosis of other cyst forming condition like echinococcosis, brain tumor, tuberculoma etc. (14, 15)

Garcia et al 2003 (4) reported that upto 6% of the population in endemic village may harbor adult *T. solium* tapeworm at a given time. In most endemic areas more than

10% of general population are seropositive and the proportion can reach 25%. In population based studies 10-18% of asymptomatic individual have CT features that suggest NCC, mainly brain calcification in seronegative individual.

NCC is the leading cause of epileptic seizure in pediatric and adult age group in endemic areas (2, 5). NCC is being diagnosed with increasing frequency in developing countries because of increased migration of people with the disease (30) or tapeworm carriers and because of tourism and travel to endemic areas. In most endemic areas, more than 10% of general population are seropositive (9) and the proportion can reach 25% indicating high prevalence of the parasite infection (1). In population based studies, 10-18% of asymptomatic individual have CT features that suggest NCC mainly brain calcification. Recent epidemiological evidence suggests that most common source of infective eggs is a symptoms free tapeworm carrier in the household (5).

Rates of taeniasis as determined by stool examination for ova have been reported to range between 0.1-6 percent in endemic countries (17).

In context of Nepal, although periodic deworming is practiced in under 5 children, the disease is endemic because of lack of proper sanitary measures, unavailable of clean and safe water, traditional pig-rearing technique, lack of health education, and also the contributing factors are inaccessibility to proper health care, lack of awareness by the medical community and difference in quality and availability of services. Rate of taeniasis of 50% was reported from an area in Nepal populated by pig rearing farmers (17). Of the 1026 CT scans done in paediatric department of Manipal Teaching Hospital from Nov 2000 to January 2010, 190 (18.5%) were diagnosed with NCC based on neuroimaging. Almost all the diagnosed cases presented with seizure disorder.

Very little studies have been conducted in Nepal, therefore adequate data regarding prevalence, morbidity and mortality are lacking.

According to study done by Basu et al (21) in western Nepal, 124 cases of NCC were diagnosed in period of 4 years (2000-2003) and 47.6% of these cases were carriers of Taenia species on stool examination. The incidence of admitted NCC patient was highly significant ($P<0.001$) among the hospital. The prevalence of human taeniosis in the Sarki and Magar communities of Syanja district was found 47.7% (Gaire, 2000). Out of total 23,402 general surgery specimens, 0.26% were diagnosed as cysticercosis by histopathology in Patan Hospital, Lalitpur (Amatya and Kimula, 1999). A research conducted at Model Hospital in Kathmandu showed 73% of epileptic patients were neurocysticercosis (NCC) cases (Dhakal et al., 2005). However more investigations are required to determine the prevalence and the importance of *T. solium* cysticercosis in Nepal.

CHAPTER-IV

RESEARCH METHODOLOGY

4.1 Research Design

The design of the research is exploratory and descriptive and also analytical. It is based on qualitative questions, the laboratory data and imaging studies.

4.2 Study Variables

Neurocysticercosis cases are identified on the basis of classification scheme proposed by Del Brutto, (8) Table 1. Following is the list of socio-demographic and other descriptive data and also the analytical data of each case that are included in the study.

a) Patient's characteristics: Age, sex, education, occupation, family history, geographical area.
b) Lifestyles: dietary habits, personal hygiene, sanitary condition.
c) Medical history: associated illness, history of intestinal parasites infection, prior seizure history.
d) Neuroimaging findings, lab results (serum ELISA for anti-cysticercal antibody), routine blood examination and stool routine and microscopy to detect cyst/ova of parasite.

4.3 Type of Study

Cross-sectional analytical study

4.4 Study Site and its Justification

The study site is Manipal Teaching Hospital (MTH), Pokhara. It is the tertiary care centre in the western region and most of the seizure case, the possible etiology of which could not be established and the seizure disorder which could not be managed in other centers are referred to this hospital.

4.5 Target Population

Pediatric population of age group from 1year to 15 years of age.

4.6 Sampling Methods

Non-probability Sampling. The patients of pediatric age group (age 1 to 15 years) who visit to the pediatric department either as an out-patient or in-patient are included in the study by the order of presentation to the hospital. The cases are the suspected cases of neurocysticercosis based on clinical presentation, epidemiological evidence and positive neuroimaging findings. The patients who do not meet these criteria are excluded from the study. Newly diagnosed cases are only included in the study. The controls are matched hospitalized population with the diagnosis unrelated to neurocysticercosis. And without intestinal parasitic infestation. Control sample who is found to have intestinal parasite on stool examination are excluded from the study.

4.7 Sample Size

Total sample size of 200 (100 cases and 100 controls)

i. 100 cases of Neurocysticercosis (case definition based on clinical presentation, epidemiological evidence and positive neuroimaging finding)
ii. 100 matched hospitalized controls with a diagnosis unrelated to Neurocysticercosis. (50 with seizure disorder and 50 without seizure)

4.8 Sampling Frame and Sampling Process including Criteria for Sample Selection

Patients (aged 1 to 15 years) attending to paediatric out-patient-department and those admitted in pediatric ward of Manipal Teaching Hospital (MTH) are enrolled in the study. The participants are included in the study by the order of presentation to the hospital (non-random sampling) and no restriction made as to the clinical form or

stage of infection. Our case definition for the NCC is based upon the clinical findings, epidemiological history and the diagnosis being supported by the positive neuroimaging findings (calcification or cystic lesion consistent with neurocysticercosis).

Controls were matched hospitalized patients of age group1year to 15 years with a diagnosis unrelated to NCC. Controls are without epidemiological or clinical history for taeniasis / cysticercosis. Controls are grouped as; diagnosis unrelated to NCC but with seizure and those without seizure at presentation.

4.9 Tools and Techniques for Data Collection

After recruitment of the participants, an informed oral consent is taken and details of demographic data, relevant medical history and examination findings were recorded in pre-designed Proforma.

4.9.1 Neuroimaging (CT scan)

It is done by the patient himself as a part of investigation, as it is done in all seizure cases. CT scan is done only in the suspected NCC cases but not in the controls. CT scan is done both plane and with administration of contrast medium. Radiologist read the CT scan without regard for or knowledge of the study. The presence or absence of finding relevant to the study from the radiology reports was then derived.

4.9.2 Serology (ELISA)

Blood sample is taken from all the participants (cases and controls) after being used for routine diagnostic procedure; the remaining volume (5 ml) of blood is kept undisturbed for about 2-3 hours at room temperature, the serum is separated and preserved at -20^0C until use. Each bottle is given a specific code and when 30 samples are collected, the serology is performed. The serum from each patient is tested for cysticercal antibody by Enzyme-Linked-Immunosorbent-Assay (ELISA). The ELISA

kit used is UBI MAGIWELL™ CYSTICERCOSIS for detection of IgG anti-cysticercal antibody.

4.9.2.1 Principal of procedure

This Cysticercosis test kit is a solid phase enzyme linked immunosorbent system employing plastic wells coated with *Taenia solium* antigens. Incubation of serum samples in the coated wells results in the binding of anti-*Taenia solium* antibodies to the immobilized antigens. Subsequent addition of the enzyme conjugate, comprised of horseradish peroxidase, results in the immobilization of peroxidase in direct proportion to amount of *Taenia solium* antibody present in the serum sample. Unbound enzyme conjugate is washed from the wells and a substrate and chromogen solution is added. The intensity of the color formed as a result of enzyme activity is a direct measure of the anti-*Taenia solium* antibody present in the serum samples and may be quantified by use of a photometric wells reader at 450 nm wavelength.

4.9.2.2 Specimen collection and handling

Blood is collected by venipuncture. It is then allowed to clot and the serum is separated by centrifugation at room temperature. The sera are then stored at -20^{0}C for at least three months. When required samples are obtained then all the reagents and samples are brought to room temperature (20-25^{0}C) and mix gently before beginning the test.

4.9.2.3 ELISA Reading:

OD of Blank =0.023

OD of negative control=0.024

OD of high positive control = 1.939

OD of low positive control = 0.641

Absorbance reading at 450 nm by Humareader

4.9.2.4 Interpretation of results

Specimens yielding absorbance readings greater than Low Positive at 450 nm is reported as positive for antibodies against *Taenia solium*. Absorbances of less than Low Positive are found with specimens having no prior immunological experience with *Taenia solium*.

4.9.3 Stool Examination

The stool sample is collected in multipurpose container from all the participants (200) both cases and controls on the day of admission before any anti-parasitic drugs are given. The sample is sent to Microbiology lab in MTH for routine stool examination for detection of cyst/ova of intestinal parasite.

Procedure of stool processing: formalin ether concentration method is used for stool processing to detect intestinal parasite

CHAPTER-V

RESULTS

The study was conducted at Manipal Teaching Hospital, a tertiary care centre at Western Region, Nepal. During the study period from January 2008 to January 2010, a total of 200 samples of serum for serology and Stool for microscopy was collected and microbiologically processed from hospitalized and OPD patients visiting the Paediatric department of Manipal Teaching Hospital, Pokhara.

Out of 200 samples of serum, 100 were from the cases with diagnosis of Neurocysticercosis. Case definition for NCC is based on classification scheme proposed by Del Brutto, based on clinical findings, CT scan findings and Epidemiologic evidence suggestive of NCC. The other 100 serum samples were from the matched hospitalized control, with a diagnosis unrelated to NCC and without intestinal parasites.

Most of the patients belong to low and middle socio-economic groups. Sanitation in terms of human disposal, environmental cleanliness around the house according to the interview was below standard in most cases (30%). Almost all had mixed dietery habits i.e. consumed both vegetables and meat (goat and pork). All the cases consumed one or other raw foods (vegetables) grown in their field. No documented history of parasitic infestation was seen in any of the cases.

Pig rearing was seen in 26% of the cases. And 30% (8) of pig rearing children had harbor taenia species in intestine.

5.1 Demographic profiles

Out of 100 cases of NCC, 64% of the samples were in the age group of 8 to 14 years. No cases were identified below 2 years. There is a steady rise in number of cases with the age. 24% of the cases occurred in age group 10-12. Mean age of presentation was 10.92 (SD=2.9). The most commonly affected age group was 8 to 12 years.

Table 5.1: Distribution of cases according to age group

Age group in year	Patients(N=100)	Percent
<2	0	0.0
2-4	2	2.0
4-6	6	6.0
6-8	14	14.0
8-10	20	20.0
10-12	24	24.0
12-14	20	20.0
>15	14	14.0
Total	**100**	**100.0**

Table 5.2: Distribution of cases according to Sex

Gender	Frequency	Percent
Female	40	40.0
Male	60	60.0
Total	100	100.0

60% of the cases were male. Male to female ratio was 1.5.

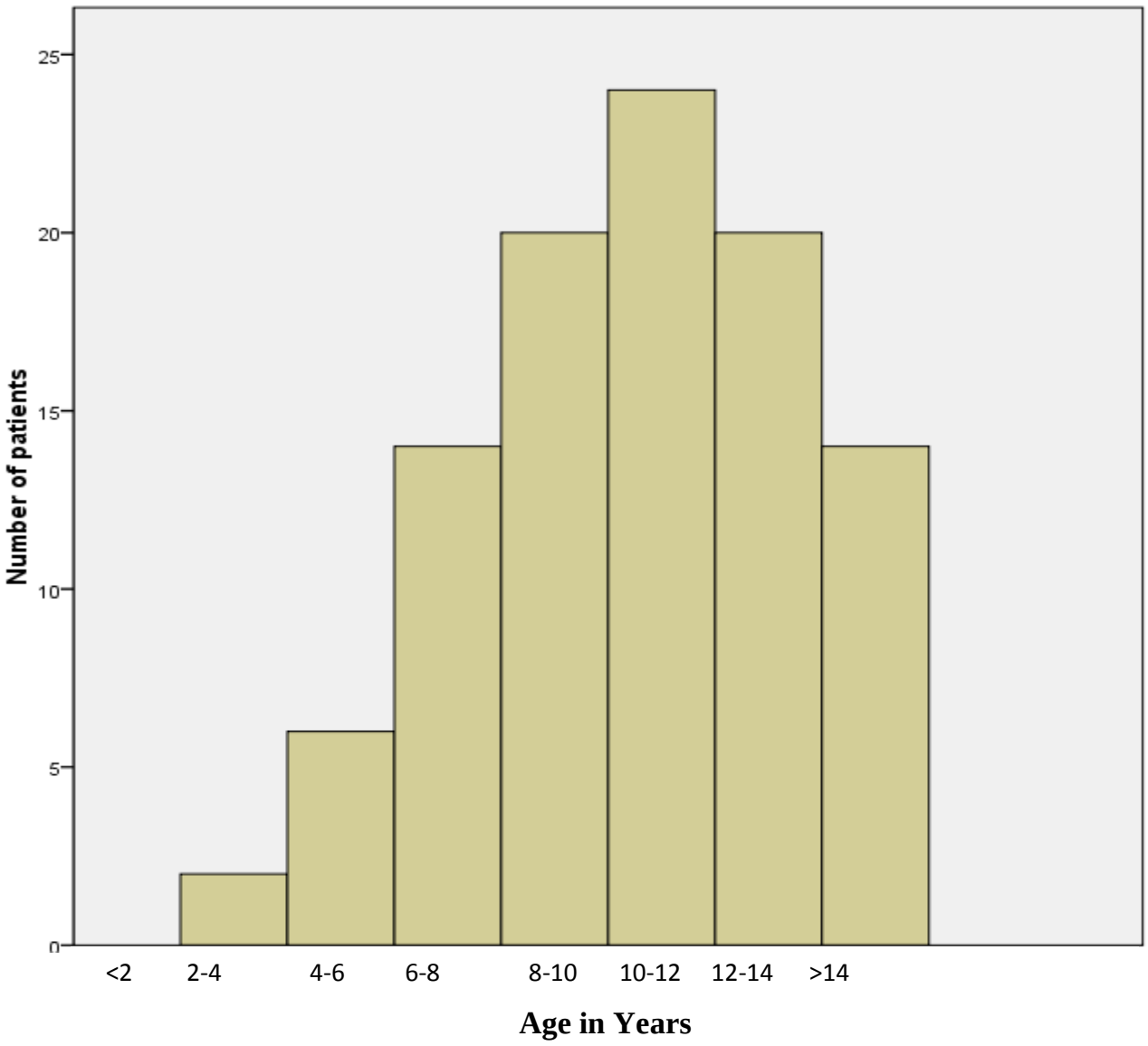

Fig 5.1: Distribution of NCC cases according to age groups

5.2 Clinical Presentation in Cases of NCC.

The most common presenting symptoms was Seizure (68%). simple partial seizure being most common (50%). Other types of seizure were complex partial, Generalized and status epilepticus. Number of seizure episodes varied from single to as many as six. Features of raised intracranial tension (headache with or without vomiting) was seen in 30% cases. Other presenting symptoms include neuropsychiatric manifestation like learning disabilities, behavioural abnormality (12%). Behavioural abnormalities included abnormal speech, emotional instability, aggressive behavior, language problem and other psychotic features. Other symptoms like focal weakness (paresis), unexplained sudden loss of consciousness in 8%. In 24% of the cases more than one presenting symptoms (combination of symptoms) was present.

Table 5.3: Presenting Symptoms of Neurocysticercosis patients

Symptoms	**N(=100)**
Seizures	68
Simple partial	34(50%)
Complex partial	12
Generalized	20
Status epilepticus	2
Features of raised intracranial tension(headache+/- vomiting)	30
Focal neurologic deficit	12
Neuropsychiatric manifestation	12
Others symptoms	8
Combination of symptoms	24

The duration of symptoms varied from few minutes to days and months. Acute symptoms were present for seizure disorder. Seizure occurred as a single episode, or in frequent intervals or in status. Whereas neuropsychiatric and behavioural symptoms were usually of long durations.

5.3 Isolation of Intestinal parasites from the stool samples

Stool microscopy of all the cases of NCC was performed. Concentration method of stool processing was done to avoid false negative results. A single morning stool sample was collected before giving any antihelmethics. Intestinal parasites were detected from Stool microscopy in 36% of the cases. Of these majority 38.9% (14/36) harbor Taenia species. Other parasites include Giardia, Ascaris, Hookworm, Trichuris, H. nana, and Cryptosporidium. Few of the cases had more than one parasites in stool examination.

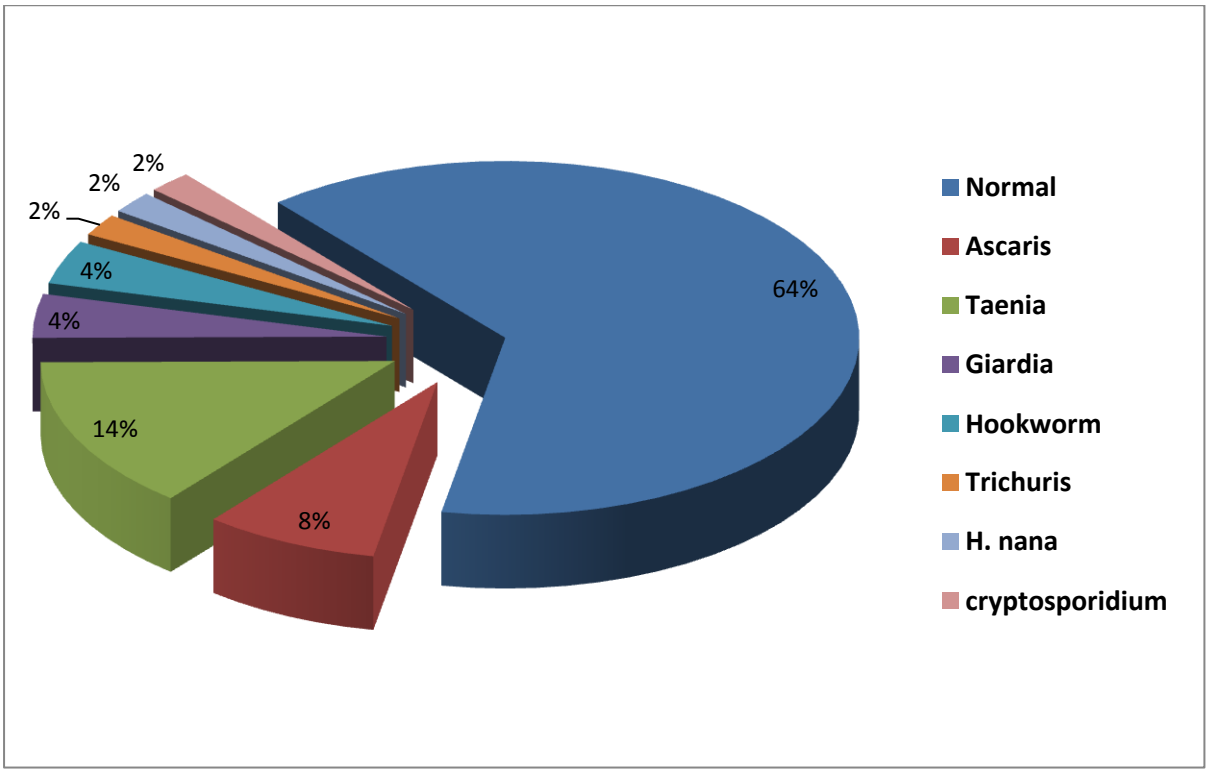

Fig 5.2: Stool microscopy findings in patients with Neurocysticercosis

5.4 Intestinal parasites in different age groups

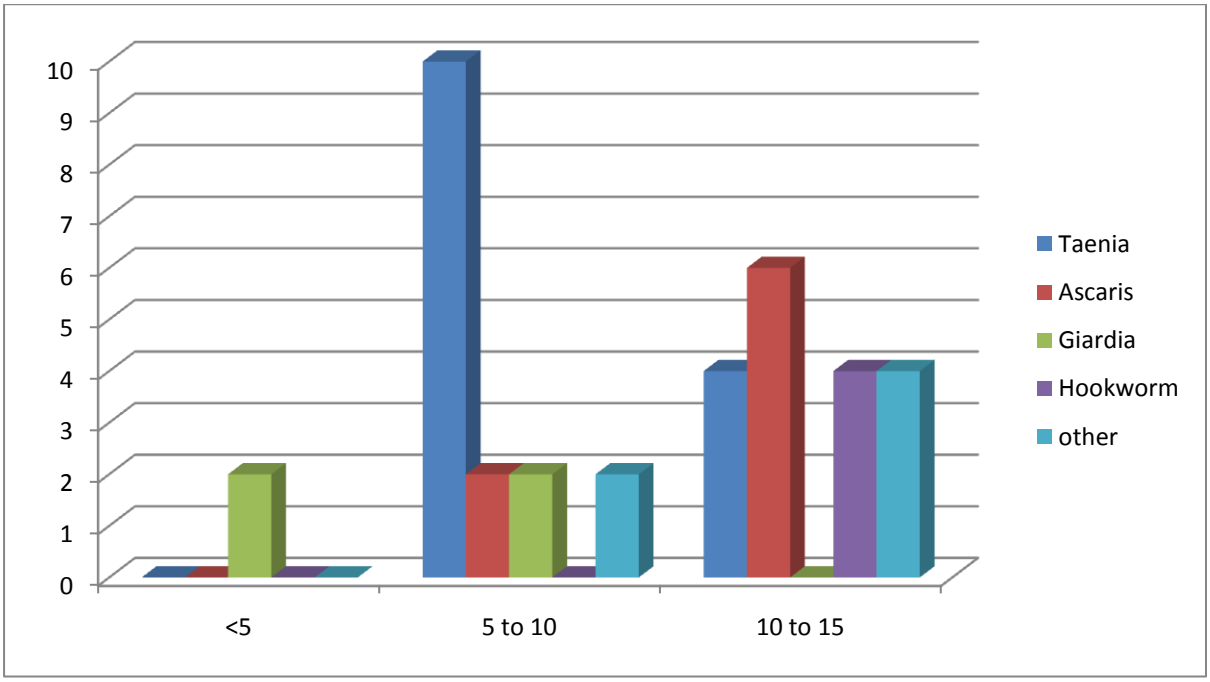

Fig. 5.3: Age-wise distribution of intestinal parasite

Different types of intestinal parasitic infestation were identified in the cases by stool examination. Parasites were commonly found in child aged >5 years. Taenia species was the commonest among the age group 5 to 10 years. Whereas age group 10-15 had mixed parasitic infestations with almost equal occurrence. Intestinal worms were not seen in under-5 child, though Giardia was identified in this age group.

5.5 Computed Tomography features of Neurocysticercosis

Plain and contrast enhanced computed tomography was done in all the cases. Abnormal neuroimaging was seen in 100% of the cases whereas confirmation of diagnosis by neuroimaging alone could be made only in 38% of the cases based on diagnostic criteria. Single ring enhancing lesion was the most common finding (58%) in CT scan. The most common site of occurrence of the lesion within the brain was

Parietal in 46% followed by frontal in 26%, occipital 12%, temporal 10%. The other sites were cerebellar and intraventricular. Maximum diameter of lesions in CT scan was 22mm.

Of these 40% of the lesions were active which showed well demarcated cyst with minimal ring enhancement on contrast CT scan. Transitional lesion was seen in 44% of the cases which showed ill defined cyst with marked ring enhancement. These cysts both active and transitional usually contain scolex within them. Scolex was positively identified in 38% of the all cases. Perilesional edema was present in 77%. Inactive calcified lesion was present in 4% of the cases.

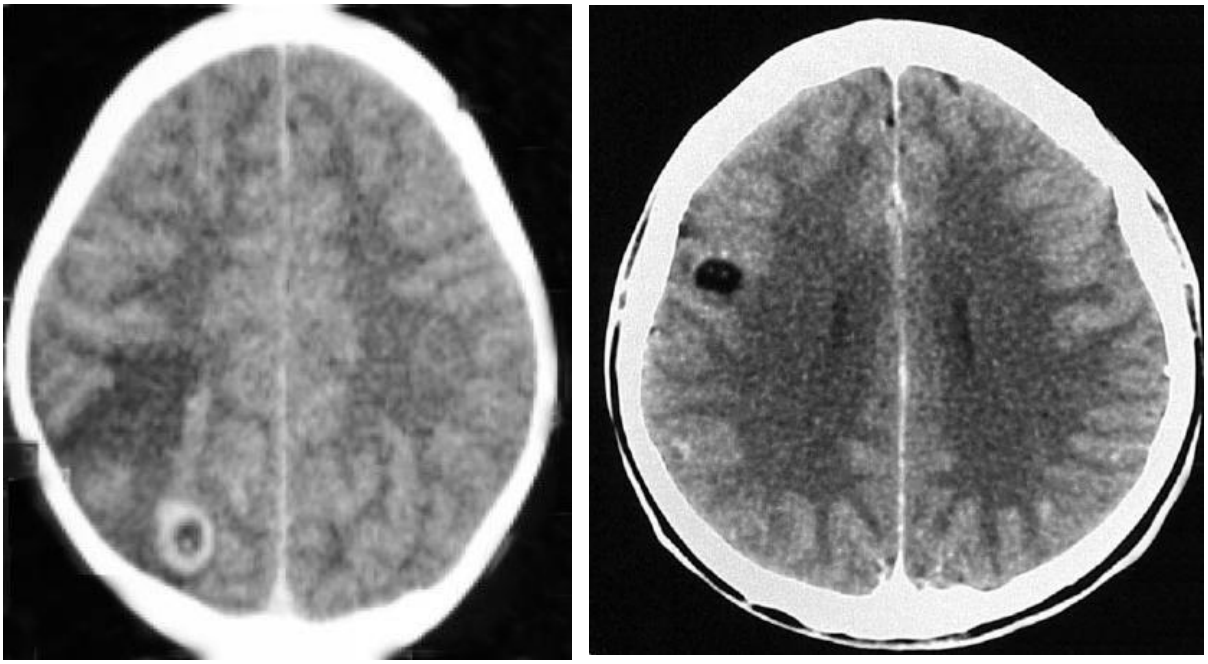

Fig 5.4: Single nodular ring enhancing lesion on Contrast enhanced CT scan (Central dot represents scolex)

Table 5.4: Number of Parenchymal lesions on CT scan Head

Number of Lesions		**N (100)**
	Single	58
	Multiple	42

Table 5.5: Distribution of Intracranial cysticercus according to CT scan

Site of Lesion		**N (100)**
	Parietal	46
	Frontal	26
	Occipital	12
	Temporal	10
	Cerebellar	4
	Intraventricular	2

Table 5.6: Stages/Nature of Parenchymal neurocysticercosis on CT scan

Nature of Lesions	**N (100)**
Transitional	44
Active	40
Mixed	12
Inactive	4

5.6 Comparision of ELISA in cases and controls

Cysticercus antibody was tested by ELISA in the sera of all the cases of NCC and controls. ELISA detected antibodies in 87% cases of NCC. Control population showed ELISA positivity in 16% of the samples. The ELISA sensitivity was 87% and Specificity 84%. Positive predictive value of 84.47% and Negative predictive value of 86.6%.

ELISA detected antibody in 96.3% (52/54) of CT scan confirmed cases. Other cases with CT scan features suggestive of NCC showed ELISA positivity in 73.9% (34/46).

The performance of ELISA also depended on number and type of lesion. ELISA had higher sensitivity in cases with multiple lesions compared to those with single cyst. And also higher sensitivity in cases with active lesions.

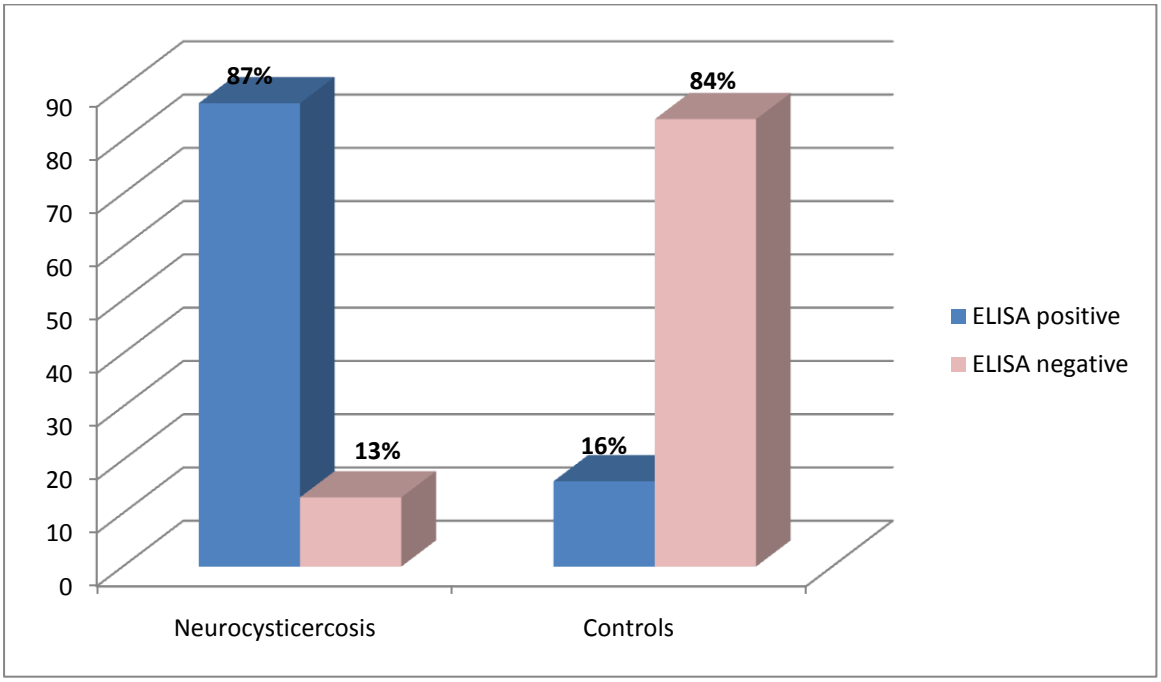

Fig 5.5: ELISA findings in Cases and Controls

Table 5.7: Relationship between ELISA and number of lesion on CT scan

		Number of Lesions		Total
		Multiple	**Single**	
ELISA	**Positive**	40	47	87
	Negative	2	11	13
Total		42	58	100

The sensitivity of ELISA for serum anti-cysticercus antibodies in cases of NCC with multiple parenchymal lesions was 95.24%, showing strong positivity in contrast to NCC with single parenchymal lesion, with sensitivity of 81.03%. Comparative studies of the sensitivity of ELISA between NCC cases with single and multiple parenchymal lesions were significant ($p<0.05$).

5.7 Relationship between ELISA and other parameters

Table 5.8: Relationship between Nature of Lesion on CT scan and ELISA

		ELISA		Total
		Positive	negative	
Nature of Lesions	Active	39	1	40
	Inactive	1	3	4
	Mixed	12	0	12
	Transitional	35	9	44
Total		87	13	100

The sensitivity of ELISA is higher in active lesions (97.5%) as compared to inactive and transitional lesions.

CHAPTER-VI

DISCUSSION

Cysticercosis, the infection caused by the larval stage of the tapeworm *Taenia solium*, is the most common parasitic disease of the nervous system in humans and the single most common cause of acquired epileptic seizures in the developing world, where prevalence rates of active epilepsy are twice those in developed countries. Millions of persons are affected by *T. solium* taeniosis/cysticercosis in Latin America, Asia, and Africa where the disease is a factor in the extremely high prevalence rates of epilepsy. Countries where *T. solium* infection is endemic, it is also an important cause of seizures in paediatric age groups.

The diagnosis of neurocysticercosis is based on clinical, epidemiologic, laboratorial, and neuroimaging data. The clinical manifestations of neurocysticercosis are nonspecific and vary, depending on the number, localization, and developmental stage of the *T. solium* cysticerci in the CNS. Neuroimaging studies are usually abnormal but, in most cases, not pathognomonic. Serologic tests have been developed to support the diagnosis. However, older tests had low specificity and current assays have decreased sensitivity in patients with single lesions.

Taenia solium, is becoming an increasing problem in Nepal with high prevalence of porcine cysticercosis and human taeniasis/cysticercosis detected in epidemiological studies undertaken in different parts of the country. It is one of the commonest cause of neurologic morbidity in countries like Nepal where the parasite *Taenia solium* is endemic.

In patients with NCC, clinical manifestations and the results of neuroimaging procedures vary widely and often do not facilitate a definite diagnosis. In clinical practice, absolute criteria are not available in majority of cases, and a probable diagnosis must depend on different, more indirect approaches with special emphasis on the recognition of a wide variety of clinical manifestations and neuroimaging

results. The availability of noninvasive diagnostic confirmation in cases of suggestive clinical and imaging data has become even more important. Antibody detection by various test procedures such as Enzyme-linked immunosorbent assay has been used with variable results.

Diagnosis of neurocysticercosis till date in Nepal is mainly focused on Imaging technique (CT/MRI). But it is rarely confirmatory. And also most of the health facilities lack these imaging techniques. Serologic test, EITB has higher sensitivity and specificity in diagnosing and confirming the Disease, but still this test is costly and cannot be performed in all the health facilities. But appropriate utilization of ELISA/dot-ELISA is a useful alternative in diagnosing cysticercosis in rural settings also. This can also be applied to any cases where CT scan is inconclusive. Stool examination to identify intestinal parasites in any cases with cysticercosis is important from the public health point of view. Screening every cases of NCC with stool microscopy and treating accordingly helps in reducing the burden of Taeniasis and ultimately the burden of cysticercosis. The most common source of transmission of cysticercosis is an intestinal carrier.

Very little study has been done in Nepal to identify the sensitivity of the diagnostic tests and comparative evaluations. In this study attempt was made to identify seroprevalence of cysticercosis and comparative study on diagnostic tools like neuroimaging, ELISA and stool microscopy. The present cross-sectional study was carried out during a 2 year period from Jan 2008 through Jan 2010 at Manipal Teaching Hospital, Pokhara.

Distribution of NCC

The study was conducted in age group less than 15 years, focusing mainly on paediatric population. During the study period, total of 100 cases of neurocysticercosis and 100 controls were included in the study. Among the 100 cases of NCC, 42% of the cases were from admitted and 58% from outpatients.

The disease was common in age group 10 to 12 years. This age group accounted for 24% of the cases. The frequency of disease increase steadily with advancing age i.e. higher incidence was seen in adolescent than at preschool age, and school age. Current study showed less than 10% incidence of NCC in preschool-age children. Nearly 80% of children were older than 7 years. There were no patients younger than 2 years. And among all the cases, 60% of the cases were male while 40% were female. Thus male:female ratio was 1.5. The findings from this study correlates with the finding of Basu et al (21), Thakur et al (33) and Shrestha BM (34) where they showed a higher incidence in late childhood. Lower incidence of NCC in the preschool-age children is because of the prolonged incubation period (as long as 5 years) of *T. solium* as well as the nutritional habits of young children's.

Clinical presentations of the disease

68% of the cases presented with seizures, mostly focal in nature (50%). This finding is similar to those reported by other studies (4,21,22,34,35). Solitary parenchymal lesions mostly produce focal seizures and focal neurological deficit (4,9,10)). Headache and vomiting, which may be the features of raised intracranial pressure, were present in 30% of cases in the current study. Similar presentations were seen in other studies (4,21, 34). More than one symptom at presentations was present in 24 % of the cases and few cases 12% had focal neurological deficit which was transient and these deficit improved completely within 24 hours. The frequency and durations of symptoms varied widely among the patient, this may be due to lack of awareness by the parents, lack of adequate medical facilities and lack of awareness by the paramedics at rural areas.

Cysticerci cause symptoms because of mass effect or by blocking the circulation of cerebrospinal fluid, but most symptoms in neurocysticercosis are the direct result of the inflammatory process that accompanies cyst degeneration. Clinical manifestations are thus related to individual differences in the number, size, and topography of lesions and in the severity of the host's immune response to the parasites (4,6) Symptoms and signs are varied and non-specific.

Routine blood examinations in this study showed Eosoniphilia in 22% of the cases. But this tests is neither sensitive nor predictive of the diagnosis of NCC, however it may indicate the ongoing parasitic infestation.

Neuroimaging (CT) findings

Neuroimaging (CT scan) was abnormal in 100% of the cases. Single parenchymal lesion was the most common findings. Diagnosis could be confirmed based on CT scan only in 38% of the cases. Other 62% showed some abnormality but not were pathognomonic of NCC. In this study maximum lesions were seen in parietal region 46%, followed by frontal 26%, occipital 12% and temporal 10%. This is in accordance with Basu et al (21). Imaging studies showed single parenchymal ring enhancing lesion in 58% of cases, with perilesional edema in 77% of the cases and calcified lesion in 4%. This finding was in contrast with the finding from similar study done by Shrestha BM (34), which showed 84 % of cases with single ring enhancing lesion and 90% with perilesional edema. Similar study done by Joshi DD (36) showed 77.2% case with seizure and single ring enhancing lesion in 63.6% of cases. And in their study parietal region was the commonest site of occurrence of the lesion. The finding from our study was consistent with that of study by Del Brutto (28).

Neuroimaging techniques, including computed tomography (CT) and magnetic resonance imaging (MRI), have improved the accuracy of the diagnosis of neurocysticercosis by providing objective evidence on the number and topography of lesions, their stage of involution, and the degree of inflammatory reaction of the host against the parasites (3). Computed tomography has contributed to a more accurate diagnosis and a better understanding of the pathophysiology of neurocysticercosis (10). However, because of their high cost and restricted availability, these procedures may be of limited use in developing countries with high rates of infection. Computerized tomography (CT) indicates structural disease but misses brain stem and intraventricular neurocysticercosis lesions and, if not performed with enhancement,

misses those that are isodense. In addition, the accuracy of CT diagnosis is extremely dependent on the judgement of the reader, which may be highly variable (22).

Serology

All the cases and controls were tested for IgG antibody against *T. solium*/cysticercosis by ELISA methods. Specimens yielding absorbance readings greater than Low Positive (OD=0.641) at 450 nm is reported as positive for antibodies against *Taenia solium.*

Among the total of 200 samples of sera, ELISA was positive in 51.5% of the samples. ELISA detected antibodies in 87% of the cases of neurocysticercosis, whereas 16% of the control population had antibodies against *T. solium*/cysticercosis. The sensitivity, specificity, positive predictive value, and negative predictive value are 87%, 84%, 84.47% and 86.6% respectively. The seroprevalence among control population was 16%. The possibility that there were cases of NCC within the control group is low, but we could not discard the possibility that they harboured extracerebral cysticerci or had been in contact with the parasite without acquiring infection.

The sensitivity of the ELISA was higher in cases with active neurological lesion (97.5%) and in cases with multiple parenchymal lesions (95.25%). A low sensitivity was observed with ELISA in cases with low number of cysts or calcified lesions only. Table 6.1 shows the sensitivities of ELISA in relation to type and localization of parasite in brain.

Table 6.1: Sensitivities of ELISA in relation to type and localization of parasites

Patient group	N	% of samples positive by ELISA
NCC with multiple cyst	39	97.4
NCC with single cyst	57	82.4
NCC with calcified lesions	4	25
Total	100	

ELISA sensitivity among cases without intestinal parasite was 85.9% and among cases with Taeniasis was 85.7%. The difference is not statistically significant.

Study done by Rosas (12) has shown the ELISA sensitivity of 75-87% and specificity of 75%. Similar study done by Mandal et al (13) on children have found the sensitivity, specificity, positive and negative predictive values of 89%, 81%, 79%, 90% respectively, this finding is in accordance with this study. According to Pawlowski (37) Human seroprevalence assessed using a variety of techniques and suggesting exposure to Taenia eggs, but not necessarily an actual cysticercosis, has been described as follows: Colombia (1.8-2.2%), Brazil (3.0-5.6%), Mexico (1.3-10%), Peru (7.1-26.9%), Guatemala (10-17%). Over all these countries the average seroprevalence is 10%. Prevalence of *T. solium* taeniasis seldom exceed 4% although, in some countries prevalence upto 7% have been reported. According to A. Carpio (9) Immunological assay detect positivity for human cysticercosis in 8-12% of people in some endemic region which indicate the presence of antibody against the parasite but not necessarily active or CNS infection. But in contrast to the present study Serology sensitivity in cases with multiple cysts was 94% and of only 28% was found in cases with single cyst in brain (29).

Serum antibody detection assays, particularly those based on purified or fractionated

parasite glycoproteins, are highly specific and sensitive. Test sensitivity is good in individual with two or more viable cysts but lower in individual with single or calcified cysts (37). Significant variations are often observed in the ELISA results for neurocysticercosis. These variations are probably related to several factors, including the heterogeneity of the patients studied, the immune status of the patients at the time of CSF collection, the intrinsic properties of the techniques, the mode of antigen preparation, the quality of the conjugate and substrate used in the assays, and the method of calculating the cut-off value.

Stool examination

Among the 100 cases of stool examinations revealed abnormal findings in 36% of cases. The commonest intestinal parasite was Taenia species 38.9% (14/36). No sub species of Taenia was determined. Other parasites were Ascaris (8%), Giardia (4%), Hookworm (4%) and Trichuris, H.nana and cryptosporidium (2% each). Few of the cases had more than one parasite in stool. Intestinal parasites were frequently identified in age group older than 5 years. Taeniasis was common in age-group 5 to 10 years.

Garcia et al (16) demonstrated that tapeworm carriers are at high risk of developing massive brain infection with viable *T. solium* cysticerci. 25% of the patients with cerebral cyst were found to harbor adult *T. solium* in their intestine or have history of such infection. Garcia et al 2003 (4) reported that upto 6% of the population in endemic village may harbor adult *T. solium* tapeworm at a given time. Study done by Gilman (18) has shown Up to 15% of patients harboring a tapeworm at the time of diagnosis of neurocysticercosis.

Taenia carriers are extremely potent sources of NCC, endangering everyone coming in contact with them. These Tenia carriers are also at higher risk of developing massive brain infection with viable *T. solium* cysticerci. Adult tapeworms cause almost no symptoms in carriers and that medical advice is seldom sought because of the presence of proglottids in the stools; this makes it difficult to identify cases and

provide treatment (1). And also Taenia are infrequently shed in stool, thus routine stool microscopy without concentration method may show false negative results. Every Physician should realize that every newly diagnosed patient with NCC has probably been infected by someone harboring a tapeworm in the patient's immediate environment. Epidemiological studies demonstrating clustering of cases of NCC around taeniasic individuals, strongly suggest a major role for direct contamination.

In clinical practice, absolute criteria are not available in majority of cases, and a probable diagnosis must depend on different, more indirect approaches with special emphasis on the recognition of a wide variety of clinical manifestations and neuroimaging results. The availability of noninvasive diagnostic confirmation in cases of suggestive clinical and imaging data has become even more important. Antibody detection by various test procedures such as Enzyme-linked immunosorbent assay can been used. No single test is confirmatory but proper integration of data provided by immunologic tests and neuro-imaging findings and epidemiologic data allow an accurate diagnosis in most cases.

Thus the diagnostic tests like ELISA for serology is highly effective in endemic regions also and can be used in screening and confirming the diagnosis of Neurocysticercosis. Neuroimaging is useful for the identification of stages, type, nature and localization of parasite within the brain. Stool for microscopy when done with concentration method is reliable tool in detecting the intestinal carriers of the parasite and it should be done in every suspected case of neurocysticercosis.

CHAPTER-VII

SUMMARY AND RECOMMENDATION

7.1 SUMMARY

- Altogether 200 clinical samples were collected from outpatients and admitted patients attending Paediatric department of Manipal Teaching Hospital, Pokhara, among which a total of 100 were from the cases of neurocysticercosis.
- Among 100 cases of neurocysticercosis, 60% were from males while 40% were from females.
- The samples were collected from the cases of age group below 15 years.
- Almost all the cases had mixed dietary habits, consumed both vegetable and meat (goat and pork).
- The most commonly affected age group was 8-14 years. Mean age of presentation was 10.92 years (SD=2.9). No cases were identified below 2 years of age.
- Seizure was the commonest presenting symptoms in 68% of cases. Among 68 cases of seizure focal seizure occurred in 50% cases.
- More than one symptom at presentation was seen in 24% of the cases.
- The frequency and duration of symptoms varied widely among the cases.
- 26% of the cases practiced pig rearing and were in direct contact with pigs. 30% of these children with pig rearing harbored Taeniasis.
- Stool examination revealed intestinal parasites in 36% of the cases. Of these majority 38.9% (14/36) harbor Taenia species. Taeniasis was commonly found in age group 5 to 10 years.
- Abnormal neuroimaging was seen in 100% of the cases but confirmation by neuroimaging alone could be made in only 38% of the cases. Findings seen in

these 38% cases were pathognomonic of neurocysticercosis.

- Single ring enhancing was the most common finding in contrast enhanced CT scan.
- Parietal region of the brain was the commonest site of cyst localization (46%) followed by frontal in 26% cases.
- 40% of the lesions were active in CT scan and transitional lesion was seen in 44% cases, indicating ongoing host parasitic interaction.
- Perilesional edema in contrast CT scan was seen in 77% cases and calcified intracranial lesion was present in 4% cases.
- IgG antibody against *T. solium*/cysticercosis in serum was tested by ELISA and was positive in 87% of the cases and 16% of control population.
- ELISA sensitivity was 96.3% in CT scan confirmed cases.
- ELISA sensitivity, specificity, positive predictive value and negative predictive value were 87%, 84%, 84.7% and 86.6% respectively.
- ELISA sensitivity was higher in cases with multiple intracranial cysts (sensitivity of 95.24%) and in cases with active lesions.
- Low sensitivity was observed in single nodular lesion and in calcified lesions.

7.2 RECOMMENDATIONS

Neurocysticercosis should be suspected in any cases with neurological symptoms (especially partial seizure) in Taenia endemic regions.

Neuroimaging should be done in cases with clinically and epidemiologically suspected Neurocysticercosis to identify the type, location, nature/stages and number of intracranial lesions.

ELISA should be done in every case of suspected neurocysticercosis where neuroimaging is inconclusive, or in areas where neuroimaging facilities are not available but serology is available.

EITB to detect antibody against *T. solium*/cysticercosis is gold standard, but in settings where EITB is not available or not feasible, ELISA or dot-ELISA in serum can be done with almost similar efficacy.

Presence of antibody against *T. solium*/cysticercosis in asymptomatic individual in endemic regions do not warrant immediate treatment but these individual may require further evaluation to rule out cysticercosis if they have high risk of acquiring the disease.

Serology mostly has a screening or confirmatory role and should be used in conjunction with neuroimaging if available, as the clinician needs to know the number, location, size and stage of intracranial parasites.

Stool routine and microscopy to detect intestinal parasite should be done in every cases with cysticercosis.

Concentration methods of stool processing yield higher identification of intestinal parasite than simple microscopy as the parasite is shed in stool infrequently.

To the Researcher:

Population based epidemiological survey on seroprevalence of cysticercosis and intestinal carriers of parasite is required to know the burden of disease in different regions and in whole country.

Further study on dot-ELISA and ELISA should be done to evaluate the significance of these tests in population survey.

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APPENDICES

Appendix I: Chart of diagnostic criteria and degree of diagnostic certainty for human cysticercosis

Diagnostic criteria & degrees of certainty	Criteria
Absolute criteria	• Histologic demonstration of the parasite • Direct visualization of the parasite by fundoscopic examination. • Evidence of cystic lesions showing the scolex on CT or MRI.
Major criteria	• Evidence of lesion suggestive of NCC on neuroimaging studies. • Positive immunologic tests for the detection of anticysticercal antibodies. • Plain X-ray films showing "cigar-shaped" calcifications in thigh and calf muscles.
Minor criteria	• Presence of subcutaneous nodules (without histologic confirmation) • Evidence of punctuate soft-tissue or intracranial calcifications on plain X-ray films. • Presence of clinical manifestations suggestive of Neurocysticercosis. • Disappearance of intracranial lesions after a trial with anticysticercal drugs.
Epidemiologic criteria	• Individuals coming from or living in an area where cysticercosis is endemic. • History of frequent travel to cysticercosis endemic areas. • Evidence of household contact with *Taenia solium* infection.

Degrees of certainty

Definitive diagnosis	Presence of one absolute criterion Presence of two major criteria Presence of one major plus two minor and one epidemiologic criterion.
Probable diagnosis	Presence of one major plus two minor criteria Presence of one major plus one minor and one epidemiologic criterion. Presence of three minor plus one epidemiologic criterion.
Possible diagnosis	Presence of one major criterion. Presence of two minor criteria. Presence of one minor plus one epidemiologic criterion.

Appendix II: Revised diagnostic criteria for neurocysticercosis

Categories of criteria	Criteria
Absolute	1. Histologic demonstration of the parasite from biopsy of a brain or spinal cord lesion
	2. Cystic lesions showing the scolex on CT or MRI
	3. Direct visualization of subretinal parasites by funduscopic examination
Major	1. Lesions highly suggestive of neurocysticercosis on neuroimaging studies*
	2. Positive serum EITB† for the detection of anticysticercal antibodies
	3. Resolution of intracranial cystic lesions after therapy with albendazole or praziquantel
	4. Spontaneous resolution of small single enhancing lesions‡
Minor	1. Lesions compatible with neurocysticercosis on neuroimaging studies§
	2. Clinical manifestations suggestive of neurocysticercosis\\
	3. Positive CSF ELISA for detection of anticysticercal antibodies or cysticercal antigens
	4. Cysticercosis outside the CNS¶
Epidemiologic	1. Evidence of a household contact with *Taenia solium* infection
	2. Individuals coming from or living in an area where cysticercosis is endemic
	3. History of frequent travel to disease endemic areas

* CT or MRI showing cystic lesions without scolex, enhancing lesions, or typical parenchymal brain calcifications.

† Enzyme-linked immunoelectrotransfer blot assay using purified extracts of *Taenia solium* antigens, as developed by the Centers for Disease Control and Prevention (Atlanta, GA).

‡ Solitary ring-enhancing lesions measuring less than 20 mm in diameter in patients presenting with seizures, a normal neurologic examination, and no evidence of an active systemic disease.

§ CT or MRI showing hydrocephalus or abnormal enhancement of the leptomeninges, and myelograms showing multiple filling defects in the column of contrast medium.

\\ Seizures, focal neurologic signs, intracranial hypertension, and dementia.

¶ Histologically confirmed subcutaneous or muscular cysticercosis, plain X-ray films showing "cigar-shaped" soft-tissue calcifications, or direct visualization of cysticerci in the anterior chamber of the eye.

ELISA = enzyme-linked immunosorbent assay.

Appendix III: Revised degrees of certainty for the diagnosis of neurocysticercosis

Diagnostic certainty	Criteria
Definitive	1. Presence of one absolute criterion
	2. Presence of two major plus one minor and one epidemiologic criterion
Probable	1. Presence of one major plus two minor criteria
	2. Presence of one major plus one minor and one epidemiologic criterion
	3. Presence of three minor plus one epidemiologic criterion

Appendix IV: ELISA procedure

Assay procedure

i. Prepare 1:101 dilutions of test samples by adding 5 uL of sample to 0.5 mL sample dilution (buffered and stabilized protein solution) in the separate glass tubes.
ii. Secure the desired number of coated wells (Isolated *Taenia solium* antigen coated wells) in the holder with sample identification.
iii. Dispense 100 uL of the Sample Diluent (buffered and stabilized protein solution) into well #1 as a blank,
iv. the Negative Control (Diluted human serum containing buffer and preservative into well #2, the
v. High Positive Control (Diluted Serum Containing anti-*Taenia solium* antibodies, buffer and preservative) into well #3, the
vi. Low Positive Control(Diluted serum containing anti-*Taenia solium* antibodies, buffer and preservative) into well #4, and
vii. The diluted patient samples into the remaining wells.
viii. Incubate for 10 minutes at room temperature.
ix. Wash five times with Washing Buffer.
x. Dispense 100 uL Enzyme Conjugate (Conjugate to horseradish peroxidase) into each well.
xi. Incubate for 15 minutes at room temperature.
xii. Wash five times with the Washing Buffer [Concentrate (20x): (50 mL) Prepare working solution by adding purified water to 1 liter].
xiii. Dispense 100 uL of Buffer solution containing peroxidase and 100 uL of Tetramethylbenzidine Solution.
xiv. Incubate for 15 minutes at room temperature.
xv. Stop reaction by adding 50 uL of Stop Solution (2 N HCl) to each well.
xvi. Zero a microwell reader on the blank and measure the absorbance of each well at 450 nm.

ELISA Reading:

OD of Blank =0.023

OD of negative control=0.024

OD of high positive control = 1.939

OD of low positive control = 0.641

Absorbance reading at 450 nm by Humareader

Interpretation of results

Specimens yielding absorbance readings greater than Low Positive at 450 nm is reported as positive for antibodies against *Taenia solium*. Absorbances of less than Low Positive are found with specimens having no prior immunological experience with *Taenia solium*.

Appendix V: Stool processing for Microscopy

Formalin Ether Concentration technique

Reagents: 10% formalin

Diethyl ether

Procedure:

i) Transfer about 0.5 gm of stool to 10 ml of 10% formalin in a 15 ml test tube.

ii) Mix and let it stand for 30 min for adequate fixation

iii) Strain the faecal specimen through 2 layers of gauze in a funnel into a centrifuse tube. Add about 3-4 ml of diethyl ether. Close the tube with glass stopper and shake well for 30 sec.

iv) Centrifuse the tube for 2-3 min at 500rpm.

v) Four layers are found

   a. Small amount of sediment at the bottom of tube containing parasite.
   b. Layer of formalin
   c. Plug of faecal debris
   d. Layer of ether at the top

vi) Loosen the plug of debris from the side of tube using applicator stick.

vii) Rapidly invert the tube to pour off the ether, the debris and the formalin.

viii) Transfer all the sediment on to a slide. Cover with a coverslip and examine under microscope for parasites.

ix) Count the number of each type of parasite.

Appendix VI: Questionnaire for data collection

1) Name……..……..

   Age……. Sex…….. H.No. .....

   Hospitalization- □, for …….days.

2) Address……. DOA…….

3) Presentation: Duration of symptoms……days

   Seizure- □ If Yes. Focal □ / Generalized □ No. of Episodes……..

   Headache- □ Focal weakness- □

   Behavior abnormality- □ Ocular complaints- □

   Others……...

Milestones – age appropriate □ / inappropriate □

Past H/O seizure- □

Family H/O Seizure- □

Pig rearing- □

Pork consumption- □

Raw food consumption-□

H/O parasite infection- □……..

Deworming done- □ If yes when……...back

Socioeconomic status ….....

Sanitation……

Diet: mixed □ / veg. □

Co morbidities- □ If yes specify ……..…..

4) On Examination:

   Residual weakness- □ No positive findings- □

   Relevant positive findings …….

Complication in hospital Y□ / N□, if yes ……..……

5) **Investigations:**

i) CBC: Hgb …...

WBC…….....

N…. L.… E.… M.…

ii) Stool microscopy-

Pus cells ……..….

Ova/cyst/parasite ……..….

iii) CECT head:

Lesion- Single□/Multiple□

Site …….....

Calcification-□

scolex □

Associated abnormality Y□ / N□ ….....

CT diagnosis ….....…

iv) EEG …….....…..

v) Serum for IgG ELISA …….....….

vi) Other …….....…..

6) Outcome: Improved□ / Cured□ / Referred□ / Death□