1. INTRODUCTION

1.1 Background

Zoonoses is defined as the diseases and infections that are naturally transmitted between vertebrate animals and humans. The zoonotic pork tapeworm, *Taenia solium* has become a serious neglected zoonotic helminth (Dorny et al. 2009) and increasing problem in Nepal (Poudel et al. 2014). Neurocysticercosisis a major cause of symptomatic epilepsy in areas where pork tapeworm *Taenia solium* is endemic.

T. saginata, T. solium and *T. asiatica* live as adult tapeworms in human intestines and cause taeniasis. An infection with the cysticercus larva of the cestode *Taenia solium* is called cysticercosis (Garcia et al. 2003, Joshi et al. 2007). The parasite needs two hosts to complete its life cycle. Pigs act as intermediate hosts and harbor cysticerci larva in different parts of the body, mostly in the skeletal, cardiac muscles and the brain. Primarily, human act as the definitive host but also can act as intermediate host which is the dead end of the parasite's lifecycle. The cysticerci larva migrate mostly into muscle causing cysticercosis or brain tissue causing neurocysticercosis (NCC). NCC is still common in developing countries and is considered as an emerging disease in many developed countries due to increased migration from endemic areas (Rodriguez-Carbajal et al. 1983 and Sorvillo et al. 2007). It is a human-to-human infection acquired by the ingestion of *T. solium* eggs shed in the faeces of human carriers (Angels et al. 2003). WHO has classified it as the most important neurologic disease of the parasitic origin in humans (Joshi et al. 2007). The disease also affects many people of productive age group (White 2000). In Nepal, Rajbhandari (2003) reported that the epilepsy cases has been increased and 7.3 per 1000 populations suffer from epilepsy and almost 50% of total epilepsy are due to NCC. The study of Joshi et al. (2011) as well as other review clearly showed that NCC is the major contributory factor in the occurrence of epilepsy in Nepal. NCC is rare in Eastern and central Europe, North America with exception of South Coast of USA and Australia, Japan, New Zealand and not found in Israel and in Muslim countries of Africa and Asia (Del Brutto et al. 1998).

1.2 Taeniasis and cysticercosis

T. solium is endemic in many countries where livestock and consumption of pigs are common. The intestinal infection caused by adult tapeworms *Taeniasolium* and *T. saginata* and also by *T. asiatica* is called taeniasis. The most important human tapeworm infections are caused by *Taenia solium* (pork tapeworm) and *T. saginata* (beef tapeworm). Taeniasis due to *T. saginata* usually has a minor impact on human health. Sheep, deer, dogs and cats are seldom infected. Water, wind, flies, and other indirect means of infection play little part in transmission (Joshi et al. 2001).

Cysticercosisis a common public health problem especially in developing countries especially in South East Asia, Latin America, Sub-Saharan Africa, Eastern Europe (Garcia et. al. 2003, Rajshekhar et al. 2003). Human and porcine taeniasis/cysticercosis has been reported one of the major zoonotic diseases in Nepal (Poudel 1998 and Thapa 2000). Cerebrum and cerebellum region of brain are common sitesof infection but larvacan migrate to any organ in the bodysuch as,eyes, muscles or subcutaneous tissues (Handique et al. 2008). Human beings acquire cysticercosis through faecal-oral contamination with *T. solium* eggs from tapeworm carriers (Garcia and Del Brutto 2000 and Garcia et al. 2003). Thus, vegetarians and other people who do not eat pork as well as beef can acquire cysticercosis (Garcia et al. 2003).

1.3 Neurocysticercosis and Epilepsy

The infestation of the Central Nervous System (CNS) with the larval stage of the pork or beef tapeworm is called neurocysticercosis (NCC). A review of Christina and Herbert (2009) indicated that the clinical stages of NCC are active (vesicular), transitional (colloidal and granular nodular) and inactive (calcified) stage. The process of degeneration of the cyst of parasite has been categorized by Escobar (1983) into the vesicular (the cyst containing live larva), colloidal (the cyst starting to degenerate), nodular-granular (the thickening of cyst membrane and calcification) and calcified vesicular stage (the cyst fully calcified and necrotized).

The WHO has classified NCC as the most important neurologic disease of the parasitic origin in humans. It is one of the cause of serious morbidity in areas where *T. solium*is endemic and known to be a leading cause of epilepsy (Garcia and delBrutto 2000, Garcia et al. 2003). According to White (1997), the frequency of NCC has increased with the increase of migration of tapeworm carriers. The disease accounts for the 50% of patients presenting with partial seizures (Rajshekhar et al. 2003). In some countries like Peru and Mexico, NCC contributes 30% of seizures (Sorvillo 2007).

Unlike developed countries, disease has been ignored in most parts of Asia (Mamkin 2007). In South India, 48.3% epileptic cases were due to NCC (Singh and Sappal 2012). In the rural area of Northern Vietnam, 10% of the epileptic cases were due to NCC (Trung et al. 2013). Similarly, up to half of patients with seizure disorders were due to NCC (Tsang and Wilson 1995). In Asia, including Bali and Korea, a large proportion of epileptic patients had the disease (White 1997). In Nepal, NCC patients rate is 2.34 per 1000 OPD patients visiting neurological departments of hospitals and NCC per epileptic admission episodes is between 13.3-31.7% (Joshi et al. 2004). A research conducted at Model Hospital in Kathmandu showed 73% of epileptic cases were due to NCC cases (Dhakal et al. 2005). The burden of the disease in patients with epileptic seizures is about 70-90% (Prasad et al. 2008). In most endemic villages, more than 10% of the general populations havebeen found to be seropositive (Roman et al. 2000, Garcia et al. 2003). In population-based studies, 10–18% of asymptomatic individuals had NCC (Roman et al. 2000). Seizure was found to be the commonest clinical presentation observed in 100% patients of NCC according to the hospital based study in India (Kotokey 2006). A study from India suggested that seizures/epilepsy is common in children than in adult population (Singhi and Singhi 2009).

Epilepsy is a state in which a person has recurrent seizures and a seizure is a paroxysmal event caused due to abnormal, excessive, hyper synchronous discharge from an aggragate of CNS neurons (Piya et al. 2005). It is considered as the only clinical manifestation of NCC (Carabin et al. 2006) which occurs in about 80% of the patient and more frequent in between 2nd and 5th decade of life and the patients with parenchymal disease (Del Brutto et al.1998). It occurs when a cyst is in an active viable or degenerating form (Rajshekhar et al. 1995) or

in a calcified form (Del Brutto et al. 1992). Seizures, due to NCC, are most commonly generalized tonic, clonic or simple partial and few cases show complex partial or myoclonic seizures (Nicoletti 2002). In patients with epilepsy, parenchymal cysts appeared to be common (Shandera et al. 1994) and the single calcifications are considered to be found often in patients with seizures (Rajshekhar et al. 1995). The calcified lesions are much more common than viable cysts, and they are more prevalent in patients with epilepsy than in asymptomatic patients (Nash et al. 2004). Increased intracranial pressure, hydrocephalus, headache, cerebrovascular complications, psychiatric disorders are other clinical menifestations of NCC.

1.4 Diagnostic Aspects

The diagnosis of cysticercosis can be done by radiological, serological, molecular and histopathological examinations (Yamasaki 2007).

1.4.1 Radiological diagnosis

The radiological diagnosis of NCC is done through imaging techniques. The modern neuroimaging techniques are computed tomography (CT) and magnetic resonance imaging (MRI)which detect active, transitional and inactive lesions (Garcia and Del Brutto 2003). Moreover, it has helped in the development of clinical classifications of NCC (Garcia et al. 2002). In neuroimaging tests, the cysts appear as the images with definite edges (Nash and Neva 1984) but in the degenerating cyst, the edges become irregular and shrink and it appear as whitish, round, calcified nodule with the surrounding parenchyma whose most common location is cerebral hemisphere (Garcia et al. 2003). Calcified cysts can also be detected by radiography (White 2000). For the detection of muscles cyst, X-rays can be used (IOE 2004).

1.4.1.1 Brain CT scan

CT scan has been claimed to have sensitivity and specificity of 95 % for diagnosing NCC (Nash and Neva 1984). The sensitivity of MRI for the detection of calcified lesions has been considered to be less, so CT has been reported to be the better neuroimaging procedure for NCC suspected patients (Garcia and Del Brutto 2003). The cysticerci within the basilar cisterns are missed by CT scan which can be visualized with MRI. CT scans are usually or commonly used while, MRI is used in cases in which axial skeleton is involved (Parasca et

al. 2006). Colloidal cysticerci appear on CT and MRI as ill-defined lesions surrounded by edema (Garcia and Del Brutto 2003).

1.4.1.2 MRI of Brain

MRI has been considered accurate for the diagnosis of NCC but costs of MRI are high and the equipment is scarcely available in many endemic countries (Garcia and Del Brutto 2000). Garcia et al. (1994) stated that CT images are rarely diagnostic for the disease. As reviewed by Christina and Herbert (2009), ventricular cysts shifted within the ventricular cavities due to person's head movement can also be better observed by MRI than with CT scan but MRI cannot detect parenchymal calcification of cyst (Teitelbaum et al. 1989). Hence, MRI is more sensitive and is superior to CT scans in finding multiple cystic lesions and protoscoleces (Ng et al. 2000).

1.4.2 Serological diagnosis

Different techniques have been described to detect antibody or antigen to *T. solium*. For the detection, either an ELISA (Enzyme-Linked Immuno-Sorbent Assay) or EITB (Enzyme-Linked Immuno-Electro Transfer Blot) assays are the most commonly employed. In developing countries ELISA is preferred because of better availability, simplicity and lower cost compared with EITB and serum sensitivity of 75-87% and specificity of 75% has been reported (Rosas et al. 1986).

1.4.2 .1 Enzyme-linked immuno-sorbent assay (ELISA)

ELISA measures specific IgG antibody to partially purified antigen from cysticercal fluid (Mandal et al. 2006). Serologic test is considered to be quiet useful for confirmation of neuroimaging finding for differential diagnosis of other cyst forming condition (Del Brutto et al. 1996). When serum samples are used ELISA has sensitivity and specificity 50% and 70% respectively (Dorny et al. 2003). On the other hand, those are 87% and 95% when CSF samples used for testing respectively (Garcia et al. 2000). Carod et al. (2012) detected the sensitivity under 72% and specificity above 60% in his study. A comparative study of ELISA and dot-blot assay using blood samples from children with multiple brain lesions was found to be both sensitive and equally effective (Mandal et al. 2006). The detection of antibody in serum indicates the parasitic exposure but not the established infection (Garcia 2000).

Moreover, antibodies have been found to exist even after the parasite has been eliminated by antiparasitic therapy (Dorny et al. 2003) which may lead to misdiagnosis (Bern et al. 1999) and the misuse of antiparasitic drug even (Garcia et al. 2000).

While, the antigen detecting ELISA tests shows the presence of viable parasites (Deckers et al. 2010). After the successful antiparasitic therapy, the antigen level also drops (Deckers et al. 2010). Two monoclonal antibody based tests (B158/B60 Ag-ELISA and HP10 Ag-ELISA) have been validated and are used routinely for the detection of parasite antigens (Erhart et al. 2002 and Rodriguez et al. 2009). Garcia and others have used Ag-ELISA based on the use of a monoclonal antibody (HP10) that reacts with a repetitive carbohydrate epitope found in excretory/secretory and surface antigens of living cysticerci (Christina et al. 2009). Moreover, the sensitivity of Ag-ELISA for detecting NCC patients has been reported to be 92% (Deckers et al. 2010), 82.60% (Kotokey et al. 2006) and 85% (Garcia et al. 2000) for viable cyst. Corona et al. (1986) found 87% sensitivityof ELISA test in CSF and serum with a specificity of 100% and 90% respectively. Serologic test can be very useful for confirmation of neuroimaging finding for differential diagnosis of other cyst forming condition (Del Brutto 2012). It has better sensitivity with the use of CSF specimens as compared with serum samples; however, CSF collection requires lumbar puncture which is highly invasive and risky (Rodriguez et al. 2009).

1.4.2.2 Enzyme-Linked Immuno-Electro Transfer Blot (EITB)

EITB assay has been used worldwide for the diagnosis of individuals with NCC (Garcia et al. 1991). The test was considered to be 100% specific and 98% sensitive (Tsang et al. 1993). However,Wilson et al. (1991) demonstrated it to be only 28% sensitive when applied to serum samples collected from patients with single cysts. The serum EITB is more sensitive than that of CSF EITB (Wilson et al. 1991). This tests shows negative result after the cyst dies (Garcia et al. 1997). Its demrit is that it can be positive in patients with taeniasis and the presence of antibodies may indicate only previous exposure to or infection with the parasite (Garcia et al. 1997). Most of the serologically positive individuals have no identifiable lesions on CT scans (Schantz et al. 1998). Thus, the presence of antibodies does not reveal direct evidence of a living parasite within the host (Hauser and Hesdorffer 1990). Moreover,

the EITB assay is nearly 100% sensitive for patients witheither multiple cysts (Wilson et al. 1991).

1.4.3 Molecular diagnosis

The direct use of molecular techniques for NCC diagnosis was first reported in 2006 and demonstrated *T. solium* DNA in the CSF of patients, using a PCR with primers against pTsol9, specific for *T. solium* (Almeida et al. 2006). A study done by using primers against HDP2, specific for *T. saginata*, also found parasite DNA in CSF (Hernandez et al. 2008). A recent comparative study done by using pTsol9PCR, showed that PCR had the best sensitivity and its specificity was 80% and only calcified stage are PCR-positive (Michelet et al. 2011). In Nepalese patients molecular diagnosis of NCC was first performed by Pant et al. (2011). Moreover, the clinical studies on NCC have so far been limited to imaging studies (Piryani et al. 2007). In Nepal, because of the limited availability and high cost of molecular diagnosis of NCC, it has remained impractical (Pant et al. 2011). However, the DNA-based technology has contributed in determining the genotype of the genus *Taenia*, which has served in the genus identification and production of antigenic molecules for vaccines production (McManus 2006) but this methods have been considered unsuitable for diagnosing NCC in clinical settings (Rodriguez et al. 2012).

1.4.4 Histopathological diagnosis

Histopatholological diagnosis helps to diagnose the skeletal cysticercosis. Biopsy of brain tissues or subcutaneous tissue reveals the *T. solium*scolex with hooks. One of the rapid tools for the subcutaneous cysticercosisdianosis is FNAC (Fine Needle Aspiration Cytology).The diagnostic role of FNAC in cysticercosis was first emphasized by Kung et al. (1989) which is a well-recognized diagnostic procedure for the evaluation of inflammatory nodules caused by parasites. As reviewed by Del Brutto (2012), microscopic visualization of the scolex with its characteristics four suckers and a double crown of hooks, or the presence of parasitic membranes, confirm the diagnosis of NCC. However, biopsy of many granular and almost all calcified cysticerci may not confirm the diagnosis since the scolex and the membranes are not present in most of these (Del Brutto 2011).

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. NCC is the commonest cause of seizure in Nepal (Shrestha et al. 1989). Thus, base-line schematic for intervention of the parasite's life cycle is required. Most of the patients suffering from NCC belong from the low economic group of the society where pigs have access to untreated faeces. This is especially true in countries such as Nepal. So, prevention is most essential.

1.5 *Objectives*

1.5.1 General Objective:

To determine the sero-prevalence of neurocysticercosis along with Knowledge, Attitude and Practices among the patients visiting Neurology Department of Institute of Medicine, Tribhuwan University Teaching Hospital of Nepal.

1.5.2 Specific Objectives:

- To determine the ethnicity, sex, age and region-wise sero-prevalence of neurocysticercosis among the symptomatic and asymptomatic patients.
- To assess the knowledge, attitude and practice regarding neurocysticercosis among the patients.

2. LITERATURE REVIEW

Neurocysticercosis is a serious neurological problem particularly in developing countries (Lightowlers 2010). The disease is one of the important cause of epilepsy leading to fatal consequences. In some cases, it may be fatal but it is usually preventable. About 50 million people are infected worldwide and 50 thousands die from NCC yearly (Roman et al. 2000). Due to various steps taken like strict meat inspection, awareness programmes, better hygiene and sanitary facilities, the occurrence of NCC has decreased in developed countries. Moreover, in Nepal, no enough research activities and awareness programmes has been carried out and the information about the disease is very limited (Rajshekhar 2003, Agrawal 2011). The occurrence of cysticercosis is considered as a "biological marker" of the social and economic development of a community (Pal et al. 2000). Hence, this review basically focused on the distribution of NCC cases in different continents particularly in National context.

Scenario of Neurocysticercosis in the Context of World

In Asian Continent:

The disease has been ignored in most parts of Asia (Mamkin 2007). Cysticercosis is highly prevalent in the northern States of Bihar, Orissa, Uttar Pradesh and Punjab state of India where pig farming and pork consuming communities are prevalent (Singh and Sappal 2012)

Human cysticercosis is more prevalent in the northern states of India than the southern states (Singh et al. 2002). Few cases of NCC has been reported from Jammu and Kashmir, a predominantly Muslim state of India (Singh et al. 2002). In a rural pig farming community of Mohanlalganj block, Lucknow district, Uttar Pradesh, the prevalence of taeniasis was found to be 18.6% with active epilepsy cases 5.8% of the populations where 48.3% epileptic due to NCC (Singh and Sappal 2012). NCC has been reported in various states of India like Kerela (Singh et al 2002), Uttarakhand (Goel et al. 2011) and Assam (Kotokey et al. 2006). According to Heap (1990), about 80% epileptic cases among the Gurkhas working in Hong Kong confirmed the cerebral cysticercosis. *T. solium* infection is not endemic in Japan (Yanagida et al. 2012). Outbreak of human taeniasis due to *T. solium asiatica*, has been reported in Tokyo, Japan (Yamasaki et al. 2012). During 1994-2010, 35 NCC, 7

subcutaneous (or muscular) (SCC), and 2 ocular cysticercosis (OCC) cases has been reported from Japan (Yanagida et al. 2012).

NCC is non-endemic in Sri-Lanka and Thailand but some cases (11%) has been reported from several hospitals of Thailand (Singh et al. 2002). Anantaphruti et al. (2010) reported some cases of NCC in Central Thailand. A hospital-based study done by Cao et al. (1997) showed that 158 NCC cases were diagnosed from a hospital in Beijing, China. The seroprevalence of cysticercosis was reported to be 3.2% in Shangdon, China (Cao et al. 1996). In the rural area of Northern Vietnam, the seroprevalence of NCC in epileptic patient population was found to be less than 10% (Trung et al. 2013). Taeniasis and cysticercosis cases have been reported to be frequent in Vietnam (Willingham et al. 2003 and Van De et al. 2014). Neurocysticercosis is uncommon in Singapore but because of people or foreign workers from developing countries some cases have been diagnosed (Foo et al. 2008). The prevalence of NCC in Muslim countries, especially in the Gulf region like Qatar has increased because of labourers from highly endemic areas (Khan et al. 2011). In Malaysia, cysticercosis cases has been reported to be in increasing trend (Willingham 2008). It has been documented that the disease has been eradicated from South Korea (Pawlowski, 2006). Taeniasis/cysticercosis has been recorded from several provinces of Indonesia (Surso et al. 2006). In Bangladesh NCC is not endemic because Muslims mostly who don't consume pork live there (Diaz et al. 1992). According to Agrawal (2011), NCC is not found in Turkey, Iran and Israel, Muslim countries of Asia. The age-wise prevalence of active epilepsy in tropical countries was recorded to be 0.01% and about 50-70% of all patients with NCC present seizures (Roman et al. 2000). A field survey conducted in Irian Jaya and Wamena of Jayawijaya District, Indonesia, it has been found that cysticercosis infection was high (Wandra et al. 2003).

In African Continent:

It has been reported that *T. solium* cysticercosis is the main cause of acquired epilepsy in human population and has caused public health problems and economic loss in Africa (Assana et al. 2013). Poor sanitary conditions, free-roaming of domestic pigs and lack of awareness of the disease play an important role in the perpetuation of the *Taenia solium* taeniosis and cysticercosis in Africa (Assana et al. 2013).

Nguekam et al. (2003) reported very high prevalence of NCC from rural communities of Western Cameron, Central Africa and according to Foyaca-Sibat et al. (2009) NCC is prevalent in South Africa. The prevalence of NCC in persons with epilepsy visiting St. Elizbeth clinic NCC was 37% (Foyaca-Sibat et al.2009), in Iringa rural district that was significantly higher (62.7%). Mason et al. (1992) reported NCC cases to be prevalent in Zymbabwe. In West Africa, *T. solium* cysticercosis in pigs and man has been reported in Benin, Burkina-Faso, Ghana, Ivory Coast, Senegal and Togo While, In some regions of Nigeria, the prevalence of porcine cysticercosis (20.5%) and human taeniosis (8.6%) is reported to be high (Zoli et al. 2003). Few cases of human NCC had been recorded from in Kenya (Isaac et al. 2003).

A prevalence of NCC was 10.8% was reported in hospitalized epileptic patients in the capital Lome (Grunitzky et al. 1995). Rwanda has for a long time been considered as being hyperendemic for taeniosis and cysticercosis (Brandt 1997). A review done by Semie at al. (2015) showed that human taeniasis and cysticercosis are highly prevalent in Ethiopia. NCC has been recognised as an emerging public health problem and in sub-Saharan Africa and prevalence of NCC was 30-50% of acquired epilepsy (Winkler et al. 2009). In the southern highlands of Tanzania, *T. solium* infection in humans is found to be highly endemic (Mwanjali et al. 2013). Similarly, in Mozambique, very high sero-prevalence (51%) of NCC was recorded (Afonso et al. 2011).

In European Continent:

Favourable conditions for local *T. solium* transmission is found in eastern parts of Europe despite of modernization and intensification of the pig sector (Devleesschauwer et al. 2015). The prevalence of NCC has been expressed to be in increasing trend in the non-endemic areas of Europe and has been documented increasing especially in Spain and Portugal (Devleesschauwer et al. 2015). In Eastern Europe, on the other hand, cysticercosis has remained endemic (Del Brutto 2012).

A review performed by Del Brutto (2012), on NCC patients in Western Europe between 1970 and 2011 showed that out of the 371 cases, almost half were European non-travellers. According to Fabiani and Bruschi (2013), human cysticercosis has been prevalent in 17

European countries Austria, Belgium, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Sweden, United Kingdom, and Croatia, Norway, Switzerland. In Portugal, prevalence has been shown similar to that in endemic areas like Greece and Spain (Fabiani and Bruschi 2013). Zammarchi et al. (2013) reported that mostly NCC is prevalent in Portugal (70%), Serbia (15%), and Spain (7%). Moreover, *T. solium* taeniasis cases has been reported in Poland, Albania, and Italy (Alexander et al. 2014).

In American Continent:

NCC is a cause of premature death in the USA and the cases has increased because of immigrants from endemic to non-endemic regions (Serpa and White 2012). NCC is rare in North America (Agrawal 2011). In Panama, NCC case was documented in the people with no sanitary facilities (Garcia et al. 1990). NCC was endemic in Haiti with similar prevalence as that reported in other Latin American and African countries (Raccurt et al. 2009). The presence of *T. solium* antibodies in pig breeders was 8.7% and in the pigs was 20.9% in Columbia (Aqudelo-Florez et al. 2009). The seroprevalence of NCC was found to be 9.1% in Canoabo, 6.1% in Sanare, and 5.7% in Rio Tocuyo communities of Venezuala (Ferrer et al. 2003). In the United States, the disease was found in immigrants from Mexico, Central and South America (Singh and Sappal 2012).

In Australian Continent:

NCC is not frequent in Australia (Garcia et al 2003) and most cases occur in adult (McKelvie and Goldsmid 1998). Walker et al. (1991) reported NCC cases from Sydney in the immigrants or visitors from countries like Chile and India. Some cases has been documented by Crimmins et al. (1990) from hospitals in eastern Australia and the patients were immigrants from Eastern Europe, Asia or Central America. A study of Del Brutto and Garcia (2012) revealed that epidemiological of NCC in Australia has increased during the past two decades (Del Brutto And Garcia 2012). Further, NCC is an unusual cause of seizure in the New Zealand (Pybus and Heron 2012).

Scenario of Neurocysticercosis in the context of Nepal

Human cysticercosis in Nepalese people has been described since the early 1990s, mainly neurocysticercosis (NCC), ocular cysticercosis and muscular and soft tissue cysticercosis has been reported (Devleesschauwer et al. 2015). From Nepal, there are very limited data on epidemiology of cysticercosis. Here, very few research works has been carried out, and very few research articles have been published which makes us clear that the disease has been established in the country (Karna and Joshi 2009 and Sah et al. 2012). NCC prevalence is nearly 5% in patients with chronic headache (Sah et. al. 2012).

Different hospital records and survey has also been conducted to determine the prevalence of cysticercosis in human in which NCC is the most common (Joshi et al. 2007). Hospital-based studies of Nepal showed 7% to 73% NCC prevalences in seizure patients and in the most cases, neuro-imaging was used as diagnostic tool (Devleesschauwer et al. 2015). A retrospective study done at the Kathmandu Model Hospital reported the prevalence to be 61% out of total epileptic cases (Pia et al. 2005). According to study done by Basu et al (2007) 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. Similarly, twenty cases of NCC has been reported from Shree Birendra Hospital, 40 (7.18%) from Patan hospital (Choudhary 2006). Twenty- four (43.7%) NCC cases have been recorded from Om Hospital (Sharma 2006). Similarly, 66 cases of NCC were reported from TU Teaching Hospital (Agrawal 2006). Moreover, Bista determined the prevalence of human cysticercosis to be ranges from 0.002-0.1% in general population of Kathmandu (Bista 2005). According to Joshi et. al. (2004) human cysticercosis cases reviewed on the basis of hospital-based data was reported to be 62, 11 and 4 in Patan hospital, Kanti children and Bir hospital hospital respectively. Similarly, Ojha et al. 2015 in Neurology Department, Bir Hospital, Kathmandu, Nepal reported several cases of NCC from 2012 to 2014 and revealed that 21 out of 131 seizure patients admitted were due to NCC.

Several hospital based studies and the reviews shows prevalence of NCC among the patients visiting various hospitals of Nepal such as Nepalgunj medical college (Piryani et al. 2007), Manipal College of Medical Sciences and Manipal Teaching Hospital, Pokhara (Basu et al. (2007). From the survey of 5 hospitals of Kathmandu valley TUTH, Bir, Patan, Norvic and

Nepal Medical College, Karna et al.(2009) presented that the rate of NCC patients was 9.8% (179/1839) of epilepsy patients. Ale (2010) revealed the prevalence rate of NCC cases from Institute of Medicine (IOM), Maharajgunj to be 13.63%. The study of Joshi *et. al*. (2011) as well as other review clearly showed that NCC is the major contributory factor in the occurrence of epilepsy in Nepal. NCC per 1,000 admission episodes was the highest (5.27) in Norvic Hospital followed by College of Medical Sciences Teaching hospital and the lowest (0.26) in Patan Hospital (Joshi et al. 2011). The general prevalence of NCC among the patients visiting Neurology ward of TUTH, Maharajgunj was to be 10.6% (Lama 2012). Shrestha et al. (2013) reported 49 cases of NCC in Gandaki Medical college and Teaching Hospital, Pokhara with the mean age of 10.6 years. A study of Shrestha (2013) in western region of Nepal showed that the disease occured more in Kaski district (28.6%) followed by Tanahun (26.53%) and Baglung (12.24%). NCC cases have been recorded from the patient of Sunsari district (38.6%), Morang (12.3%) visiting BPKIHS, Dharan (Sah et al. 2014). According to Sah et al. (2014), a study conducted in Lumbini Zonal Hospital showed NCC prevalence frequent among childrens between 2 and 14 years.

Scenario of *Knowledge*, Attitude and Practices (KAP) of Neurocysticercosis

A KAP survey was done to gather information about what respondents know about NCC, what they think about NCC and perform several activities related to NCC.

Knowledge:

Epilepsy, a neurological disorder has been heard by majority of the people of the world. But the level of knowledge regarding risk factors associated was varied. The tapeworm has been found to be endemic in many parts of the world including Southeast Asia among the non-vegetarian people particularly, pork/beef consuming community. The disease has been ignored in most parts and the epidemiological aspects of the disease are poorly worked out in most Asian countries (Singh and Sappal 2012). About 12% of people in India epilepsy is caused due to worm while, more than half were aware of the link between worms and epilepsy (Synkhyan et al. 2015). Similarly, 12.9% of Kerela, India people know that worm is the causative agent (Girotra et al. 2011). Moreover, 21% people of South India know that infected pork leads to the taeniasis and most of them knew about the NCC. Chacha et al.

(2014) reported that 35% people of Africa had knowledge of tapeworm. Cysticercosis was a challenge for European care providers, since they are often poorly aware of this infection and had less knowledge about managing the disease (Zammarchi et al. 2013). In Kenya, most of the residents had knowledge of themselves or others in the community having tapeworm infections (Isaac et al. 2003). Mostly people are known about the commom zoonotic diseases like rabies. An exploratory questionnaire-based survey carried out in Tanga and Arusha regions, northern Tanzania by Swai et al. (2010), showed that rabies, tuberculosis and anthrax were considered the three most common zoonotic diseases.

In Kenya, most of the residents had knowledge of themselves or others in the community having tapeworm infections. Most homesteads lack toilets and the few that have do not always use them. In India it is expressed that 35.7% of participants believed that NCC spreads by eating pork and 30% in the control group believed that it spreads by drinking contaminated water (Radhakishan et al. 2005). Similarly, about half of the people had knowledge that NCC spreads by eating pork and knowledge regarding use of cysticidal drugs was also low (Radhakishan et al. 2005). Most of the people of Tanzania had knowledge about the symptoms of epilepsy (Mwidunda et al. 2015). There is limited knowledge on the management and prevention of epilepsy in Iringa rural (Chacha et al 2014). Only some of the people (35%) in Africa had knowledge that contaminated food and water are the source of Taeniasis/cysticercosis (Chacha et al. 2014). Moreover, the knowledge about mode of infection and spreading is rarely observed in the people of Tanga and Arusha, Northern Tanzenia (Swai et al. 2010). A study done by Florez et al. (2009) in the village of Andagoya, Colombia showed that participants had partial knowledge of the taeniasis-cysticercosis complex. They considered taeniasis to be an illness resulting from faecal transmission and cysticercosis was not caused after consuming pork. The knowledge regarding cause of NCC was found to be lacking among the people of Mexico and Delhi (Alexander et al. 2014). Nevertheless, no respondents in Tanzania, had knowledge that human faeces is a source of cysticercosis (Mwidunda et al. 2015).

The general information related to personal food hygiene was known to majority of the teachers (respondents) but knowledge of taeniasis/cysticercosis and seizure prevention was lacking in

India (Mishra et al. 2007). In Mbulu District, northern Tanzania the knowledge about the transmission and prevention of porcine cysticercosis among respondents was good (Nqowi et al. 2008). Participants of Mbulu district, Northern Tanzania had good knowledge of epilepsy symptoms, and seizures, falling down accompanied with loss of consciousness and froths from the mouth emerged as theme ideas (Mwidunda et al. 2015).**Attitude:**

The most of the patients visiting epilepsy clinic in India, believed that the disease cysticercosis is acquired by eating cabbages rather than by consuming pork (Sankhyan et al. 2015). A study done by Girotra et al. (2011) in North west India showed different traditional attitude regarding NCC, 31% and 27% thought epilepsy was a hereditary disorder and a result of poor sanitation, respectively. Similar view or concept of patients (40%) was found in a study of Chacha et al. (2014) in Africa. The people of Colombia belived that NCC is caused due to contaminated food and water and not by infected pork consumption (Floez et al. 2009). Regarding prevention of NCC, most of the people believed that by drinking clean water spread of NCC can be prevented and few believed that NCC can be controlled by not consuming pork (Radhakrishan et al. 2005). A large proportion of NCC patients (45.7%) in Kerela, India believed in ayurvedic treatment also, majority of the respondents were not aware of any treatment for NCC (Radhakishan et al. 2005). Most of the respondents strongly believed that keeping pigs indoors was a measure to prevent pigs from acquiring cysticercosis (Mwidunda et al. 2015).

Practices:

A study showed that in Syangja district about 87% of households did not have a toilet and open defecation is common (Gaire 2000). Similarly, in Botetar, Tanahun district none of the households have been using a toilet but by contrast, some of the people of Baireni, Atreuli, and Dumsi have been using safe latrines (Thapa 2000). Most of the households did not have a proper latrine or sewage disposal system and had a very low level of sanitary practice in the western part of Nepal (Shrestha et al. 2013). In Eastern terai of Nepal, open defecation was significantly more common among Dalits and Terai Janjati than among hill Janjati and high-

caste Hindus (Devleesschauwer et al. 2012). Moreover, most of the people in Kenya lack toilets and the few have but do not always use them (Isaac et al. 2003).

Only few (16%) of households had latrines in northern Lao PDR and was found that there was lack of water access, poor building techniques and poverty (Bardosh et al. 2014). In Africa about 22.7% slaughtered pigs at home and sold uninspected pork and the majority of the surveyed population 89.8% eat pork while, for epileptic symptoms, mostly, the hospital was the preferred place for treatment and some used traditional healer and few used both hospitals and traditional healing (Chacha et al. 2014). Similarly, open-field defecation in the general community in India was more (Alexander et al. 2012).

A survey in northern Lao PDR showed a remote ethnic minority village the risk behaviours like consumption of raw pork, and poor latrine coverage have been found (Cao et al. 1997). In Nepal, the Janjati are found to raised pigs significantly more frequently than the other Dalit while, so called high caste Hindus do not raise pigs (Devleesschauwer et al. 2012). Moreover, weekly pork consumption has been found to be more frequent among the Dum than among any other ethnic group (Devleesschauwer et al. 2012). The Banjar community of Bali, (An Indonesian Island) consumption of Lawar, minced uncooked pork with blood has been found frequent (Ito et al. 2005). In Africa, free-roaming of domestic pigs and lack of awareness of the disease has been reported (Assana et al. 2013). In Mexico, the risk factors associated with human taeniasis and cysticercosis was the eating of infected pork and close proximity to a carrier of *T. solium* (Rodrequez- Canul et al.1999).

3. MATERIALS AND METHODS

3.1 Study Area

This was a hospital based study conducted by visiting Neurology Department of Institute of Medicine (IOM), Tribhuwan University Teaching Hospital (TUTH), Maharajgunj, Kathmandu. Kathmandu is a district of mountaineous region and is the capital of Nepal and is situated in Central Development Region. The district covers an area of 395 sq. km. The average altitude is about 1400 m above sea level. It is located at the longitude of 27'43''E and latitude of 85'21''N. The fluctuation of temperature is between 32 degree celcius in summer and -2 degree celcius in winter. The average rainfall is 176:4 ml annually. Tribhuvan University Hospital is the largest hospital in country established in 1984 with support from JICA. It provides new tertiary-level health service to the Nepalese people. This hospital is the site of teaching and research activities of IOM (Institute of Medicine). IOM was established in 1972 under Tribhuvan University.

3.2 Study design

The study was divided into two phases to fulfil the objectives.

(i) General screening of patients with neurological problem visiting Neurology Department of IOM, TUTH to determine the prevalence of NCC.

(ii) Questionnaire survey to assess the knowledge, Attitude and Preventive Practices (KAP) of the patients towards NCC.

3.3 Study Period

The study was conducted from July 2014 to July 2015.

3.4 Sample Size

A total 80 blood samples were collected from the symptomatic as well as asymptomatic patients visiting Neurology Department of the Hospital for the serological test.

3.5 Sampling Procedures

3.5.1 Sample Collection and Transport

About 2 ml of the blood samples of the patients were collected in sterile, clean and leak-proof viles and labeled properly. Then blood tubes were centrifused for 15 minutes. Then

serum was separated and pipette out in a sterile eppendorf tubes and were frozen at -20^{o}C till analysis. The samples were taken to the laboratory of National Zoonoses and Food Hygiene Research Centre, Tahachal Kathmandu for the further diagnosis and were tested by Enzyme Linked Immunosorbent Assay (ELISA) method .

3.5.2 Serological study

ELISA was conducted at NZFHRC, Kathmandu for the detection of circulating *T. solium* antigens. The sandwich Ag-ELISA, as described by Dorny et al. (2000) and adapted by Dorny et al. (2004) was used. It was performed in polystyrene 96-well microplates. All unknwown serum samples and two positive control samples were test in duplicate. The protocol of ELISA is described below.

3.5.2.1 Protocol of ELISA for the detection of circulating T. solium antigens (Ag-ELISA)

The protocol manufactured by the Prince Leopold Institute of Tropical Medicine, Department Animal Health Antwerpen (Antwerp), Belgium was followed for the ELISA process. First of all serum samples were pretreated with trichloroacetic acid (TCA). Pretreatment was done to break down immune complexes to obtain free antigen.

- For, treatment, 150 µl of each sample were vortexed and were mixed with150 µlof TCA followed by vortexing.
- These mixtures were then incubated for 20 minutes at room temperature then,these were centrifuged for 9 minutes at 1200 rpm.
- 100 µl of the supernatants was then neuralizedby adding 100 µl of neutralizing buffer.
- The monoclonal antibody (B158C11A10) was diluted at 5 µg/ml in carbonate buffer (0.06 m, pH 9.6) for coating the ELISA plate and all the wells except two wells for the substrate control were coated with the capturing antibody.
- After coating, the plate was incubated for 30 minutes at 37°C while shaking.
- The plate was washed once with washing buffer.
- All the wells were then blocked with 150 µl of blocking buffer and it was incubated for 15 minutes at 37°C while shaking.

- 100 µl of the pretreated samples were kept in designated wells. For substrate and conjugate controls, wells were filled with blocking buffer. For positive controls and unknown samples, 2 wells per sample. Then incubation was done for 15 minutes at 37°C while shaking.
- The plates were then washed 5 times with washing buffer.
- 100 µl of detecting antibody was poured in B60H8A4 in all wells except the 2 wells for substrate control. In these 2 wells blocking buffer was kept.
- Then incubation was done for 15 minutes at 37°C while shaking.
- The plates were then washed 5 times with washing buffer.
- 100 µl of peroxidase labelled streptavedin in all wells except 2 wells for substrate control and then it was incubated done for 15 minutes at 37°C while shaking.
- The plates were then washed 5 times with washing buffer.
- 100 µl of ortho-phenylenediamine) OPD was put in all wells and left for incubation at room temperature for 15 minutes in the dark without shaking.
- To stop the reaction, 50µl of H_2SO_4 was added in each well.
- Then plate was read by using ELISA reader.

3.5.2.1 Interpretation of Results

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

$$\text{average OD} = \frac{OD_{well\ 1} + OD_{well\ 2}}{2}.$$

The ELISA values were expressed as a ratio by dividing the OD of the test sample by the OD of the cut off value The cut-off values was calculated on the basis of OD's negative samples variation of the student test. With the help of cut-off value, a ratio was calculated:

$$\text{ratio} = \frac{\text{average } OD}{\text{cut off}}.$$

The ratio is greater than 1, was considered positive with 99.9% of certainity.

3.6 Quality control

Strict quality control was maintained to obtain the reliable results. Aseptic condition was maintained throughout the lab work. 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 OPD can change colour due to a specific circumstances. The result was intepreted on the basis of specificity and sensitivity of this test using statistical technique (Dorny et al. 2004). According to this statistical technique, the sensitivity is estimited at 76.3 to 86.7% and the specificity at 84.1 to 98.9% (Dorny et al. 2004).

3.7Questionnaire survey to assess KAP towards NCC

An structured questionnaire was firstly pre-tested among the colleagues and tested among the patients and was modified where the they felt uneasy to answer the questions. Then questionnaire was applied to total 200 patients, admitted as well as OPD patients visiting the Neurology Department during the study period.

The questions were based on Knowledge, Attitude and Preventive Practices (KAP) towards NCC and face to face interview was performed among the patients Details about knowledge on NCC like parts of the body affected by larva of *Taenia,* mode of transmission, clinical symptoms and nature of disease were included in the questionnaire. The questionnaire also contained questions related to the knowledge of the participants on preventive method, transmission and also their practices and attitude towards NCC.

3.8 Statistical Analysis

After data collection, data were checked for the completeness. Microsoft Excel- 2007 and Statistical Package for Social Sciences (SPSS) version 21 for windows was used to analyze the data. The association between categorical variable was assessed by chi-square ($\chi 2$) test. A result was considered significant at a P value <0.05. The analyzed data were summarized in table and percentages.

Photo Plates

Photo no. 1 Centrifuging the Samples

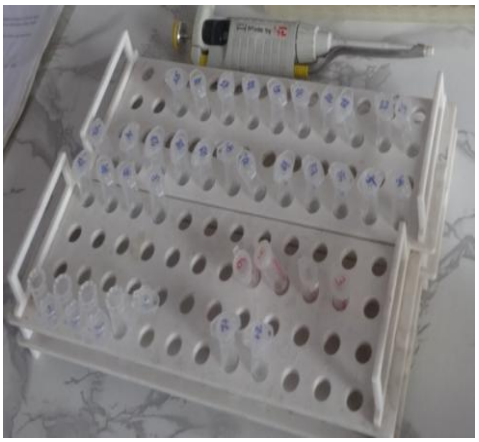

Photo no. 2 Serum extraction

Photo no. 3 Vortexing of sample

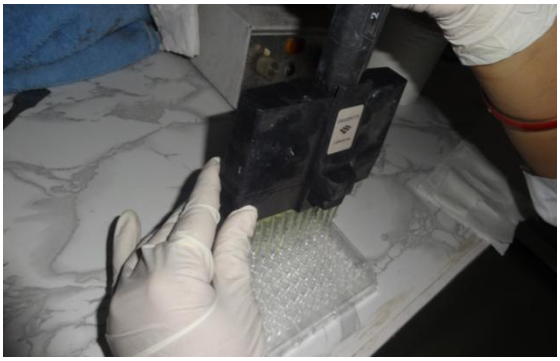

Photo no. 4 Pretreatment of sample

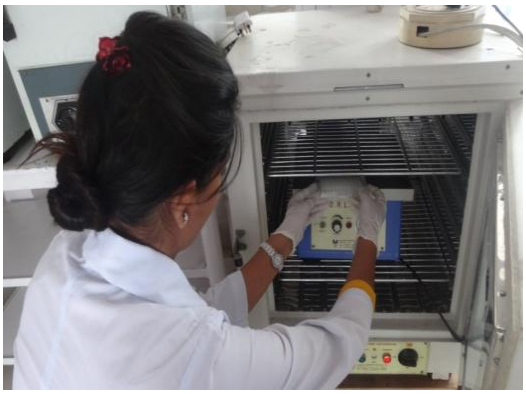

Photo no. 5 Incubating the samples at 37°C.

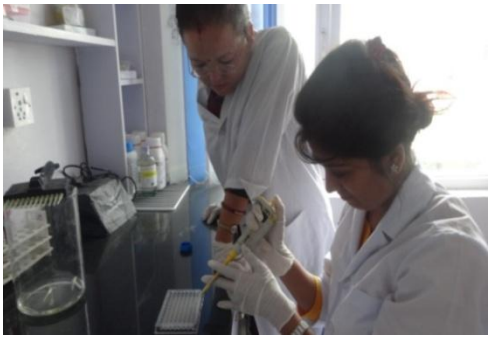

Photo no. 6 Washing the sample

Photo no. 7Arresting the reaction

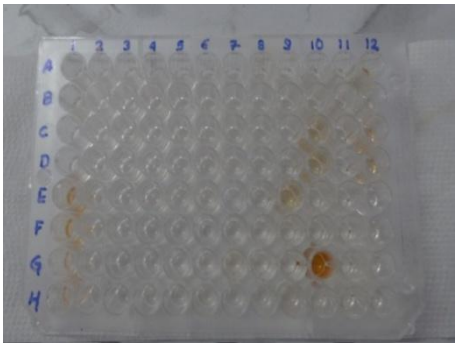

Photo no. 8 Interpretation of ELISAresult

Photo no. 9 ELISA reader reading OD value

Photo no. 10 Questionnaire with OPD Patient

4. RESULT

4.1 Sero-prevalence of Neurocysticercosis Among the Patients Visiting Neurology Ward, TUTH

General prevalence of Neurocysticercosis

A total 80 blood samples were collected from patients visiting Neurology ward of Tribhuwan University Teaching Hospital (TUTH), Maharajgunj. Out of 80 samples, tested for IgG antibody using Ag- ELISA, 5(6.25%) were positive for *Taenia solium* metacestode circulating antigens (Fig. 1).

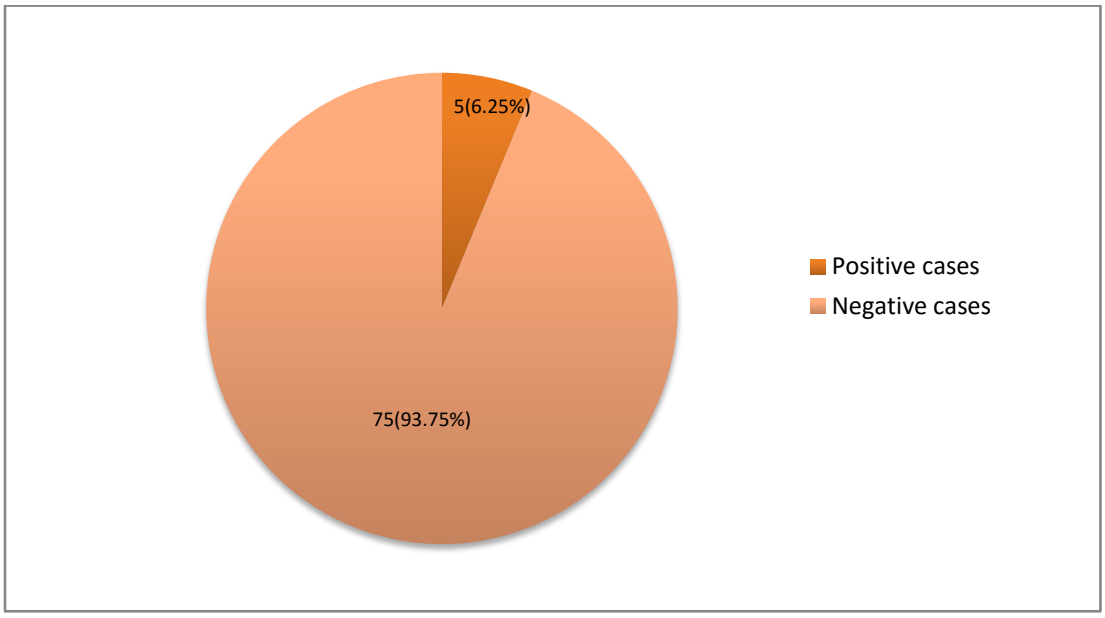

Figure 1: *General prevalence of neurocysticercosis*

The distributions of neurocysticerosis cases were analysed on basis of their socio-demographic characters like ethnicity, gender, age-groups and the regions (Table 1). Out of total 80 samples, 26 (32.5%) were brahmin, 19 (23.75%) were chhetri, 23 (28.75%) were janajati, 3 (3.75%) were dalit and 9(11.25%) were madhesi. The highest sero-positivity rate of NCC was found among the madhesi ethnic group (22.2%) followed by janajati (8.7%) and the lowest chhetri (5.3%). Statistically no significant association was found ($P > 0.05$).

Table: 1 *Ethnicity, sex , age and region-wise sero-positivity distribution of neurological patients visiting TUTH*

Variables	Frequency $n = 80$	Positive (%)	Value of χ^2	d.f	*P*-value
Ethnicity					
a) Brahmin	26 (32.5%)	0 (0.0%)			
b) Chhetri	19 (23.75%)	1 (5.3%)			
c) Janajati	23 (28.75%)	2 (8.7%)	6.118	4	0.190
d) Dalit	3 (3.75%)	0 (0.0%)			
e) Madhesi	9 (11.25%)	2 (22.2%)			
Sex					
a) Male	37 (46.3%)	3 (8.1%)	0.485	1	0.486
b) Female	43 (53.7%)	2 (4.6%)			
Age					
a) 0-10	9 (11.25%)	0 (0.0%)			
b) 10-20	14 (17.5%)	2 (14.3%)			
c) 20-30	21 (26.25 %)	3 (14.3%)			
d) 30-40	13 (16.25%)	0 (0.0%)			
e) 40-50	7 (8. 75%)	0 (0.0%)	6.525	7	0.480
f) 50-60	9 (11.25%)	0 (0.0%)			
g) 60-70	5 (6.25 %)	0 (0.0%)			
h) 70-80	2 (2.5%)	0 (0.0%)			
Region					
a) Mountain	4 (5.0%)	0(0.0%)	1.940	2	0.379
b) Hill	50 (62.5%)	2(4.0%)			
c) Terai	26 (32.5%)	3(11.5%)			

Out of the total samples included in the study, the highest sero-positivity rate of neurocysticercosis was found in male as compared to female. But association revealed insignificant ($P > 0.05$). The patients included in the study were of different ages ranging from 3 to 71 years. The neurocysticercosis cases were found only within the age group 10-20 and 20-30 with equal prevalence (14.3%) which is statistically insignificant ($P > 0.05$). The maximum patients were from Terai region (62.5%) compared to mountain (32.5%) and hilly region (5.0%). Among them, the sero-positivity rate of neurocysticercosis was found highest among the people of terai region followed by hilly region and no positive case was found among the people of mountain region with insignificant association.

4.2 Assessment of Knowledge, Attitude and Practices Among the Patients Visiting Neurology Ward of TUTH

4.2.1 *Socio-demographic Characteristics of the Respondents*

The questionnaire survey was carried out among 200 patients visiting neurology ward of TUTH to asses their knowledge, attitude and practices using structured questionnaire.

The distribution of respondents ethnic wise and gender-wise equal almost. Among all the respondents, majority (40.5%) belong to early adulthood followed by midlife 37.0% and others [Table 2]. Twenty-two percent of the respondents were illiterate while rest other were literate. While, the maximum 65.5% were engaged in some kind of work or occupation like farming or other kind of services like teaching, running own business etc.

Table 2: *Socio-demographic characteristics of the respondents*

Variables	Characteristic	Frequency (*n* = 200)
Ethnicity	Janajati	96 (48.0 %)
	Brahmin/chhetri	104 (52 .0%)
Sex	Male	103 (51.5 %)
	Female	97 (48.5 %)
Age group	Adolescence (1-20)	15 (7.5%)
	Early adulthood (21-35)	81 (40.5%)
	Midlife (36-50)	74 (37%)
	Mature adulthood (51-80)	30 (15.0%)
Education	Illiterate	44 (22.0 %)
	Below secondary	75 (37.5 %)
	Above secondary	81 (40.5 %)
Occupation	Not engaged in work	69 (34.5 %)
	Farmer	84 (42.0 %)
	Service	47 (23.5 %)

4.2.2 Assesment of Knowledge towards Neurocysticercosis

Among 200 respondents, majority of the respondents (64%) had knowledge about epilepsy which was insignificant statistically among various ethnic group and gender. Similarly,

epilepsy knowledge was insignificantly associated with various level of education, occupation as well as different age groups. About half of the respondents had heard about tapeworm which is significantly associated with gender (χ^2=3.907, df=1, P<0.005), education (χ^2=8.316, df=2, P<0.005) and age group(χ^2=14.618, df=6, P<0.005).

Table 3: Knowledge of respondents towards neurocysticercosis

Variables	Frequency (*n* = 200)	Socio-demographic characters				
		Ethnicity (*P*-value)	Gender (*P*-value)	Education (*P*-value)	Occupation (*P*-value)	Age-group (*P*-value)
Knowledge about epilepsy a) Yes b) No	128 (64%) 72 (36%)	0.272	0.451	0.427	0.315	0.362
Ever heard or infected with Tapeworm a) Yes b) No	101 (50.5%) 99 (49.5%)	0.121	**0.048**	**0.016**	0.911	**0.023**
Knowledge about Zoonotic diseases a) Yes b) No	102 (51%) 98 (49%)	0.187	0.127	0.361	0.725	0.435
Ever heard about NCC a) Yes b) No	71 (35.5%) 129 (64.5%)	0.133	0.108	0.160	0.281	0.096
Knowledge about mode of infection of NCC a) Contaminated food and water b)Infected pork consumption c)Both a and b d)Don't know	5 (7.0%) 32 (45.07%) 32 (45.07%) 2 (2.06%)	0.066	0.163	0.211	0.271	0.419
Knowledge about consequence of NCC a) epileptic symptom b) death c) Nothing d) Don't know	33 (46.48%) 14 (19.72%) 5 (2.06%) 19 (7.04%)	0.653	0.169	0.330	0.132	0.225
Heard or seen measly pork a) Yes b) No	94 (47%) 106 (53%)	0.271	0.062	0.444	0.491	0.910
Measly pork looks like a) White rice grain b) White nodules c) Small white balls	21 (11%) 57 (28.5%) 16 (8.0%)	0.341	0.056	0.364	**0.030**	0.653

Out of total respondents, 51% were known or had heard about the zoonotic diseases but mostly they had idea about very common zoonotic diseases like rabies. Mojority of the respondents had not heard about NCC and most of them don't know proper mode of infections. Among them who had heard about NCC, only 5 (7%) knew that it spreads by contaminated food and water.

Ninety-four (47%) of the people has seen or heard about measly pork and among them, 28.5% put the view that it looks like white nodules, some told it looks like small white balls while other told that it looks like white rice grain [Table 3]. The association is significant with the occupation of the people (χ^2=14.001, df=6, P<0.005). Mostly, people involved in farming or agriculture had some idea about measly pork.

4.2.3 Assesment of Attitude towards Neurocysticercosis

Regarding NCC, 36% (*n* =72) of respondents thought that the pork trade should be banned to stop spreading of such animal-borne diseases while other thought that promotion of meat trade should be done instead people should be more conscious about personal cleanliness and hygienic food habits. Mostly, brahmin/chhetri ethnic group thought that pork trade should be stopped ($P < 0.05$). But according too the service holders instesd of banning the po trade, healthy food habits should be promoted ($P<0.05$). Even though 71 (35.5%) respondents had heard about NCC, they don't have proper idea about treatment and control measure of the disease [Table 4]. Among the respondents known or having idea about NCC, few thought that epilepsy is caused due to NCC. Statistically, the association is significant with ethnicity (χ^2=, df=, P<0.005). Especially, janajati had view that epilepsy is caused by NCC.

Regarding treatment of NCC, 18.3 % (*n*=13) respondents still have traditional view that witch doctors or dhaami/jhankri can treat it. Significantly, there is association between this traditional thought and the age and gender of the respondents (χ^2=6.713, df=2, P<0.005 and χ^2=9.032, df=12, P<0.005 respectively). Respondents of age above 50 years and female respondents had this type of thought.

Table 4: Attitude of respondents towards Neurocysticercosis

Variables	Frequency (*n* =200)	Socio-demographic characters				
		Ethnicity (*P*-value)	Gender (*P*-value)	Education (*P*-value)	Occupation (*P*-value)	Age-group (*P*-value)
Epilepsy is caused only due to NCC a) Agree b) Disagree	 20 (28.2%) 51 (71.8%)	**0.012**	0.227	0.339	0.429	0.275
NCC disease of non-vegetarians a) Agree b) Disagree	 36 (50.7%) 35 (49.3%)	0.233	0.058	**0.048**	0.372	0.143
Witch doctors can treat NCC a) Agree b) Disagree	 13 (18.3%) 58 (81.7 %)	0.189	**0.035**	0.158	0.454	**0.004**
Pork/beef trade should be totally banned a) Agree b) Disagree	 72 (36.0%) 128 (64.0%)	**0.002**	0.134	0.537	**0.032**	0.652
Control measure of NCC is a) Personal hygiene b) Consumption of well cooked meat c) Both d) Dont know	 2 (2.8%) 28 (39.5%) 39 (54.9%) 2 (2.8%)	**0.000**	**0.045**	0.272	0.544	0.402

4.2.4 Assesment of Practice towards Neurocysticercosis

The practices that can be the risk factors for *Taenia solium* infection included the type of meat consumed such as pork consumption (8.3%), open defeacating practice (7%), hand washing with nothing after using toilets (8.3%). There is significant association between the materials used by the respondents for washing hands with and their age. Mostly, people above 50 years use either nothing or anything to wash hands after using toilet. Almost all the male respondents were non-vegetarian by food habit while, some females were vegetarian. Majority of the meat consuming respondents consume all kinds of meat also most of them consume meat except pork. There is significant association between gender of the respondents and type of meat consumed (χ^2=23.689, df = 3, P<0.005). Most of the female don't have practice of having pork while male are most

Table 5: Practices of respondents towards Neurocysticercosis

Variables	Frequency (n = 200)	Socio-demographic characters				
		Ethnicity (*P*-value)	**Gender (*P*-value)**	**Education (*P*-value)**	**Occupation (*P*-value)**	**Age-group (*P*-value)**
Food habit a) Non-vege b) Vege	168 (84%) 32 (16%)	0.092	**0.004**	0.311	0.249	0.882
Types of meat consumed a) Pork b) Buff c) All d) Others	14 (8.3%) 19 (11.3%) 68 (40.5%) 67 (39.9%)	0.183	**0.005**	0.295	0.440	0.791
Toilet ownership a) Yes b) No	186 (93%) 14 (7%)	0.206	0.321	0.087	0.180	0.447
Open defeacation a) Jungle b) Open area c) River side	12 (85.7%) 2 (14.3%) 0 (0.0%)	0.222	0.355	0.443	0.167	0.182
Washing hands after using toilet a) Yes b) No	195 (97.5%) 5 (2.5%)	0.030	0.700	0.558	0.337	0.068
Washing hands with a) Ash b) Soap c) Soil d) Nothing e) All	8 (4.1%) 149 (76.4%) 3 (1.5%) 5 (2.5%) 30 (15.5%)	0.146	0.255	0.698	0.214	**0.025**
Ever done stool test a) Yes b) No	58 (29%) 142 (71%)	0.833	0.294	0.642	0.590	0.886
Parasitic infection history a) Ascaris b) Tapeworm c) Hookworm d) Don't know	2 (3.5%) 0 (0.0%) 0 (0.0%) 56 (96.5%)	0.837	0.379	0.381	0.875	0.775

likely to have all type of meat. Some of the respondents have the record of medical history of stool test (29%), but they don't have idea of the parasitic infection. Only 3.5% knew that they had *Ascaris* infection. No history of *Taenia solium* was found (0%).

5. DISCUSSION

Nepal is an agricultural country where most of the people depend on agricultural activities. In Nepal, pig farming has been increasing day by day with the annual growth rate of 4.55% (CLDP 2003). Pork consumption is highest (38%) in world followed by poultry (30%) and beef (25%) (Bhattarai 2005). The socioeconomic status and poor personal hygiene are the major cause for the transmission of the zoonotic pork tapeworms *Taenia solium* and *Taenia saginata asiatica* (Sah et al. 2014**).** Human and porcine taeniasis/cysticercosis are reported to be among the major zoonotic diseases in Nepal (Poudyal 1998, Gaihre 2000, Thapa 2000, Joshi et al. 2001, 2003, 2005).

Neurocysticercosis (NCC) is the most common cause of epilepsy and neurological morbidity in many developing countries (Prasad et al. 2008). This disease has serious impact on the public health and agricultural systems of pig producing and pork consuming countries like Nepal, where most of the people are farmers by occupation. In Nepal, 2.73 per 1000 lives are lost annually due to NCC associated seizures or epilepsy (Dorny 2010). Though pork meat is considered rich in thiamine, with the increment in the pork trade, porcine and human cysticercosis has become endemic in Nepal.

Two monoclonal Ab-based tests (B158/B60 Ag-ELISA and HP10 Ag-ELISA) have been validated and are used for the detection of the parasites antigens (Lama 2012). The sensitivity and specificity of this ELISA are generally high (Poudel et al. 2002, Dorny et al. 2004 and Lama 2012). In human cysticercosis, clinical symptoms are lacking. Ag-ELISA is used for determining infection with few cysts and was under an experimental setting able in detecting even a single cyst (Nguekam et al. 2003 and Dorny et al. 2004). Thus, antigen detection in Neurocysticercosis cases is useful that tool would support clinical diagnosis when neuroimaging tools are unavailable or are inconclusive (Dorny et al. 2003). Computed tomography (CT) and magnetic resonance imaging (MRI) are two neuroimaging techniques that are used for accurate diagnosis of the disease in recent years (Bhattarai 2010). The chemotherapeutic treatment for NCC associated epilepsy was found to be analgesics, corticosteroids, and/or a combination of both. Albendazole was found the most used and effective anthelminthic drug for the treatmentof multiple lesion (Joshi et al. 2014).

This study determined the seroprevalence of neurocysticercosis in the patients with neurological problems. A total 80 samples 9from the patients with neurological problem visiting Neurology Department of IOM and Ag-ELISA was used for the detection of viable metacestode antigens. Among total samples, 5 were found to be positive for metacestode circulating antigens. The prevalence rate of neurocysticercosis was found to be 6.25%. Previously, Ale (2010) reported 13.63% prevalence and Lama (2012) reported 10.56% prevalence among the suspected patients the department of IOM. But the sample size of the suspected patients were varied as 22 (Ale 2010), 142 (Lama 2012) and present study it was 80.

Other hospital based studies also showed similar results like 7.18% in Patan Hospital (Choudhary 2006), 5% in patients with chronic headache in BPKIHS, Dharan (Sah et. al. 2014), 16% in Bir Hospital (Ojha et al. 2015), 0.10% in Nepalgunj Medical College (Piryani et al. 2007) and overall rate of 9.8% among epilepsy patients in the five hospitals of Kathmandu valley TUTH, Bir, Patan, Norvic and Nepal Medical College, (Karna et al. 2009) .

Globally, about 11% and 10.8% prevalence has been reported from several hospitals of Thailand (Singh et al. 2002) and capital Lome, Africa (Grunitzky et al. 1995) respectively. Several community based studies showed the similar prevalence such as 3.2% in Shangdon, China (Cao et al. 1996), 10% in Los Angeles and 6% in New Mexico (Garcia et al. 2003), 9.1% in Canoabo, 6.1% in Sanare, and 5.7% in Rio Tocuyo communities of Venezuala (Ferer et al. 2003), 1.8% in rural area of S. California (De Giorgio et al. 2005), 10% in the rural area of Northern Vietnam (Trung et al. 2013), 7% in Spain (Zammarchi et al. 2013) etc .

Ethnic-wise, in present study, prevalence of NCC was highest among Madhesi (22.2%) was found with higher cysticercosis infestation followed by janajati (8.7%) and very less proportion of others Chhetri caste 5.3% but no prevalence in Brahmin. But the association was insignificant (P>0.05) as shown by Sah et al. (2012). A study conducted by Rai et al (2002) showed that parasite positive rate was higher in Dalits (known as low socio-economic group of Nepal) than other ethnic groups with insignificant association.

In present study, the sero-positivity of neurocysticercosis was higher in male (60%) than in female (40%) among the patients visiting Neurology Depart of TUTH. Several studies carried out in other countries indicated similar result such as in TUTH (Lama 2012), Chitwan Medical college (Karna 2009), a teaching hospital of Kaski district (Shrestha et al. 2013) , Shree Birendra Hospital, Kathmandu (Joshi et al. 2007) and Manipal Hospital, Pokhara (Sapkota 2015), BPKIHS Dharan (Sah et al. 2014), Bir Hospital, Kathmandu, (Ojha et al. 2015) etc. The study of Wandra et al. (2003) in Indonesia, Willingham et al. (2003) in Vietnam showed similar result but the prevalence of infection was statistically not significant by sex. Though many studies showed the high prevalence of disease in male, there is no significant association between the disease and the patient's gender ($P>0.05$) and its co-incident (Sapkota 2015). In contrast to these results, some studies have shown that females are more affected compared to male. A study conducted in Nepal by Shrestha et al. (2013) in Charak hospital Pokhara, Sah (2014) in BPKIHS, Dharan showed that there were more girls suffering from NCC as compared to boys. The studies done by Ruiz-Garcia et al. (1997), Kossoff (2003) and Thakur et al. (1999) also showed similar result.

Age-wise scenario showed that the maximum incidence of neurocysticercosis was found in the age group between 21 and 30 years in Assam Medical College and Hospital (Kotokey et al. 2006). The present result coincide with this result. Similarly, higher prevalence in similar age-group was found in Manipal Teaching Hospital, Pokhara, (Sapkota 2015), TUTH (Lama 2012 and Agrawal 2006), Chitwan Medical College (Karna 2009), Northwest, India (Girotra et al. 2012) etc. However, association between the disease and the patient's age was insignificant ($P>0.05$). The study from Northwest India showed no significant association (Girotra et al. 2012). In majority of cases NCC are mostly seen after the age of seven (Shrestha et al. 2013). The reason could be because of their tendency to intake outside food and poor hygiene.

The present study showed the highest sero-positivity rate of neurocysticercosis among the people of terai region followed by hill. The association between the region from which the patients belong and the prevalence of the disease revealed insignificant ($P> 0.05$). Similarly, (Sah et al. 2014) determined that the prevalence of the disease was more in Terai (Sunsari district followed by Morang District). But, the study conducted by Shrestha (2013) in

western region of Nepal showed that the disease occurs more in Hilly districts, Kaski district (28.6%) followed by Tanahun (26.53%) and Baglung (12.24%).

Knowledge, Attitude And Practices towards Neurocysticercosis

Human activities directly as well as indirectly impact the lifecycle of *T. solium / T. saginata* as man is responsible for dispersion of the parasite's egg through outdoor defecation and improper disposal of faeces. Health education to cause behavioral changes in these practices can be an effective intervention strategy (Mishra et. al. 2008).

Among 200 respondents, half of the respondents heard about NCC but most of them don't know proper mode of transmission as shown among the people of North west India (Girotra et al. 2011), Kerela, South India. The knowledge of NCC was found statistically significant between NCC patients and general population of Kerela, South India (Radhakishan et al. 2000). But in Mexico (Sarti et al. 1997), Delhi (Mishra et. al. 2008), Iringa rular district of Tanzania (Chacha et al 2014) and South Indian community (Alexander et al. 2012), only few respondents had heard about NCC. People of Kaduna metropolis, Nigeria too had very low knowledge about *T. solium* infection (Edia-Asuke et al. 2014).

In present study, only half of the respondents had heard about tapeworm with insufficient knowledge about infection. Statistically, there is significant association between the gender, education and age of the people and their association with knowledge of the tapeworm ($P<0.005$). Similar to this result, boys had more knowledge about human cysticercosis than girls in Mbulu district of northern Tanzania while the association was not significant age-wise (Mwidunda et al. 2015). The knowledge regarding the responsible causative agent for the NCC was poor in Northwest India (Girotra et al. 2011).

The present study showed about 50% respondents were known or had heard about the zoonotic diseases but mostly they had idea about very common zoonotic diseases like rabies, swine-flu etc. Similar to this study in Tanzania, rabies, tuberculosis and anthrax were considered the three most common zoonotic diseases (Swai et al. 2010). In present study, only few respondents knew that it spreads by contaminated food and water. A study conducted by Maharjan and Gaihre (2010) also showed that the pig farmers of Magar communities in Syangja had no idea about the way of transmission of the disease. Some had

idea about symptom of NCC while some other thought it is fatal disease. But awareness regarding presenting symptoms was greater among respondents and the knowledge regarding prevention of NCC was poor among respondents in India (Girotra 2011). Among the patients visiting an epilepsy clinic in India show that out of the 182 respondents, 12% believed that a worm was responsible for their seizures, while 52% were aware of the link between worms and epilepsy (Sankhyan et al. 2015). Different studies showed different view about the cause of disease like in Kerela, South India, few (12.9%) knew tapeworm as a causative agent for NCC (P = 0.092) and some believed that it spreads by drinking contaminated water (Radhakrishan et al. 2000), in Iringa rural people believed that epilepsy is caused by evil spirits, witchcraft, infected pork and inheritance (Chacha et al 2014).

Regarding NCC, present study revealed that some of respondents thought that the pork trade should be banned to stop spreading of such animal-borne diseases while other thought that promotion of meat trade should be done instead people should be more conscious about personal cleanliness and hygienic food habits. Mostly, brahmin/chhetri ethnic group thought that pork trade should be stopped (P< 0.05). Service holders had view that pork trade should not be banned food despite of that people's food habits should be healthy (P<0.05). Even though 49.1% (n=71) respondents had heard about NCC, they don't have proper idea about treatment and control measure of the disease. Among the respondents known or having idea about NCC, 28.2% thought that epilepsy is caused due to NCC. There is significant association between the respondents thought about epilepsy and their ethnicity (P < 0.05).

Regarding treatment of NCC, a large proportion of NCC patients (45.7%) believed in ayurvedic treatment in Kerela state of India (Radhakrishan et al. 2005) and in Northwest India, patients preferred other indigenous treatments in addition to allopathic treatment (Girotra et al. 2011) which is in contrast to this study. Statistically, significant association was revealed with the age of the respondents (P < 0.05). Respondents of age above 50 years had this type of thought. Most of them confess that they generally feel relief by visiting local witch doctors than the allopathic treatment.

The practices can be the risk factors for *Taenia solium* infection. This study showed that included the type of meat consumed such as pork consumption, open defeacating practice (7%), hand washing with nothing after using toilets (8.3%) can be some related risk factors.

Mostly, people above 50 years use either nothing or anything to wash hands after using toilet ($P<0.05$). The hill Janjati more commonly raised pigs, but tended to keep their pigs in sheds, where as the Terai Janjati and Dalit preferred the free ranging systems. Generally, most of the people don't have more practice of open defecation. But still some people from rural areas had such practices. It is more in practice among old age- people and lower caste. But there is no significant association. Similarly, most of the people of Northern Vietnam, used toilet (Le et al. 2012). In contrast to this, in South Indian community a widely prevalent practice of open-field defecation (Alexander et al. 2012), one-third of the population had toilets at their homes and only 83.3% of them use toilet, with 74% practising open defecation (Mishra et al. 2007) in the same region. In Eastern terai of Nepal, open defecation was significantly more common ($P < 0.005$) among Dalits and Terai Janjati than among hill Janjati and high-caste Hindus (Devleesschauwer et al. 2012). In Eastern Zambia, Africa, the use of toilet was found to be very less (Thys et al. 2015).

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Neurocysticercosis is considered to be an important cause of epilepsy. In order to screen NCC among the symptomatic and asympotomatic patients with neurological problems, total 80 blood samles were collected and tested by Ag- ELISA and a questionnaire suvey was done among 200 patients visiting the Neurology Department to asses the knowledge, attitudes and practices regarding the disease. The overall prevalence of NCC was found to be 6.25% with the highest sero-positivity among the madhesi ethnic group (22.2%) followed by janajati and chhetri. The higher prevalence of NCC was found in male (8.1%) as compared to female (4.6%). The sero-positivity rate was found maximum in the age group 10-30 years. Region-wise, highest serpositivity was found in the Terai followed by Hill. But statistically there was insignificant association with ethnicity, gender, age and region of the patients to that of the disease.

The questionnaire survey revealed that majority of the patients had not heard about NCC and are unknown about symptoms, route of transmission, and prevention of the NCC. Though the majority of the respondents had heard about epilepsy but they were unknown about its cause. About tapeworm maximum male respondents had heard and about half of the respondents had heard about very common zoonotic diseases such as rabies. Among the respondents known or having idea about NCC, few thought that epilepsy is caused due to NCC which is statistically significant with ethnicity. Especially, janajati had view that epilepsy is caused by NCC. The assessment of attitude showed that mostly, brahmin/chhetri ethnic group thought that pork trade should be stopped but according to the service holders instead of banning the trade, healthy food habits should be promoted. Some of the old ages respondents especially, those of ages above 50 years and female respondents had traditional view regarding treatment of epilepsy. The practices that can be the risk factors for *Taenia solium* infection included the type of meat consumed such as pork consumption, open defeacating practice, no use of soap for washing hands after using toilet. Mostly, people above 50 years use no soap wash hands after using toilet. Majority of the meat consuming respondents consume all kinds of meat also most of them consume meat except pork while male are most likely to have all

type of meat. Though some of the respondents have the record of medical history of stool test, they don’t have idea of the parasitic infection. In general, people's knowledge and attitude showed that they are unknown and ignorant about the epilepsy and NCC. It is a neglected but important neurological problem in our country.

6.2 Recommendations

- Symptomatic and asymptomatic neurological patients must be screened for NCC as routine diagnostic test.
- In order to indicate the healthy food habits and to break parasite's life cycle, government should strictly inforce animal slaughtering and meat inspection act.
- NCC is still neglected disease in the country, in order to reduce the morbity and mortality due to NCC the disease needs to be included in priority list and preventive and control programs should be formulated through out the country particularly in terai region.

REFERENCES

Afonso, S.M., Vaz, Y., Neves, L., Pondja, A., Dias, G., Willingham, A. L., et al. 2011. Human and porcine Taenia solium infections in Mozambique: identifying research priorities. Animal Health Research Review **12**(1):123-129.

Agapejev, S. 1996. Epidemiology of neurocysticercosis in Brazil. Revista do Instituto Medicina Tropical Sao Paulo **38**(3):207-216.

Agrawal, J.P. 2006. Clinical aspects of neurocysticercosis in teaching hospital, Institute of Medicine. Proceedings of present situation challenges in treatment and elimination of Taeniasis/cysticercosis in Nepal,edited by Joshi DD, Sharma M, Rana S. National Zoonoses & Food Hygeine Research Centre, Kathmandu:15-19.

Agrawal, J.P. 2011. Neurocysticercosis in Nepal and it's Global Perspective. Kathmandu University Medical Journal **9**(2):1-2.

Agudelo-Flórez, P., Restrepo, B.N. and Palacio, L.G. 2009. Knowledge and practices concerning taeniasis-cysticercosis in Colombian pig-breeders. Rev Salud Publica (Bogota) **11**(2):191-199.

Ale, A. and Joshi, D.D. 2010. Prevalence of *Taenia solium* Cysticercosis in swine and Neurocysticercosis in human in Kathmandu valley an its impact on public health. Zoonosis and Food Hygiene News **16**.

Alexander, A.M., Mohana, V.R., Muliyila, J., Dorny, P. and Rajshekhar, V. 2012. Changes in knowledge and practices related to taeniasis/cysticercosis after health education in a south Indian community. International Health **4**:164– 169.

Allan, J.C., Avila, G., Brandt, J., Correa, D.O., Del Brutto, H., Dorny, P., et al. 2005. WHO/FAO/OIE Guidelines for the Surveillance, Prevention and Control of Taeniosis/Cysticercosis. 131-139.

Almeida, C.R., Ojopi, E.P., Nunes, C.M., Machado, L.R., Takayanagui, O.M., Livramento, J. A, et al. 2006. *Taenia solium* DNA is present in the cerebrospinal fluid of neurocysticercosis patients and can be used for diagnosis. European Arch Psychiatry Clin Neuroscience **256**:307–310.

Anantaphruti, M.T., Okamota, M., Yoonuan, T., Saguankiat, S., Kusolsuk, T. Sato, M., et al.. 2010. Molecular and serological survey on Taeniasis and cysticercosis in Kanchanaburi Province, Thailand. Parasitology International Journal **59**:326-330.

Angels, D., Urbani, C., Belotto, A., Meslin, F., and Savioli, L. 2003. The Control of human (neuro)cysticercosis: which way forward? Acta Tropica **87:**117-182,

Assana, E., Amadou,F.,Thys,E., Lightowlers,M. W., Zoli, A.P., Dorny, P., and Geerts, S. 2010. Pig-farming systems and porcine cysticercosis in the north of Cameroon. Journal of Helminthology **84**(4): 441–446.

Assana, E., Lightowlers, M.W., Zoli, A.P., Geerts, S. 2013. Taenia solium taeniosis/cysticercosis in Africa: risk factors, epidemiology and prospects for control using vaccination. Veterinary Parasitology **195**(1-2):14-23.

Bardosh, K., Inthavong, P., Xayaheuang, S. and Okello A.L. 2014. Controlling parasites, understanding practices: The biosocial complexity of a One Health intervention for neglected zoonotic helminths in northern Lao PDR. Social Science & Medicine**120:** 215–223.

Basu S, Ramchandran U and Thapliyal A.C. 2007. linical profile and outcome of pediatric neurocysticercosis: A study from Western Nepal. Journal of Pediatric Neurology **5**(2007): 45–52.

Bern, C. Garcia, H.H., Evans, C. Gonzalez A.E., Verastegui. M., Tsang, V.C., et al. 1999. Magnitude of the disease burden from neurocysticercosis in a developing country. Clinical Infectious Diseases **29:**1203–1209.

Bhattarai, R. 2010. Estimating the Burden of Neurocysticercosis in Mexico. Office of Graduate Studies of Texas A&M University.

Bhattarai, R., Budke, C.M., Carabin, H., Proaño, J.V., Flores-Rivera, J., Corona, T., et al. 2012. Estimating the non-monetary burden of neurocysticercosis in Mexico.PLoS Neglected Tropical Diseases **6**(2):e1521.

Bhattarai, T.C. 2005. Nepalese Poultry Industries and strategies for sustainable development. Proceedings: Technical seminar and workshop, National Poultry Expo-**2005**:166-171.

Carabin, H., Krecek, R.C., Cowan, L.D., Michael, L., Foyaca-Sibat, H., et al. 2006. Estimation of the cost of *Taenia solium* cysticercosis in Eastern Cape Province, South Africa. Tropical Medicine and International Health **11**: 906-916.

Carod, J.F., Randrianarison, M., Razafimahefa, J., Ramahefarisoa, R. M., Rakotondrazaka, M., Debruyne, et al. 2012. Evaluation of the performance of 5 commercialized enzyme immunoassays for the detection of *Taenia solium* antibodies and for the diagnosis of neurocysticercosis. Diagnostic microbiology and infectious disease **72**(1):85-89.

Chacha, M., Yohana, C., Nkwengulila G. 2014. Indigenous Knowledge, Practices, Beliefs and Social Impacts of Porcine Cysticercosis and Epilepsy in Iringa Rural. Scientific Research Publishing **6:** 2894-2903.

Chaudhary, S. 2006. Present situation of Neurocysticercosis in Patan Hospital , In: Preceedings of present situation challenges in treatment and elimination of TaeniasisL Cysticercosis in Nepal, Kathmandu. Organized by Natioanl Zoonoses and Food Hygiene Research Centre (NZHRC), Chagal, Kathmandu, Nepal. Pp. 155-161.

Christina, M., Coyle, H. and Tanowitz. B. 2009. Diagnosis and Treatment of Neurocysticercosis. Interdisciplinary Perspectives on Infectious Diseases **2009:** 1-9.

Corona, T., Paxol, D., Gonzalez Barranco, D., Abad, P., Landa, L., Estanol, B. 1986 Anticysticercosis antibodies an serum and cerebrospinal fluid an patients with cysticercosis. Journal of Neurology Neurosurgery Psychiatry **49**:1044-1049.

Crimmins, D., Collignon, P. I., Dwyer, D. and Gytis, D. 1990. Neurocysticercosis: an under-recognized cause of neurological problems. The Medical Journal of Australia **152:**434-438.

Deckers, N., and Dorny. P. 2010. Immunodiagnosis of *Taenia solium* taeniosis/ cysticercosis. Trends in Parasitology **26:**137-144.

DeGiorgio, C.M., Medina, M.T., Durón, R., Zee, C., Escueta, S.P. 2005. Neurocysticercosis. Epilepsy Currents **4**(3):107-111.

Del Brutto**,** O**.**H**.** 2012**.** A review of cases of human cysticercosis in Canada. Canadian Journal of Neurological Sciences **39**(3)**:**319-322.

Del Brutto, O.H., Wadia, N.H., Dumas, M., Cruz, M., Tsang, V.C. and Schantz, P.M. 1996. Proposal of diagnostic criteria for human cysticercosis and neurocysticercosis. Journal of . Neurology Science **142**:1–6.

Del Brutto, O.H. 2012. Diagnostic criteria for neurocysticercosis, revisited. Pathogens of Global Health **106**(5):299-304.

Del Brutto, O.H., García, H.H. 2012. Neurocysticercosis in Nonendemic Countries: Time for a Reappraisal. Neuroepidemiology **39:**145–146.

Devleesschauwer, B., Allepuz, A., Dermauw, V., Johansene, M.V., Laranjo-González, M., Suzanne, A.G. et al. 2015 *Taenia solium* in Europe: Still endemic? Acta Tropica **3704**:1-4.

Devleesschauwer, B., Aryal, A., Joshi, D.D., Rijal, S., Sherchand, J.B., Praet, N. et al. 2012.Epidemiology of Taenia solium in Nepal: is it influenced by the social characteristics of

the population and the presence of Taenia asiatica? Tropical Medicine and International Health **17**(8): 1019-1022.

Diaz, J.F., Verastegui, M., Gilman R.H., Tsang, V.C., Pilcher, J.B., Gallo, C., et al. 1992. Immunodiagnosis of human cysticercosis (Taenia solium): a field comparison of an antibody-enzyme-linked immunosorbent assay (ELISA), an antigen-ELISA, and an enzyme-linked immunoelectrotransfer blot (EITB) assay in Peru. The Cysticercosis Working Group in Peru (CWG). The American Journal of Tropical Medicine and Hygiene **46** (5):610-615.

Dorny, P., Brandt, J., Zoli, A. and Geerts, S. 2003. Immunodiagnostic tools for human and porcine cysticercosis. Acta tropica **87**: 79-86.

Dorny, P., Phiri, I.K., Vercruysse, J., Gabriel, S., Willingham, A.L. 3rd, Brandt, J., Victor, B., Speybroeck, N. and Berkvens, D. 2004. A Bayesian approach for estimating values for prevalence and diagnostic test characteristics of porcine cysticercosis. International Journal of Parasitology **34**(5):569-76.

Dorny, P., Praet, N., Deckers, N. and Gabriel, S. 2009. Emerging foodborne parasites. Veterinary Parasitology **163**:196–206.

Dorny, P., Vallée, I., Alban, L., Boes J, Boireau P, et al. 2010. Development of harmonised schemes for the monitoring and reporting of Cysticercus in animals and foodstuffs in the European Union. European Food Safety Authority.

Edia-Asuke, A.U., Inabo, H.I., Umoh, V.J., Whong, C.M., Asuke, S., Edeh, R. E. 2014. Assessment of sanitary conditions of unregistered pig slaughter slabs and post mortem examination of pigs for Taenia solium metacestodes in Kaduna metropolis, Nigeria. Infectious Diseases of Poverty **3**(1):45.

Erhart, A., Dorny, P., Van De, N., Vien, H.V., Thach, D.C., Toan, N.D., et al. 2002. Taenia solium cysticercosis in a village in northern VietNam: seroprevalence study using an ELISA for detecting circulating antigen. Transactions of the Royal Society of Tropical Medicine and Hygiene **96**(3):270-272.

Fabiani S and Bruschi F. 2013. Neurocysticercosis in Europe: Still a public health concern not only for imported cases. Acta Tropica **128**(1):18-26.

Ferrer, E., Cabrera, Z., Rojas, G., Lares, M., Vera, A., de Noya, B. A. et al. 2003. Evidence for high seroprevalence of Taenia solium cysticercosis in individuals from three rural communities in Venezuela. Transactions of Royal Soceity of Tropical Medicine and Hygiene **97**(5):522-516.

Figueroa, J.J., Davis, L.E., Magalhaes, A. 2011. Extraparenchymal neurocysticercosis in Albuquerque, New Mexico. Journal of Neuroimaging **21**(1):38-43.

Florez, N., Martí-Bonmatí, L., Forner, J., Arana, E., Moratal, D. 2009. Normal values for cerebrospinal fluid flow dynamics in the aqueduct of Sylvius through optimized analysis of phase-contrast MR images**.** Radiologia **51**(1):38-44.

Foo, S.S., Selvan, V.S., Clarke, M.J. and Shen, E.J. 2008. Unusual cause of seizures in Singapore: neurocysticercosis. Singapore Medical Journal **49**(6):147-150

Freitas, F.I., Meza-Lucas, A., Lima, C.B., Costa, Wd., Melo, A. 2005. Cysticercosis research in epileptic patients dwelling in towns of the western Cariri in the State of Paraíba, Brazil. Arquivos de Neuropsiquiatria **63**(3A):656-660.

Gaihre, Y.K. 2000. Prevalence of intestinal helminth parasites in general, ascariasis in detail in Sarkies and Magars community of Tindobate VDC, Syangja, Kathmandu, Nepal. M. Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu Nepal.

Garcia, H.H. and Del Brutto, O.H. 1999. Heavy nonencephalitic cerebral cysticercosis in tapeworm carriers. Neurology **53**(7):1582-1584.

Garcia, H.H., Evans, C.A.W., Nash, T.E. et al. 2002. Current consensus guidelines for treatment of Neurocysticercosis. Clinical Microbiology Reviews **15**:74-76.

Garcia, H.H., Herrera, G. And Gilman, R.H. 1994. Discrepancies Between Cerebral computed tomography and western blot in the diagnosis of neurocysticercosis patients. American Journal of Tropical Medicine and Hygiene **50**:152-157.

García, H.H., González, A.E., Del Brutto, O.H., Tsang, V.C., Llanos-Zavalaga, F., Gonzalvez, G.et al. 2007. Cysticercosis Working Group in Peru. Strategies for the elimination of taeniasis/cysticercosis. Journal of Neurological Sciences **262**(1-2):153-157.

Garcia, H.H. and Del Brutto O.H. 2000. Taenia solium cysticercosis, Infectious Disease Clinics of North America **14**:97–119.

Garcia, H.H., Gilman, R.H., Horton J ., Martinez, M., Her-Rera, G., Altamirano, J ., et al. 1997. Albendazole Therapy For Neurocysticercosis: A Prospective Double-Blind Trial Comparing 7 Versus 14 Days Of Treatment. Neurology **48**:1421- 1427.

Garcia H.H., Parkhouse R.M.E., Gilman R.H., Montenrgro T., Bernal T., Martinez S.M., et al. 2000. Serum Antigen Detection In The Diagnosis, Treatment, And Follow- Up Of Neurocysticercosis Patients. Transactions of The Royal Society Of Tropical Medicine And Hygiene **94**:673-676.

Garcia, H.H. and Del Brutto, O.H. 2003. Imaging findings in neurocysticercsis. Acta tropica **87**:71-78.

García, H.H., Gonzalez, A..E, Evans, C.A.W., Gilman, R.H. and for the Cysticercosis Working Group in Peru: 2003. *Taenia solium* cysticercosis **362**:547-556**.**

Garcia-Noval, J., Moreno, E., de Mata, F., Soto de Alfaro, H., Fletes, C., Craig, P.S., et al. 2001. An epidemiologicalstudy of epilepsy and epileptic seizures in two rural Guatemalan communities. Annals of Tropical Medicine and Parasitology **95**(2):167–175.

Girotra, M., Gera, C., Abraham, R.R., Gauba, R., Bansal, T., Kaur,P. et al. 2011. Awareness of neurocysticercosis: A study from northwest India. Annals of Indian Academy of Neurology **14**(1):27–30.

Goel, D., Dhanai, J.S., Agarwal, A., MehlotraV. and Saxena, V. 2011. Neurocysticercosis and its impact on crude prevalence rate of epilepsy in an Indian community. Neurology India **59**(1):37-40.

Gonzales, I., Miranda, J.J., Rodriguez, S., Vargas, V., Cjuno, A., Smeeth, L., et al. 2015. Cysticercosis Working Group in Peru. Seizures, cysticercosis and rural-to-urban migration: the Peru Migrant study. Tropical Medicine and International Health **20**(4):546-552.

Gracia, F., Chavarria, R., Archbold, C., Larreategui, M., Castillo, L., Schantz, P., Reeves, W. C. 1990. Neurocysticercosis in Panama: preliminary epidemiologic study in the Azuero region. The American Journal of Tropical Medicine and Hygiene **42**(1):67-69.

Handique, S.K., Das, R.R., Saharia, B., Das, P., Buragohain, R., Saikia, P. 2008. Coinfection of Japanese encephalitis with neurocysticercosis: an imaging study. American Journal of Neuroradiology **29**(1):170–175.

Hauser, W.A. and Hesdorffer, D.C. 1990. Epilepsy: frequency, causes, and consequences: Epilepsy Foundation of America Landover, MD.

Heap, B.J. 1990. Cerebreal Cysticercosis as a Common Cause of Epilepsy in Gurkhas in Hongkong. Journal of the Royal Army Medical Corps **136**:146-149.

Hernandez, M., Gonzalez, L.M., Fleury, A., Saenz, B., Parkhouse, R.M., Harrison, L.J., et al. 2008. Neurocysticercosis: detection of *Taenia solium* DNA in human cerebrospinal fluid using a semi-nested PCR based on HDP2. Annual Tropical Medecine Parasitology **102**:317–323.

Isaac, K.P., Ngowi, H., Afonso, S., Matenga, E., Boa, M., Mukaratirwa S., et al. 2003. The emergence of Taenia solium cysticercosis in Eastern and Southern Africa as a serious agricultural problem and public health risk. Acta Tropica **87**:13-23

Ito, A., Minoru Nakao, M., Wandra, T., Suroso, T., Okamoto, M., Yamasaki, H. et al. 2005 Taeniasis And Cysticercosis In Asia And The Pacific: Present State Of Knowledge And Perspectives. Southeast Asian Journal of Tropical Medicine and Public Health **36**(4):123-130

Joshi, D.D., Bista, P.R., Ito, A. and Yamasaki, H. 2007. Present situation of Porcine Taeniasis and Human Cysticercosis in Nepal. Southeast Asian Journal of Tropical Medicine and Public Health **38**:144-150.

Joshi, B., Pant, B., Rajbhandari, P., Aganja, R.P.,Malla, R., Manandhar, K.D., and Sreerama, L. 2014.The Epidemiology and Health Burden of Neurocysticercosis in Nepal. International Journal of Tropical Disease and Health **4**(2):204-223.

Joshi, D.D., Oommen, A., Dorny, P., Bista, P.R., Rajshekhar, V., and Vercruysse, J. 2011. Sero-Prevalence of Porcine Cysticercosis and Human Taeniasis/Cysticercosis in Nepal. Nepalese Veterinary Journal **30**:110-118,

Joshi, D.D., Maharjan, M. , Johnsen, M.V., Willingham, A.L., Gaihr, Y. and Sharma, M. 2004. Taeniasis/Cysticercosis Situatin in Nepal. Southeast Asian Journal of Tropical Medicine and Public Health **25:**252-258.

Joshi, D.D., Poudyal, P.M., Jimba, M., Mishra, P.N., Neave, L. and Maharjan, M. 2001. Epidemiological status of taenia/cysticercosis in pigs and human in Nepal. Journal of the Institute of Medicine **23**:1 –12

Karna, A.K. and Joshi, D.D. 2009. Prevalence of Taenia solium Cysticercosis in swine in Kathmandu Valley. American Journal of Tropical Medicine and Hygiene. **15**(3).

Khan, F.Y., Imam, Y.Z., Kamel, H. and Shafaee, M. 2011. Neurocysticercosis in Qatari patients: case reports. Travel Medicine and Infectious Diseases **9**(6):298-302.

Kotokey, R.K., Lynrah, K.G., and De, A. 2006. A Clinico-serological Study of Neurocysticercosis in Patients with Ring Enhancing Lesions in CT Scan of Brain.The journal of The Associationa of Physicians of India **54**:367-70.

Kung, I.T.M., Lee, D., Yu, H.C. 1989. Soft tissue cysticercosis. Diagnosis by fine-needle aspiration. American Journal of Clinical Pathology **92**(6):834–835.

Lama, S. 2012. Prevalence of *Taenia solium* Cysticercosis in pigs and human in Nepal. M.Sc. Thesis. Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal.

Le, T.T., Luu, N.H., Rheinländer ,T., Dalsgaard, A., Konradsen, F. 2012. Sanitation behavior among schoolchildren in a multi-ethnic area of Northern rural Vietnam. BMC Public Health. **12**:140.

Lee Willingham, A.L., III, Leslie, J., Harrison, Eric, M., Fèvre, and Michael, E. Parkhouse. 2008. Inaugural Meeting of the Cysticercosis Working Group in Europe. Emerging Infectious Diseases. **14**(12):e2.

Lightowlers**,** M**.**W**.** 2010**.** Eradication of Taenia solium cysticercosis: a role for vaccination of pigs. International Journal of Parasitology **40**(10):1183-92.

Mahajan, R.C. 1982. Geographical distribution of human cysticercosis. In: Flisser A, Willms K, LaClette JP, Larralde C, eds. Cysticercosis: present state of knowledge and perspectives. New York: Academic Press, **1982**:39 -46.

Maharjan, M. and Gaihre Y. 2010. Porcine Cysticercosis in the Magar Community of Syangja district. Lifescience Journo- Magazine **2**:20-26.

Mamkin, I., Sood, N. and Ramanan, S. 2007. *Taenia solium*-Neurocysticercosis. The New England Journal of Medicine **357**(16):1666-1667.

Mandal, J., Singhi, P.D., Khandelwal, N., Malla, N. 2006 Jan. Evaluation of ELISA and dot blots for the serodiagnosis of neurocysticercosis, in children found to have single or multiple enhancing lesions in computerized tomographic scans of the brain: Annals of Tropical Medicine and Parasitology **100**(1):39-48.

Martins-Melo F.R., Ramos, A.N .Jr., Cavalcanti, M.G., Alencar, C.H. and Heukelbach, J. 2015. Neurocysticercosis-related mortality in Brazil, 2000-2011: Epidemiology of a neglected neurologic cause of death. Acta Tropica **153**:128-36.Mason, P., Houston, S. and Gwanzura, L. 1992. Neurocysticercosis: experience with diagnosis by ELISA serology and computerised tomography in Zimbabwe. The Central African Journal of Medicine **38**(4):149-154.

McKelvie, P.A., Goldsmid, J.M. 1988. Childhood central nervous system cysticercosis in Australia. The Medical Journal of Australia **149**(1):42-44.

McManus, D.P. 2006. Molecular discrimination of taeniid cestodes. Parasitology International **55**:31–37.

Medina, M.T., Rosas, E., Rubio-Donnadieu, F., Sotelo, J. 1990. Neurocysticercosis as the main cause of late-onset epilepsy in Mexico. Archives of Internal Medicine **150**:325-327.

Michelet, L., Fleury, A., Sciutto, E., Kendjo, E., Fragoso, G., Paris, L., et al. 2011.Human neurocysticercosis: comparison of different diagnostic tests using cerebrospinal fluid. Journal of Clinical Microbiology **49**:195–200

Mishra, D., Kalra, V. and Agrawal, K. 2008. Awareness about taeniasis and neurocysticercosis among municipal schoolteachers in Delhi. The Journal of communicable diseases **39**(4):225-228.

Mwanjali, G., Kihamia, C., Kakoko, D.V., Lekule F, Ngowi H, Johansen MV, Thamsborg SM, Willingham AL. 2013. Prevalence and risk factors associated with human Taenia solium infections in Mbozi District, Mbeya Region, Tanzania. PLoS Neglected Tropical Diseases **7**(3):e2102.

Mwidunda, S.A., Carabin, H., Matuja, W.B., Winkler, A.S., Ngowi, H.A. 2015. A school based cluster randomised health education intervention trial for improving knowledge and attitudes related to Taenia solium cysticercosis and taeniasis in Mbulu district, northern cysticercosis? Acta Cytologica **50**(1):114–115.

Nash, T. E., Del Brutto, O.H., Butman, J.A., et al. 2004. Calcific neurocysticercosis and epileptogenesis. Neurology **62**(11):934–1938.

Nash, T.E. and Neva, F.A. 1984. Recent advances in the diagnosis and treatment of Cerebral Cysticercosis. The New England Journal of Medicine **311**:1492-1496.

Ng, S.H., Tan, T.Y., Fock, K.M. 2000. The value of MRI in the diagnosis and management of neurocysticercosis. Singapore Medicine Journal **41**:132-134.

Nguekam, Zoli, A.P., Ongolo-zogo, P., Dorny, P., Brandt, J. and Geerts, S. 2003. Follow-up of neurocysticercosis patients after treatment using an antigen detection ELISA. Parasite. **10**(1):65–68.

Nicoletti A, Bartoloni A, Reggio A, Bartalesi F, Roselli M, et al. 2002. Epilepsy, cysticercosis, and toxocariasis: a population-based case-control study in rural Bolivia. Neurology **58**:1256-1261.

OIE, 2004. Manual of diagnostic tests and vaccine for terestrial animals, Cysticercosis, Paris: Part 2, Section 2.10, Chapter 2.10.1.

Ojha**,** R., Shah, D.B., Shrestha, A., Koirala, S., Dahal, A., Adhikari, K., et al. 2015. Neurocysticercosis in Nepal: aretrospective clinical analysis. Neuroimmunology and Neuroinflammation **2**(3):167-170

Otte, W.M.and Alo, C. 2014. Epilepsy prevalence, potential causes and social beliefs in Ebonyi State and Benue State, Nigeria Chijioke Osakwe. Epilepsy Research **108**:316-326

Pal, D.K., Carpio, A., Sander, J.W. A. S. 2000. Neurocysticercosis and epilepsy in developing countries.:Journal of Neurology, Neurosurgery and Psychiatry **68**(2):137-143.

Pant, B., Devleesschauwer, B., Shrestha, P., Shrestha, I., Praet, N. and Dorny, P. 2011. Intraventricular Taenia solium Neurocysticercosis: A Report of Three Cases. Journal of Nepal Medical Association **51**(184):192-195.

Parasca, I., Damian, L. and Albu, A. 2006. Infectious muscles disease. Romanian Journal of Internal Medicine **44**(2):131-141.

Piryani, R.M., Kohli, S.C., Shrestha, G., Shukla, A., Malla, T.B. 2007. Human neurocysticercosis managed at Nepalganj Medical college, Teaching Hospital, Kohalpur, Nepal. Kathmandu University Medical Journal **5**(4):518-20.

Piya, D., Manandhar, Y., Shrestha, S., Munakarmi, R. and Pokhrel, R. 2005. Prevalence pattern of types of epilepsy and prescribing pattern of antiepileptic drugs with focus on NCC in Neurosurgery Depart of Kathmnandu Model Hospital.

Poudel, D.R., Lhaden, T., and Shrestha, M. 2014. Disseminated Cysticercosis in a Child: A Rare Presentation; Case report. Journal of Institute of Medicine **36**:3.

Poudyal, P.M. 1998. Prevalence of *Taenia solium* in pigs and its public health importance in Kathmandu Metropolitan City and Dharan Municipality, Sunsari District of Nepal. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal, Master‟s thesis, pp. 50.

Pybus, M., Heron, P. 2012. Neurocysticercosis: an unusual cause of seizure in the New Zealand setting.Journal of Paediatrics and Child Health. **48**(3):119-121.

Raccurt, C.P., Agnamey, P., Boncy, J., Henrys, J.H., Totet, A. 2009. Seroprevalence of human Taenia solium cysticercosis in Haiti. Journal of Helminthology **83**(2):113-116.

Radhakrishnan, K., Pandian, J. D., Santhoshkumar, T., Thomas, S.V., Deetha, T.D. and Sarma, P.S. et al. 2000. Prevalence, Knowledge, Attitude, and Practice of Epilepsy in Kerala, South India. Epilepsia **41**:1027-1035.

Rai, S.K., Hirai, K., Abe, A. et al. 2002. Intestinal parasitosis among school children in a rural hilly area of Dhading District, Nepal. Nepal Medical College Journal **4**:54-58.

Rajbhandari, K.C.2003. Clinical Profile of epilepsy in Nepal. International Symposium on Epilepsy, Kathmandu, Nepal: NES. Pp. 69-73.

Rajshekhar, V., Chacko, G., Haran, R.P., Chandy, M.J., Chandi, S.M. 1995. Clinicoradiological and pathological correlations in patients with solitary cysticercus granuloma and epilepsy: focus on presence of the parasite and oedema formation. Journal of Neurology and Neurosurgery Psychiatry **59**:284-286.

Rajshekhar, V., Joshi, D.D., Doanh, N.Q. and Van De, N. 2003. Taenia solium taeniosis/cysticercosis in Asia: epidemiology, impact and issues. Acta Tropica **87**:53-60.

Rodriguez, S., Dorny, P., Tsang, V.C., Pretell, E.J., Brandt, J., Lescano, A.G.,et al. 2000. Detection of Taenia solium Antigens and Anti–T. solium Antibodies in Paired Serum and Cerebrospinal Fluid Samples from Patients with Intraparenchymal or Extraparenchymal Neurocysticercosis. Journal of Infectious Diseases **199**(9):1345-1352.

Rodriguez,S., Wilkins,P. and Dorny, P. 2012. Immunological and molecular diagnosis of cysticercosis. Pathogens of Global Health **106**(5):286–298

Rodriguez-Canul, R., Fraser, A., Allan, J.C., Dominguez-Alpizar, J.L., Argaez-Ruez, F., Craig, P.S. 1999. Epidemiological study of Taenia solium taeniasis/cysticercosis in a rural village in Yucatan state, Mexico. Annals of Tropical Medicine and Parasitology **93**(1):57-67.

Rodriguez-Carbaja,l J., Salgado, P., Gutierrez-Alvarado, R., Escobar-Izquierdo, A., Aruffo, C., et al. 1983.The acute encephalitic phase of neurocysticercosis: computed tomographic manifestations. American Journal of Neuroradiology **4**:51-55.

Roman G, Sotelo J, DelBrutto O, Flisser A, Dumas M, Wadia N, et al. 2000. A proposal to declare neurocysticercosis an international reportable disease; Bulletin of the world Health Organization **78**(3):399-406.

Rosas, N., Sotelo, J. and Nieto, D. 1986. ELISA in the diagnosis of neurocysticercosis. Arch. Neurol. **43**:353–356.

Ruiz-Garcia M, Gonzalez-Astiazaran A, Rueda-Franco F. 1997. Neurocysticercosis in Children: Clinical Experience in 122 Patients. Child Nervous System **13**:608-612.

Sah, R.B., Subedi, L., Pandey, N ., Sah, B.P., Jha, S., Shah, U., N Jha, N., and Pokharel, P.K. 2014. The epidemiology and health burden of neurocysticercosis in tertiary hospital of Nepal Journal of Chitwan Medical College **4**(8):13-16.

Sah, R.B., Pokharel, P.K., Paudel, I. S., Acharya, A., Jha, N. and Bhattarai. 2012. A study of Prevalence of Taenia Infestation and Associated Risk factors Among the School Children of Dharan. Kathmandu University Medical Journal **10**(39):14-27.

Sankhyan, P., Gupta, S. and Singh, G. 2015. Knowledge about, attitudes towards, practices regarding Taenia solium cysticercosis among people attending an epilepsy clinic in India. International Journal of Epilepsy **2**(1):6–10

Sapkota, S. 2015. Role Of Serlogy, Neuroimaging And Stool Examination In Diagnosis of Neurocystiercosis**.** Research Grant Section, Nepal Health Research Council (NHRC).

Sarkies and Magars community of Tindobate VDC, Syangja, Nepal. Kathmandu, Nepal.

Sarti, E., Flisser, A., Schantz, P.M., Gleizer, M., Loya, M., Plancarte, A. et al. 1997. Development and evaluation of a health education intervention against *Taenia solium* in a rural community in Mexico. The American Journal of Tropical Medicine and Hygiene **56**:127–32.

Schantz, P.M., Wilkins, P.P., Tsang, V.C.W. 1998. Immigrants, imaging, and immunoblots: theemergence of neurocysticercosis as a significant public health problem. Emerging infections **1998**:213-242.

Serpa, J.A. and White, A.C. 2012. Neurocysticercosis in the United States. Journal of Pathogens and Global Health **106**(5):256-60.

Shrestha B.M. 2008. Childhood Neurocysticercosis: clinico-radiological profile and outcome. Journal of Nepal Paediatric Society **28**(1):14-16.

Shrestha, B., Mainali, P., Sayami, S., and Shrestha, O.K. 2013.Clinico-radiological Aspects of Neurocysticercosis in Pediatric Population in a Tertiary Hospital. Journal of Nepal Medical Association **52**(191):483-488.

Shrestha, S.P., Dhungana, S. and Shrestha, A.K. 2013. Neurocysticercosis in children at GMC, Charak hospital, Pokhara. Journal of Chitwan Medical College **3**(4):20-23.

Singh, G., Prabhakar, S., Ito, A., Cho, S.Y., Qiu, D.C. 2002. *Taenia solium* taeniasis and cysticercosis in Asia. In: SinghG, Prabhakar S, eds. *Taenia solium* cysticercosis.From basic to clinical science. Oxon, UK:CABI

Singh,G. and Sappal, M. 2012. Neurocysticercosis-Indian Scenario. Medicine Update **22**:29-36.

Singhi P, Singhi S. 2009. Neurocysticercosis in children. Indian Journal of Pediatrics **76**(5):537– 545.

Sloan, L., Schneider, S. and Rosenblatt, J. 1995**.** Evaluation of Enzyme-Linked Immunoassay for Serological Diagnosis of Cysticercosis. Journal of Clinical Microbiology **33**(12):3124-3128.

Sorvillo, F.J., DeGiorgio, C., Waterman, S.H. 2007. Deaths from cysticercosis, United States. Emerging Infectious Diseases **13**:230-235.

Swai,E.S., Schoonman,L. and Daborn C.J. 2010. Knowledge and attitude towards zoonoses among animal health workers and livestock keepers in Arusha and Tanga, Tanzania. Tanzania Journal of Health Research **12**(4):280-286.

Teitelbaum, G.P. Otto, R.J., Wantanabe, A.T., Stull, M.A., Manz, H.J. et al. 1989. MR imaging of neurocysticercosis. American Journal of Roentgenology **153**:857-866.

Thakur, L.C., Ananda, K.S. 1991. Childhood Neurocysticercosis in South India. Indian Journal of Pediatrics **58**:15-19.

Thapa, R.B. 2000. Prevalence of Intestinal Helminth Parasites in General and Taenia sps. in detail, particularly in Bote and Darai communities of Vyash Municipality-5, Kumaltari, Tanahun District of Nepal. M. Sc. Thesis. Central Department of Zoology Tribhuvan University, Kathmandu, Nepal.

Thys, S., Mwape, K.E., Lefèvre, P., Dorny, P., Marcotty, T., Phiri, A.M., et al. 2015. Why Latrines Are Not Used: Communities' Percetions and Practices Regarding Latrines in a Taenia solium Endemic Rural Area in Eastern Zambia. PLoS Neglected Tropical Diseases 9(3): e0003570.

Trung, D.D., Praet, N., Cam, T.D., Lam, B.V., Manh H.N., Gabriël, S., et al. 2013. Assessing the burden of human cysticercosis in Vietnam. Tropical Medicine and International Health **18**(3):352-356.

Tsang, V.C.W., Peralta, J.M. and Simons, A.R. 1983. Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. Methods Enzymology **92**:377-391.

Tsang, V.C.W., Wilson, M. 1995. *Taenia solium* cysticercosis: an under-recognized but serious public health problem. Parasitology Today **11**:124-6.

Van de, N., Hoa Le, T., Huong Lien, P. T., and Eom, K. S.2014. Current Status of Taeniasis and Cysticercosis in Vietnam. Korean Journal of Parasitology **52**(2):125–129

Walker, J., Chen, S., Packham, D. and Mclntyre, P. 1991 Five Cases Of Neurocysticercosis Diagnosed In Sydney. Neurocysticercosis In Australia. The Southeast Asian Journal Of Tropical Medicine and Public Health **22**:242-244.

Wandra, T., Ito, A., Yamasaki, H., Suroso,T. and Margono, S. S.2003 . Taenia solium Cysticercosis, Irian Jaya, Indonesia. Emerging Infectious Diseases **9**(7): 884-885.

White, AC.1997. Neurocysticercosis: A Major cause of neurological disease worldwide. Clinical Infectious Diseases **24**:101-115

White, J.A.C. 2000. Neurocysticercosis: updates on epidemiology, pathogenesis, diagnosis,

Why Latrines Are Not Used: Communities' Perceptions and Practices Regarding Latrines in **9**(3): e0003570.

Willingham, A.L., Wu, H.W., Conlan, J. and Satrija, F. 2010. Combating *Taenia solium* cysticercosis in SoutheastAsia an opportunity for improving human health and livestock production. Advances in Parasitology **72**:235-266.

Wilson, M., Bryan, R.T., Fried, J.A., Ware, D.A., Schantz, P.M., Pilcher, J.B. et al. 1991. Clinical evaluation of the cysticercosis enzyme-lin1ked immunoelectrotransfer blot in patients with neurocysticercosis. Journal of Infectious Diseases **164**:1007-1009.
Winkler, A.S., Willingham, A.L., Sikasunge, C.S., Schmutzhard, E., and Wien, K.W. 2009. Epilepsy and neurocysticercosis in sub-Saharan Africa **3**:3-12.

Yamasaki, H., Muto, M., Morishima, Y., Sugiyama, H., Kawanaka, M., Nakamura-Uchiyama, F., et al. 2011. Taenia asiatica infection occurring in Kanto District, 2010. Japanese. Clinical Parasitology **22:**75-78.

Yanagida, T., Sako, Y., Nakao, M., Nakaya, K. and Ito, A. 2012. Taeniasis and cysticercosis due to *Taenia solium* in Japan. Parasites & Vectors **5**:18.

Zammarchi, L., Strohmeyer, M., Bartalesi, F., Bruno, E., Muñoz, J., Buonfrate, D., Nicoletti, A., García, H. H., Pozio, E., Bartoloni, A., and The COHEMI Project Study Group. 2013. Epidemiology and Management of Cysticercosis and *Taenia solium* Taeniasis in Europe, PLoS One **8**(7).e65937.

APPENDIX 1

Questionnaire Related to KAP

Name: Age:

Profession: Sex:

Family type: Joint ☐ Separate ☐ Education:

Knowledge

1. Do you know about epilepsy?

   Yes ☐ No ☐

2. If yes do you know about the causes of epilepsy?

   Yes ☐ No ☐

3. Do you know about zoonoic disease?

   Yes ☐ No ☐

4. Do you know about tapeworm?

   Yes ☐ No ☐

5. If yes, have you seen or get infected with the parasite?

   Yes ☐ No ☐

6. Do you know about measly pork or beef?

   Yes ☐ No ☐

7. If yes, how does it look like?
   i) white rice grain in meat ☐
   ii) nodules white in colour in meat ☐
   iii) small white balls in meat ☐

8. Do you know about neurocysticercous (NCC)?

   Yes ☐ No ☐

9. If yes, what type of disease is NCC?
   i) communicable and preventable ☐
   ii) Non-communicable and may be preventable ☐

10. How does human get infected with cysticercosis (NCC)?
    i) Contaminated food (vegetables), water ingestion ☐
    ii) Infected pork/beef consumption ☐
    iii) Don't know ☐

11. What is consequence of NCC?
    i) Epileptic symptom/ Neurological disorder ☐
    ii) Death ☐
    iii) Nothing ☐

Practice

12. Do you take meat?

    Yes ☐ No ☐

13. Which meat do you take frequently?

    Pork ☐ Beef ☐ All ☐

14. How do you prepare meat to eat?

    Well-cooked ☐ Half-cooked ☐ Raw-preparation ☐

15. Are you a pig/cattle (buffalo) farmer?

    Yes ☐ No ☐

16. If yes, how is the sanitation condition at your form?

    Well-maintained ☐ Satisfactory ☐ Not-maintained ☐

17. Do you have toilet at your home?

    Yes ☐ No ☐

18. If yes, what type of toilet do you have?

Temporary ☐ Permanent ☐

19. If no, where do you go?

Jungle ☐ Open area ☐ River side ☐

20. Do you wash your hands after toilet?

Yes ☐ No ☐

21. If yes, what do you use for washing your hands?

AS ☐ Soap ☐ Soli ☐ Nothing ☐

22. Have you ever checked your stool?

Yes ☐ No ☐

23. If yes, what type of parasites were found?

Ascaris ☐ Tapeworm ☐ Hookworm ☐ Don't know ☐

24. Where do you go for treatment?

Hospital ☐ Health post ☐ Witch doctors ☐

Attitude

25. Epilepsy is caused only by NCC ?

Agree ☐ Disagree ☐

26. Witch doctors can treat epilopsy (NCC)?

Agree ☐ Disagree ☐

27. NCC is the disease of all the pork/beef consumers and traders not the vegetarian ones?

Agree ☐ Disagree ☐

28. Pork/Beef meat and Pig/Buffalo trade should be totally banned?

Agree ☐ Disagree ☐

29. Human acquire cysticercus by ingesting *T. solium* eggs shed off by the person/pig suffering from taeniosis by auto infection or accidental ingestion through faecal-oral route.

Agree ☐ Disagree ☐

30. Way to control cysticercosis is:

i) Personal hxgiene and health food habits ☐
ii) Consumption of well-cooked meat ☐
iii) Both ☐
iv) Others ☐

Appendix-2

Buffers and product preparations

1. **Phosphate Buffered Saline (PBS)**
   The PBS buffer is prepared using tablets.
   1 tablet in 100 ml of RO-DI water yields a 100 ml PBS buffer, pH 7.3.

2. **Trichloroacetic acid (TCA)**
   The solution used for the "pretreatment" of the serum samples is a 5% $\left(\frac{w}{v}\right)$ solution in RO-DI water.

3. **Washing buffer**
   The washing buffer consists of PBS with 5% $\left(\frac{v}{v}\right)$ Tween 20.
   Examples 1: 1liter of PBS + 0.5 ml of Tween 20.

4. **Blocking buffer**
   The blocking buffer consists of washing buffer + 1% $\left(\frac{v}{v}\right)$ of Newborn Calf Serum (NBCS).

Examples: 49.5 ml of PBS-Tween 20 + 0.5 ml of NBCS

5. Coating buffer

The coating buffer is prepared using powder-filled capsules.
1 tablet in 10 ml of RO-DI water yields a 0.05 M carbonate/bicarbonate buffer, pH 9.6.

Alternative:
Stock solution A: Na_2CO_3 (0.06 M) = 0.159 g / 25 ml
Stock solution B: $NaHCO_3$ (0.06 M) = 0.504 g / 100 ml

10 ml A + 50 Ml B + 175 ml RO-DI water.
Carefully add A or B until pH to 9.6 is reached.
Adjust volume to 250 ml with RO-DI water.

Based on:
NSa_2CO_3: 0.06 mol/l = 0.006 mol / 100 ml = 0.0006 mol / 10 ml
$NaHCO_3$: 0.06 mol / l = 0.006 mol / 100 ml = 0.003 mol / 50 ml

Finally we have 0.0036 mol / 250 ml = 0.0144 M

Salt: Na_2CO_3 concentration of 0.0006 mol in 250 ml, hence 0.0024 M
Acid: $NaHCO_3$ concentration of 0.003 in 250 ml, hence 0.012 M

$$pH = 10.25 + \log \frac{0.0024}{0.012}$$

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°C and can be kept for a maximum of 3 months.

6. Neutralisation buffer

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

Stock solution A: Na_2CO_3 (0.61 M) = 6.466 g / 100 ml
Stock solution B: $NaHCO_3$ (0.61 M) = 50124 g / 100 ml

72 ml A + 55 ml B + 300 ml RO-DI water.
Adjust pH to 10 by adding either A or B.
Once the pH is set, adjust the volume to 500 ml with RO-DI water.

Based on:

Na_2CO_3: 0.61 mol / *l* = 0.061 mol / 100 mol = 0.044 mol / 72 ml
$NaHCO_3$: 0.61 mol / *l* = 0.061 mol / 100 ml = 0.034 mol / 55 ml

Finally we have 0.078 mol / 500 ml = 0.156 M

Calculation of the pH:

$$pH = pKa + \log \frac{[salt]}{[Acid]}$$

Salt: Na_2CO_3 concentration of 0.044 mol in 500 ml, hence 0.088 M
Acid: $NaHCO_3$ concentration of 0.064 mol in 500 ml, hence 0.068 M

$$pH = 10.25 + \log \frac{0.088}{0.068}$$

$$pH = 10.36$$

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

7. Sulfuric acid (H_2SO_4)

The acid we use comes in cartridges. Take the contents of cartridge and add RO-DI water up to 250 ml. This gives you 250 ml of H_2SO_4 4N.

8. Substrate (OPD)

The substrate used in this assay is Ortho phenylenediamine (OPD).
To prepare it, one tablet was taken and dissolve it in 6 ml of RO-DI water. Make sure you prepare it in a dark recipient or a falcon tube wrapped in aluminum foil. Just before putting the OPD on the plate, you add 2.5 ml of H_2O_2 was added.
If you need more than 6 µl of OPD, you must take 2 tablets in 12 ml and add 5 µl and so on... Do not break the tablets !

3.3 " Pretreatment" of the samples

Aim of the pretreatment:

1. Break down immune complexes to obtain free circulating atigen.
2. Reduce cross-reactions with sera of i.e. individuals infected with *Trypanosoma.*

To do this, the samples are mixed with and equal volume of trichloroacetic acid (TAC). This breaks down the immune complexes. The samples are then neutralized (= bring

pH from low the neutral) with a neutralization buffer.

Negative control sera (only 1 well required): 75 µl TCA (5%) solution + 75 µl serum sample.
Positive control and unknown sera (2 wells per sample): 150 µl TCA (5%) solution + 150 µl serum sample.

- Mix immediately be vortexing.
- Incubate for 20 min. at ambient temperature.
- Mix again by vortexing.
- Centrifuge for 9 min. at 12000 g.
- While centrifuging, prepare eppendorfs with 75µl (negative control sera) or 150 µl (Positive and unknown sera) neutralisation buffer (pH 10).
- Neutralise mixture by adding 75 or 150 µl (for negative controls or other samples, respectively,) of the supernatant into the eppendorfs with the same amount of neutralisation buffer. This results in a final dilution of $\frac{1}{4}$ of your sample(s) .

Important note: When using positive control sample K504, you must "pre-dilute" the sample before doing the TCA treatment.

- Add 5 µl K504 serum to 795 µl PBS
- Vortex well
- From this 800 µl you take 150 µl and add it to 150 µl of TCA.

Then you continue with a normal TCA treatment.

This gives the sample a final dilution of $\frac{1}{640}$ as opposed to a $\frac{1}{4}$ dilution for a normal samples.