

## Prevalence of cysticercosis of swine (*Sus scrofa domesticus*) in Nepal

H. B. Rana, Dr. K. D. Manandhar and Kashav G.C.

**Abstract:** A total of four hundred swine (*Sus scrofa domesticus*) samples were examined from January 2013 to December 2015. Out of 400 samples examined, 46 (11.5%) swine were infected with *Taenia solium* and *Ascaris suum*. The highest infection of cysticercus larvae of *T. solium* in swine were 8.43% (7/83) in Nepalganj, likewise 2.2% (2/95) in Dharan, 1.53% (1/65) in Kathmandu, 1.50% (1/66) in Butwal and infected swine was not found in Chitwan. The intensity determines the level of IgG production in the tested sample against the interacted antigenic fraction. Some remarkable bands were highly intense having higher integrated density value even though they were not consistent in different profile. Such highly intense fractions signify for the higher production of antibodies against those antigen and inconsistency refer for their low immunogenic value in NCC cases. ELISA test of 45 NCC suspected human serum samples only 37(82.22%) serum samples were positive. Seroepidemiological based on gender of the NCC cases showed 19 (51.35%) were male and 18 (48.65%) were female. Based on the ethnic groups among the 37 NCC positive patients analysed, 15 (40.54%) were Brahmin, similarly 7 (18.92%) patients were Chhetri, 6 (16.22%) Newar patients. Out of 3(8.11%) Mangolian and 3(8.11%) Dalit patients respectively. In the Madhesi and Muslim groups there were 2(5.41%) and 1(2.70%) patients respectively. Based on the clinical symptoms among the 37 NCC positive patients analyzed, 19 (51.35%) had Seizure. Similarly 16 (43.24%), 5(13.51%), 4(10.81%) and 3(8.11%) patients had Headache, Loss of conscious, Abnormal/jerky movements and Fainting attack respectively.

**Keywords:** Helminth, Cysticercosis, ELISA, Prevalence, Serum.

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### I. Introduction

Human cysticercosis is found worldwide, especially in areas where pig cysticercosis is common. Both taeniasis and cysticercosis are most often found in rural areas of developing countries with poor sanitation, where pigs roam freely and eat human faeces. Taeniasis and cysticercosis are rare among persons who live in countries where pigs are not raised and in countries where pigs do not have contact with human faeces. Cysticercosis is an infection caused by the larvae of the tapeworm, *Taenia solium*. A person with an adult tapeworm, which lives in the person's gut, sheds eggs in the stool. The infection with the adult tapeworm is called taeniasis and human is infected after eat undercooked or raw infected pork which contain the cysticercus cellulosae larva. The larvae then come out of their cysts in the human gut and develop into adult tapeworms, completing the life cycle. Pigs become infected by the ingestion of eggs of *T. solium* during scavenging that are present in the stools of infected human beings who are the definitive. Human are also accidentally infected with cysticercus larvae of *T. solium* by ingestion of eggs contaminated with food and water or regurgitating gravid proglottids from the human gut to the stomach of same individuals harboring the adult tapeworm in the small intestine. After eggs hatched in small intestine the larvae migrate throughout the body, invade skeletal muscle, subcutaneous tissue, or the central nervous system (CNS), the latter of which is known as neurocysticercosis is a crucial emerging disease in developing country like Nepal (Sako et al., 2000). The NCC is a disease directly related to poor personal hygiene and lack of food hygiene, socio-culture and lack of public health awareness. According to WHO, Cysticercosis is the most common parasitic disease across the world, and it was estimated that 50 million people were infected with it. The highest rates of infection for cysticercosis are found in parts of Africa, Asia, Mexico, and Central and South America and around 50 thousand people die each year as a result of the NCC and epilepsy secondary to NCC (ESNCC) (Roman et al., 2000). In this regard, WHO includes the NCC between neglected diseases or forgotten that cause a significant impact on the economy in several regions from all over the world. According to Sarti et al., (1992) it has been found that, it affects 4% of the population in endemic areas where hygiene, habits-food and sometimes religious trends can determine the incidence and prevalence of the disease (Savioli LS, 2010). Among thirty swine samples were examined only two swine (6.66%) samples were found to be infected with cysticercus larvae of *Taenia solium* In Chitwan valley of Nepal (Rana and Dhakal, 2006). Postmortem surveys of pigs at slaughter establishments in Kathmandu and Dharan municipality showed 14% (34/250) of pigs positive for cysticercosis. Antemortem detection of *T. solium* infection of pigs in a Syangja District community indicated 32% (136/419) of pigs positive by lingual examination while 24% (48/201) was serologically positive by Enzyme-linked Immuno-electro Transfer Blot (EITB) and 6% (12/201) showed evidence of old infection or exposure with 42 kDa and 50 kDa. A human helminthological survey in Syangja District in central Nepal indicated a very high prevalence of taeniasis, with

43% positive (77/180), while in Tanahun District 18% were positive (28/152). Taeniasis infection appears directly related to the ethnic groups surveyed and their food habits, literacy rates, and hygiene and sanitation. The prevalence of taeniasis among the ethnic groups surveyed, ie Magars, Sarkies, Darai, and Bote, was found to be 50, 28, 10, and 30%, respectively Joshi et al., 2004). The available data suggest that the prevalence ranges of neurocysticercosis was from 0.002 -0.1 % in general population in Nepal (Bista, 2006). NCC may be asymptomatic but may produce a broad range of clinical manifestations. Seizure is by far the most common clinical manifestation and occurs in 70-90% of cases. Less common manifestation included headache alone; symptoms of raised ICP and altered mentation (cysticercal encephalitis) and acute psychosis. Only a minority of patients present with cranial nerve palsies or other focal neurological deficit. The clinical spectrum of the disease depends upon the location, number and viability of the cysts as well as host response (White, 1997).

## **II. Materials and methods**

A total of four hundred swine samples were examined and collected from January 2013 to December 2015 in different slaughter houses/abattoirs of the country from Kathmandu, Chitwan, Butwal, Nepalganj, and Dharan. Samples were minutely observed and collected for zoonotic important and other parasites. During research work blood, muscles of tongue, neck, thigh, intercostals muscles and intestine also were thoroughly observed for zoonotic important helminth parasites and other parasites. All the collected specimens were preserved in PBS (Phosphate Buffer Solution), and organs which contain parasite/cyst/larva were kept in ice pack and carried into parasitological laboratory for detection of parasite/their larvae and stored at -20<sup>0</sup>C in deep refrigerator for further processes. For detection of dragger shaped rostellor hooks of cysticercus cellulosae larvae were removed from the infected muscle and put into 10 per cent KOH solution for 10 minutes then crushed between two glass slides. Ultimately crushed larvae were covered with cover slip and observed under objective 40X of compound microscope for detection of rostellor hooks.

Serological and molecular related laboratory work was conducted in the Central Department of Biotechnology, Tribhuvan University and Annapurna Neurological Institute and Allied Sciences, Kathmandu, Nepal. Forty five human serum samples of the individuals were collected from the patients visited to Annapurna Neurological Institute and Allied Sciences, Kathmandu, Nepal among them thirty five samples were positive and eight samples were negative. Random sampling was carried out for the sampling procedure based on their health complains and swine serum extracted from blood samples from slaughter houses. During human serum sample collection, the pre-historical information of the individuals was selected. The collected serum samples were taken in cold chain to the laboratory of Central Department of Biotechnology, Tribhuvan University and were stored at -20<sup>0</sup> C in a deep freeze until further procedure.

Preparation of Crude Soluble Antigen (CSA)- The collected 4-6 cysts in eppendroff tube were thawed in room temperature and were washed with PBS 4-5 times and then crushed manually in mortar and pestle using 500 µl of PBS maintaining cold chain and collected in the 2ml eppendroff tube. Approximately equal volume of lysis buffer prepared from complete protease inhibitor cocktail (Roche) was added to the above tube. The lysis was done by four alternative cycles of freezing (-20<sup>0</sup> C) and thawing (at room temperature) that is followed by sonication (sonicator-LOBA life) for 8 cycles for 30 sec. each in cold chain. The suspension was then centrifuged at 3,000 rpm at 4<sup>0</sup> C for 20 minutes. Finally, the supernatant, crude soluble antigen (CSA), was collected and stored at -20<sup>0</sup> C until use. Protein quantification was performed by Bradford assay (Bradford, 1976). Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis (SDS- PAGE) Protein concentration of CSA was estimated and mixed with equal volume of Gel loading dye/buffer (Genei, India, cat. # 105350) and was allowed to boil for 5 minutes. The CSA (25 µg/per well) was loaded in the wells casted on polyacrylamide gel of 5% stacking gel which was casted vertically on gel apparatus (Clever Scientific Ltd.). The molecular marker, Page Ruler™ Prestained Protein Ladder (Fermentas, cat. # SM0671) was loaded along with the sample in the gel. The set up was electrophoresed using running buffer (39 mM Tris, 48 mM Glycine, 0.1% SDS) at constant voltage of 100V for about 4 hour i.e. until the dye appeared to nearly run out of the gel as described by Laemmli (Laemmli, 1970) with certain modifications. The gel was taken out of the apparatus and washed once with distilled water and stained with Coomassie Brilliant Blue (CBB) for study of protein profile of cysticercus of *T. solium*. Similarly, SDS-PAGE was run loading at 45µg/well for immunoblotting. Enzyme Link Immunosorbent Assay

Assay was done as described by (Hommel et al., 1978) with some modifications. The CSA protein (100ng/well) was coated in 96 well ELISA plate (NUNC, USA) in carbonate buffer (0.1M NaCO<sub>3</sub>, 0.1M NaHCO<sub>3</sub>, pH 9.6) and incubated overnight at 4<sup>0</sup> C. The plates were aspirated and blocked with 125 µl/well blocking buffer (1% BSA, 0.1% Tween-20 in PBS) for 2 hours at room temperature. Washing was done for 5 times with washing buffer (0.1% BSA, 0.1% T-20 in PBS) and then incubated with 100 µl serum at dilution of 1:100 in dilution buffer (0.1 % BSA in PBS) in duplicate for ½ hour. One hundred microlitres (100µl) of goat antihuman immunoglobulin G (IgG) conjugated with horse reddish peroxidase (HRP) in dilution at 1:10000 were dispensed after washing as previous step and plates were incubated for ½ hour. Washing was repeated as

in previous step followed by addition of 100 µl trimethylebenzidine (TMB) substrate and incubated for 15 minutes at dark in room temperature. The reaction was stopped by addition of 1N H<sub>2</sub>SO<sub>4</sub> and OD was taken at 450 nm in ELISA plate reader (Spectromax 190). Positive controls (PC), negative controls (NC), conjugate control (CC) and substrate control (SC) along with the samples were incorporated in experiment to avoid error (Table 1.).

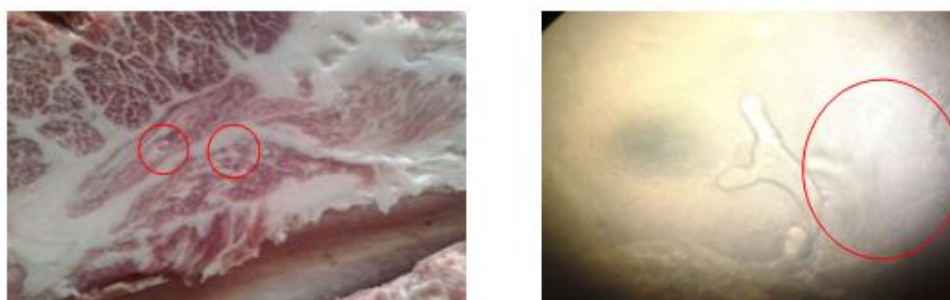
**Table 1.** ELISA steps for PC, NC, SC and CC controls

ELISA steps	PC	NC	CC	SC
Coating Ag	Coating Ag	Coating Ag	Only coating buffer	Only coating buffer
Blocking	Blocking buffer	Blocking buffer	Blocking buffer	Blocking buffer
Samples	pc	nc	Capturing antibody	Blocking buffer
Detecting antibody	Detecting antibody	Detecting antibody	Detecting antibody	Blocking buffer
Substrate	Substrate	Substrate	Substrate	Substrate
Stop solution	Stop solution	Stop solution	Stop solution	Stop solution

PC, positive control; NC, negative control; CC, conjugate control; SC, substrate control

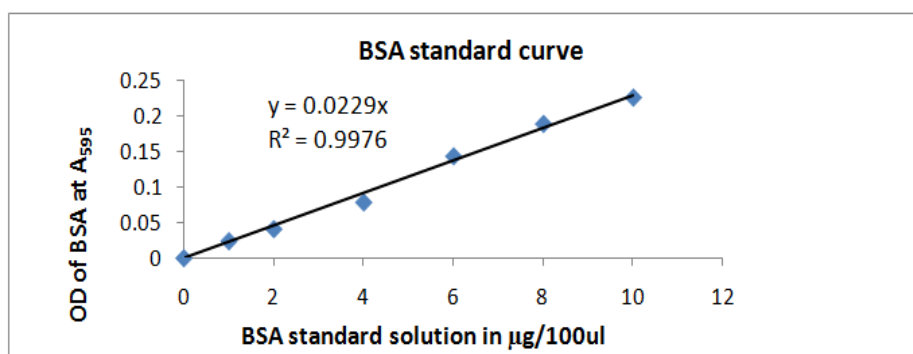
### III. Result and discussion

In case of domestic swine (*Sus scrofa domestica*) out of 400 samples examined, the total infection rate of cysticercus cellulosa larvae of *Taenia solium* was found to be 11(2.74%). The highest infection in swine were 7 (8.43%) in Nepalganj, likewise 2 (2.2%) in Dharan, 1(1.53%) in Kathmandu, 1(1.50%) in Butwal and infected swine was not found in Chitwan respectively. Dragger shaped rostellar hooks were detected from the crushed cysticercus cyst after cyst soak 10 minutes in 10 per cent KOH and confirmed that the cyst were larvae of *Taenia solium*. For ELISA test 45 human serum samples of NCC infected patients were taken from Annapurna Neurological Institute and Allied Science, Kathmandu, Nepal among 37 samples were positive and 8 samples were negative.



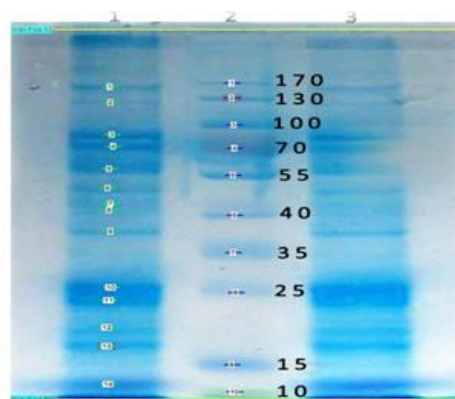
**Fig.1** Measly pork containing *Cysticercus cellulosa* **Fig.2** Microscopic view of cyst of *T. solium* showing rostellar hook (40x)

CSA collection and determination of protein concentration- Crude soluble antigen (CSA) was prepared from 6-8 cysts at a time. The volume obtained was 1.5 ml from each batch. In total 4.5 ml of sample (CSA) was prepared in three times during the work. The protein concentration was determined from the calibration curve of BSA standard solution ( $y=0.0229x$ ,  $R^2 = 0.9976$ ) obtained from Microsoft Excel 2010 (Fig. 4). The OD value of 0.217 at 595 nm using 5 µl from CSA valued the concentration of 1.89 µg/µl protein in CSA preparation of cysts of *Taenia solium*.



**Fig.3** Calibration curve of BSA standard solution for protein estimation in CSA

Protein profile of CSA of cysticerci of *T. solium*- Several protein fractions were seen on the gel after running crude soluble antigen of cysticerci of *T. solium* in SDS-PAGE and stained with CBB. A number of protein fractions were found remarkably of higher intensity. Most of the gel had about 14 clearly distinguishable protein fractions. The CSA consisting various cytoplasmic and membrane bound components were found fractionated ranging from 11 to 158 kDa. The protein polypeptide fractions as analyzed by using Alpha Imager™ 2200 were of 158, 124, 86, 72, 58, 50, 44, 42, 38, 26, 24, 20, 17 and 11 kDa (Fig.5., Table 1.).

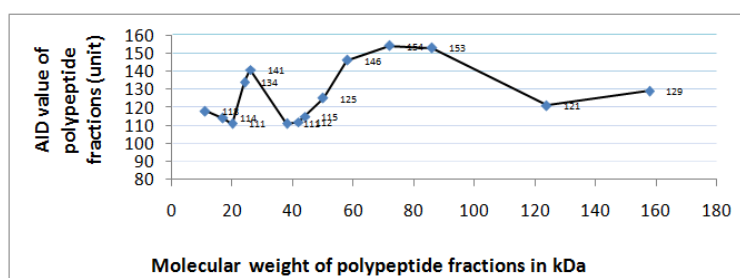


**Figure 4.** CBB-stained SDS-PAGE gel. Lane 1 and 3, CSA of cysts of *T. solium* and *Sarcocystis* sp.; lane 2, prestained mol. wt. marker and digits aside are mol. wt. of standard bands; Digit in white blocks, numbering of bands assigned by Alpha Imager™ 2200; Top horizontal line, line of initiation of resolving gel; Bottom horizontal line, dye in front.

**Table 2.** CSA profile of cysts of *T. solium* with markers obtained from Alpha Imager™ 2200

-----MARKERS-----				-----QUERIES-----			
Band	Position	Mol. Wt.	Rf	Band	Position	Mol. Wt.	Rf
1	96	170	0.145	1	102	158	0.155
2	119	130	0.185	2	126	124	0.197
3	161	100	0.258	3	176	86	0.284
4	197	70	0.321	4	194	72	0.316
5	239	55	0.394	5	229	58	0.377
6	301	40	0.503	6	259	50	0.429
7	359	35	0.604	7	283	44	0.471
8	421	25	0.712	8	293	42	0.489
9	533	15	0.908	9	326	38	0.546
10	575	10	0.981	10	413	26	0.698
				11	433	24	0.733
				12	475	20	0.806
				13	504	17	0.857
				14	563	11	0.96

The variation in intensities of these protein polypeptide fractions were further confirmed by integrated density value (IDV) analyzed by densitometry from Alpha Imager™ 2200. The IDV of polypeptide fractions was found ranging from 130869 to 181566 taking the area of 1179 unit. Thus the average integrated density (AID) values range from 111 to 154 when the highest value of total black was assigned as 255 units. The protein fractions having higher intensities and clearly distinguishable from other bands were 158, 124, 86, 72, 58, 50, 26 and 24 kDa. Among these the bands of 86, 72, 58, 26 and 24 kDa polypeptide fractions were having AID value of more than 130 (Fig.6).



**Fig.4** Average Integrated Density (AID) value of different protein polypeptide fractions calculated based on area selected (1179 units).

**Table 3.** Distribution frequency (%) of major bands detected in different study groups.

Bands (kDa)	Repetition frequency(%)		Bands (kDa)	Repetition frequency in (%)	
	NCC Patient samples (n=10)	Healthy controls (n=6)		NCC Patient samples (n=10)	Healthy controls (n=6)
128	-	17	50	90	-
127	-	50	48	-	50
124	30	33	46	70	-
120	60	-	44	40	50
112	-	50	42	40	-
110	-	33	41	-	50
108	30	17	40	70	-
105	-	50	39	100	-
100	90	-	38	80	83
95	-	50	37	60	50
93	-	17	36	50	83
92	-	33	35	80	33
88	20	33	34	20	33
86	40	-	32	70	50
85	20	33	30	30	-
82	30	-	29	40	17
81	-	33	28	30	50
78	-	33	27	20	17
76	30	33	26	80	17
72	50	17	24	100	-
70	40	-	23	20	33
68	20	50	22	60	50
66	40	17	21	50	33
65	20	33	20	40	50
64	20	-	19	20	50
63	30	-	18	70	-
62	30	17	17	40	50
60	30	50	16	60	-
58	40	-	15	40	67
56	40	17	Average no. of bands	22.4	17.33
55	40	17	Std. Dev.	1.84	2.80
54	-	83			
52	-	33			
51	20	-			

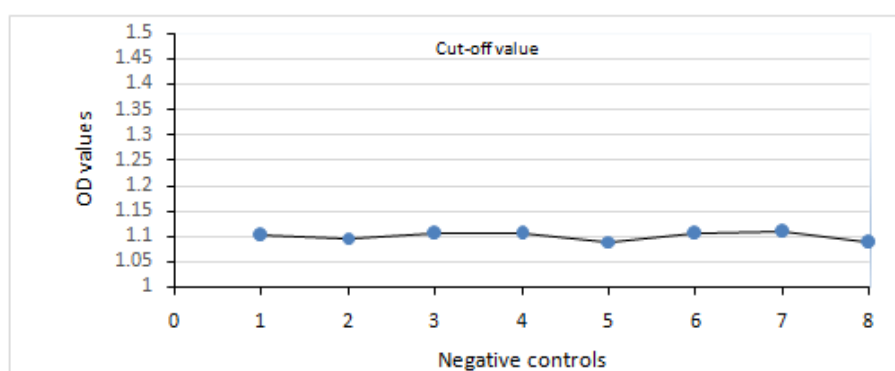
Enzyme Linked Immuno-sorbent Assay (ELISA)

The assay was performed among 45 NCC patients with 2 positive controls and 8 negative controls along with substrate control and conjugate control. The OD readings at 450nm of all the variables are given in Table 4.3 and in Appendix III. Out of 45 NCC patients, 37(82.22%) were found to be NCC positive and the rest 8(17.78%) were negative.

**Table 4.** OD Readings at 450 nm of the study variables in ELISA.

Parameters	Variables				
	Substrate control (n=1)	Conjugate control (n=1)	Negative controls(n=8)	Positive controls(n=2)	Samples(n=45)
Mean OD ± SD	0.8045±0.135057	0.8945± 0.044548	1.0995± 0.132281	3.29325 ± 0.071771	2.9831±0.62603

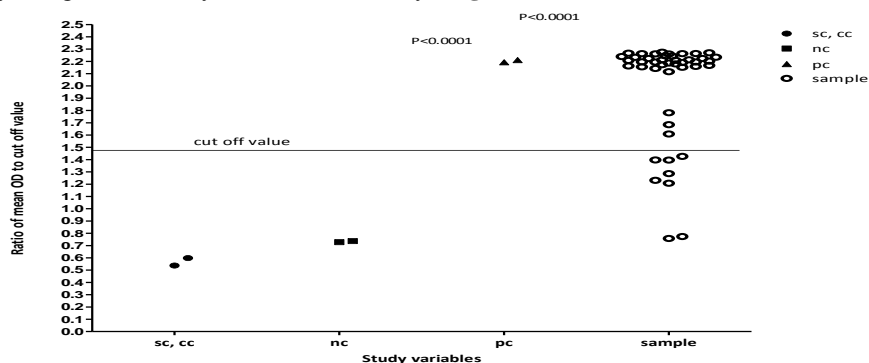
Cut-off value determination The cut-off value was determined by running negative controls and calculating the mean and standard deviation. Three times of the SD added to the mean value was taken as cut-off point. The cut-off value was determined to be 1.496 (Fig. 5).



**Fig.5** Cut-off value determination from negative controls.

### ELISA Test on NCC cases visited to hospital

Antibody based ELISA was performed in total 45 NCC cases visited to hospital that was used as test samples. Besides test samples, positive controls and negative controls were used to calculate the sensitivity and specificity of the test and substrate control and conjugate control were used for the quality control of the test. Out of 45 NCC cases visited to hospital, 37(82.22%) were found to be NCC positive and the rest 8(17.78%) were negative by using the antibody based ELISA assay (Fig. 7).



**Fig.6.** Immune response by sera of different study variables as expressed in ELISA with CSA of cysts of *T. solium*; sc, substrate control; cc, conjugate control; nc, negative control; pc, positive control and sample, patients visited to hospital.

The comparative statistical analysis between different study variables was performed. The unpaired t-test was applied between negative control and positive control and p value obtained was <0.0001 which showed their means were significantly different. Similarly, p value obtained from the unpaired t-test between negative control and sample was also <0.0001 which indicated that their means were significantly different.

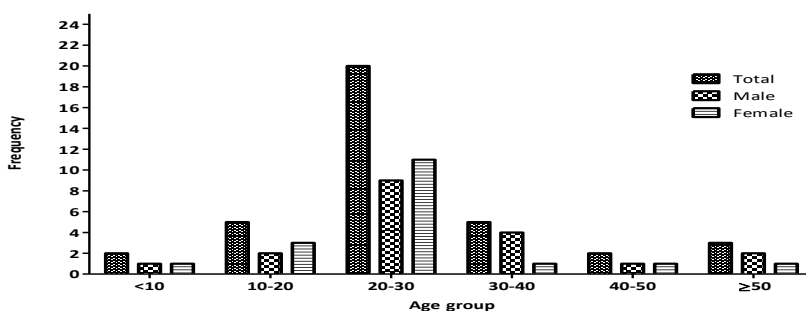
Out of 45 NCC cases visited to hospital, 37 cases were positive as displayed by antibody based ELISA. Since all the negative controls were negative in the antibody ELISA, so the specificity of the test was found to be 100%. The sensitivity of the antibody ELISA was found to be 82.22%.

### Sero-epidemiological study of confirmed patients

Seroepidemiological study was performed among the 37 NCC positive patients as depicted by ELISA assay. The sero-epidemiological study was based on the different epidemiological variables such as gender, age groups, ethnic groups and clinical symptoms. The study reflected the various prevalence rate among the different epidemiological variables under study.

#### Seroepidemiologybased on gender

Among the 37 NCC positive patients analysed, 19 (51.35%) were male and 18 (48.65%) were female. The mean age of the patient was 26.67 years with the standard deviation of 11.0631 and the range of the patient age was 4-57 years. Out of 37 NCC patient 2 patients were of age less than 10 years among them one was male and one was female. Among 5 patients between the age group 10-20, male patient were 2 and female were 3 in number. Out of 20 patients in the age group 20-30, 9 were male and 11 were female and this group occupies 54.05% of total patients. Similarly, the age group 30-40, 40-50 and more than 50 have 5, 2 and 3 patients among them 4 and 1, 1 and 1, 2 and 1 were male and female patients respectively (Fig. 6.). The patients age group showed positive correlation with the gender of the patients and the correlation was found to be significant (p<0.05) (Table 5.).



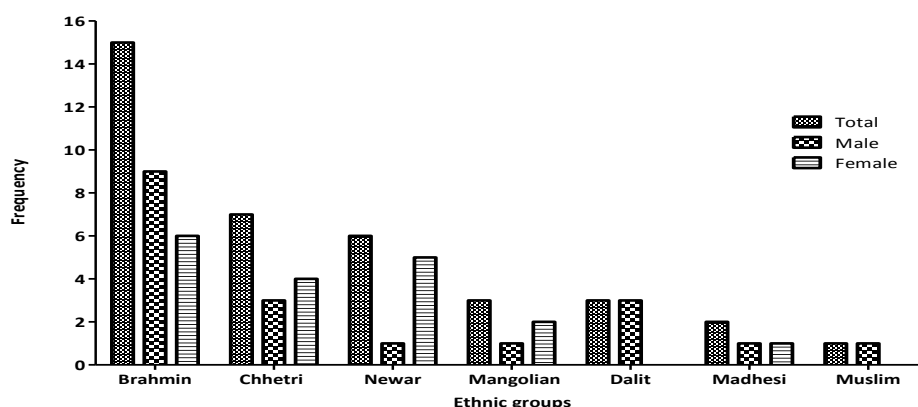
**Fig.7.** Relation between age group and gender of the patients.

**Table 5.** Correlation between age group and gender of patients with their P values.

Parameters	Total		Male - Female correlation
	Male	Female	
Correlation coefficients	0.9723	0.9838	0.9148
P values	0.0011*	0.0004*	0.0106*

\* Significant (p<0.05)

**Seroepidemiology based on ethnic groups-** Based on the ethnic groups among the 37 NCC positive patients analysed, 15 (40.54%) were Brahmin in which 9 were male and 6 were female. Similarly 7 (18.92%) patients were Chhetri among them 3 were male and 4 were female. Among 6 (16.22%) Newar patients, male patient was 1 and female were 5 in number. Out of 3(8.11%) Mangolian and 3(8.11%) Dalit patients, 1 and 2, 3 and 0 were male and female respectively. In the Madhesi and Muslim groups there were 2(5.41%) and 1(2.70%) patients in which male patient was 1 and 1, female patient was 1 and 0 respectively (**Fig. 7.**). The patients ethnic group showed positive correlation with the gender of the patients and the correlation was found to be significant (p<0.05) but within the ethnic groups the correlation between male and female patients was found to be insignificant (**Table 6.**).



**Fig.8.** Relation among various ethnic groups of patients.

**Table 6.** Correlation between ethnic groups and gender of patients with their P values.

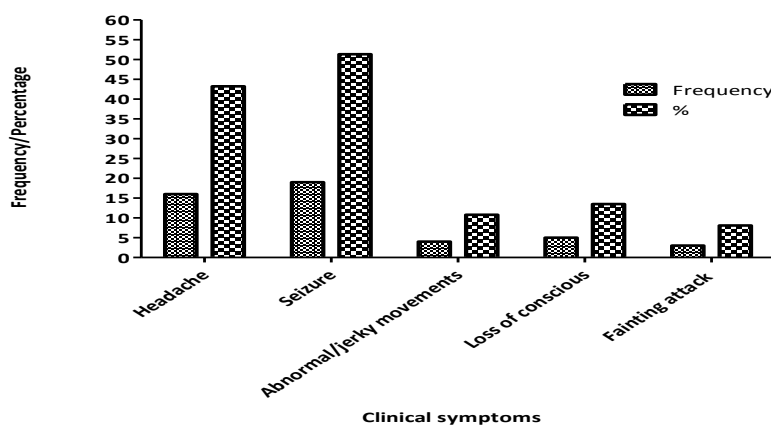
Parameters	Total		Male - Female correlation
	Male	Female	
Correlation coefficients	0.9108	0.8686	0.5867
P values	0.0043*	0.0111*	0.1662 <sup>ns</sup>

\* Significant (p<0.05)

<sup>ns</sup> non-significant(p>0.05)

**Seroepidemiology based on clinical symptoms**

Based on the clinical symptoms among the 37 NCC positive patients analyzed, 19 (51.35%) had Seizure. Similarly 16 (43.24%), 5(13.51%), 4(10.81%) and 3(8.11%) patients had Headache, Loss of conscious, Abnormal/jerky movements and Fainting attack respectively (**Fig.9.**). The relation among various clinical symptoms of patients was found to be significant (p=0.0487).



**Fig. 9.** Relation among various clinical symptoms of patients.

#### IV. Discussion

Cysticercosis in swine was caused by the cysticercus cellulosae larvae of *Taenia solium*, which is veterinary as well as public health important because eggs of *Taenia solium* is the only source of transmission in swine and the measily pork of infected swine is the only source of taeniasis in human. In life cycle man is the definitive host who usually harbour adult parasite and swine is the intermediate host which has always larval stage. Human cysticercosis or neurocysticercosis is caused by ingestion of eggs or ruptured the gravid segment of *Taenia solium* inside the small intestine of infected person. In case of domestic swine (*Sus scrofa domesticus*) out of 400 samples examined, the total infection rate of cysticercus cellulosae larvae of *Taenia solium* was found to be 11(2.74%). The highest infection in swine were 7 (8.43%) in Nepalganj, likewise 2 (2.2%) in Dharan, 1(1.53%) in Kathmandu, 1(1.50%) in Butwal and infected swine was not found in Chitwan respectively. Rana et al.,(2006) were reported 6.66 per cent swine samples were positive while recent research showed that there were not infection of *Taenia solium* in the swine of Chitwan valley due to improving farming system of pigs. Joshi et al., (2004) were reported postmortem surveys of pigs at slaughter establishments in Kathmandu and Dharan municipality showed 14% (34/250) of pigs positive for cysticercosis while in recent research showed in Dharan 1.53 per cent and Kathmandu were 1.50 per cent. Neurocysticercosis (NCC), the most important neurological disease of parasitic origin in humans (WHO, 2002) is a major cause of adult-onset epilepsy in areas where the pork tapeworm *T. solium* is endemic (García et al., 1997). NCC is characterized by a strong immune response surrounding infected neural tissues. The presence of excretory-secretory antigens and proteins released by the degeneration of the cysticerci in the host stimulates the production of specific immunoglobulins in which the isotopic predominance is related to the infection phase (Molinari et al., 2002; Abraham et al., 2004). Anti-cysticerci IgG is the one most detected in both the acute and the chronic phases of the disease. The best immunogenic proteins for NCC immunodiagnosis are in the cysticercus scolices, and these have been used to detect serum or CSF antibodies in patients with suspected NCC (Nascimento et al., 1987) while in recent CSA of whole cysticercus cellulosar of *T. solium* was used to detected antibodies in patients with suspected NCC. Therefore, in addition to the CSA, human anti-IgG antibodies were used for the immunoassays (Lopez et al., 2004). Joshi et al., (2004) had also reported that human helminthological survey in Syangja District in central Nepal indicated a very high prevalence of taeniasis, with 43% positive (77/180), while in Tanahun District 18% were positive (28/152). Taeniasis infection appears directly related to the ethnic groups surveyed and their food habits, literacy rates, and hygiene and sanitation. The prevalence of taeniasis among the ethnic groups surveyed, ie Magars, Sarkies, Darai, and Bote, was found to be 50, 28, 10, and 30%, respectively.

Neurocysticercosis is difficult to diagnose because of the dimensions, quantities and localization of the cysticerci in the CNS. The diagnosis of NCC is performed by histological techniques, neuroimaging followed by a confirmatory serological (immunological) assay. Computed tomography (CT) and magnetic resonance imaging (MRI) scans of the brain are formally indicated for diagnosing NCC, but the costs relating to these procedures make it difficult for most of the population with high infection rates in developing countries to have access to these services. Moreover, doubts concerning images may occur and auxiliary diagnostic methods are required (Del Brutto et al., 2001). The enzyme-linked immunoelectrotransfer blot (EITB), a western blot assay developed in 1989 that is 98% sensitive and 100% specific for detecting pathologically confirmed cases of NCC (Brand and Tsang, 1989) was recognized as the serological assay of choice for the detection of cysticercosis in humans and pigs by the WHO in 1995. The CSA prepared from the parasite lysate was a heterogeneous mixture as the collection of lysate was from the centrifugation at 3,000 rpm (Manandhar, 2008) containing cytoplasmic and membrane (organelles) bound protein components. CSA preparation of cysts of *T. solium* had been found to contain total protein concentration of 1.89 µg/µL. CBB dye binds most readily to arginyl and lysyl residues of different proteins (not to the free amino acids).

Proteins or polypeptides present in CSA got denatured in the presence of a detergent like sodium dodecyl sulfate and reduce in the presence of beta-mercaptoethanol. The denatured protein complexes with SDS in the ratio of 1.4 gram SDS per gram of protein giving overall negative charge to the complex. The calculated amount of CSA loaded in SDS-PAGE when fractionated applying electric force, the complexes moved towards the anode separating the proteins based on difference in charge they possesses and their molecular weight. The polypeptide fractions separated by polyacrylamide gel electrophoresis were stained with CBB. Since the method has limited range of sensitivity for the detection, it produced the detectable band possessing at least 100ng of protein. CBB staining to CSA on gel produced 14 clearly distinguishable polypetide fractions ranging from 11 to 158 kDa that include protein polypeptide fractions of 158, 124, 86, 72, 58, 50, 44, 42, 38, 26, 24, 20, 17 and 11 kDa with certain bands showing higher intensity which represented for the higher amount of the particular polypeptide in the CSA. In a similar study the electrophoresis profile for crude antigens from scolices of *T. solium* cysticerci showed at least 21 bands at 200, 180, 120, 100, 95, 80, 68, 65, 56, 53, 50, 45, 40, 38, 36, 34, 29, 26, 22, 20 and 15kDa of major proteins, for which the molecular weights ranged from 200 to 15kDa (Ludici Neto et al., 2007).



This study revealed 14 clearly distinguishable protein fraction bands out of which only 5 polypeptide bands possess remarkably high AIDV (above 130 AIDV). Similar type of work had been done in *Leishmaniadonovani* (Manandhar, 2008). Since the study was focused on the polypeptides that possessed repetition for more than 50%, there were 15 bands that had the repetition of more than 50% in the WB profiles of NCC patients. Moreover the eight distinct antigenic bands 120, 100, 50, 46, 39, 24, 18, and 16 kDa were found completely absent in the healthy controls which were the important bands among those 15 bands in NCC patients. The bands of 100, 50, 39 and 24 kDa protein fractions were found present in 90% or more in NCC patients showing their greater application in diagnostic markers for NCC cases. The absence of particular band from the sera of healthy controls clearly indicates for the absence of that antigen and its corresponding antibodies developed against that antigen. The present study showed that most of the polypeptide bands from cysticerci did not showed good reactivity and just some could be indicated as antigens for NCC immunodiagnosis. The low reactivity found in proteins bands with different molecular weights possibly occurred due to the antigenic similarity between the cysticerci, and other antigens such as the adults of *T. solium* and other helminthes. Similar study in western blotting separation of lentil-lectin glycoproteins (LLGP) results in seven distinct antigenic bands at 13, 14, 18, 21, 24, 39-42, and 50 kDa (Brand and Tsang, 1989). Antibody reactivity with any one of these bands indicates exposure to cysticercosis. In the study done by Iudici and his associates, immunogenic proteins with the molecular weights of 200, 180, 120, 100, 95, 68, 65 and 26kDa were the most reactive and among them 100, 95 and 26kDa proteins provided the best sensitivity and specificity indices when the appropriate dilutions of the sera were used (Iudici Neto et al., 2007). The 100 and 95kDa proteins are probably similar to the antigen B, which is important for inducing an immune response (Kojic and White, 2003). In a study performed by Joshi and his associates, *T. solium* specific antigenic bands corresponding to 13, 14, 16, 24, 42 and 50 kDa glycoprotein markers were obtained from the serum samples of the patients that were diagnosed with NCC by imaging techniques (Joshi et al., 2014).

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