

Full Length Research Paper

Seroprevalence of *Taenia solium* cysticercosis among people with epilepsy epileptic patients in three rural districts of Northern Uganda

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Taenia solium pork tapeworm is one of the parasites that causes serious public health and socioeconomic problems in developing countries. In Northern Uganda, extreme level of poverty, lack of sanitation infrastructure and communities' practice of free range pig farming provide suitable condition for survival of *T. solium* in the area. Additionally, increased cases of epilepsy are of serious concern. The aim of the study was to determine the proportion of patients with epilepsy who are positive for metacestodes of *T. solium* antigens and anticysticercal IgG antibodies in three districts of Northern Uganda. Forty two thousand nine hundred three participants were screened for epileptic seizures. Three hundred random samples were screened for anticysticercal IgG and circulating antigens using indirect antibody and monoclonal antibody ELISAs. Samples positive for anticysticercal IgG were confirmed using western blot. The seroprevalence of anticysticercal IgG and circulating antigens among patients samples using indirect antibody ELISA and monoclonal antibody ELISA was 15% (95% CI = 14.5-15.5 and 9% (95% CI 8.5-9.5) respectively. Thirteen, 13% (95%CI = 12.5-13.5) of patient samples were positive for *T. solium* specific glycoprotein on immunoblot. There was no significance difference ($P = 0.057$) in seroprevalence of anticysticercal IgG and circulating antigens between males and females. This finding indicates that *T. solium* infections occur among communities in three rural districts of Northern Uganda. There is a potential for proliferation of pork tape worm infections among the communities. Therefore, there is need for Health authorities to strengthen training of health workers and enforcement of public health education in the community on epilepsy associated with neurocysticercosis.

Key words: Neurocysticercosis, seroprevalence, westernblot, enzyme-linked immunosorbent assay (ELISAs), rural communities, Northern Uganda.

INTRODUCTION

Taenia (T) solium, is one of the parasites that causes huge public health and socioeconomic burden in endemic

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regions (Basem et al., 2010). Taeniasis is acquired when human eat raw or undercooked pork containing cysticerci, the larva stage of the *T. solium* (Bueno et al., 2001; Basem et al., 2010). When ingested, the cysticerci migrate to intestine mucosal where they attach and become adults. These adults worm shed proglottids containing eggs in human feces and become the source of infections that can infect other human or pigs by direct or indirect contamination of food or water (Basem et al., 2010).

T. solium causes human cysticercosis when one ingests embryonated eggs in food or water contaminated with fecal matter of persons harbouring the adult tapeworm (Bueno et al., 2005). When the human central nervous system (CNS) is infected with larval stage of *T. solium*, neurocysticercosis may occur which commonly manifests as epileptic seizures (Carabin et al., 2006). Approximately 50 million people worldwide are infected with *T. solium* parasites and 50,000 people die of cysticercosis related diseases annually (Fleury et al., 2006; Lescano et al., 2007). In developed countries particularly United States of America, cases of human cysticercosis have been reported among immigrants (Lescano et al., 2007). *T. solium* infections are prevalent in rural areas in developing countries particularly in Central and South America, Asia and Sub Saharan Africa (Ngowi et al., 2004). In Eastern Africa, the seroprevalence to anticysticercal Immunoglobulin G (IgG) of *T. solium* has been reported among human population with varying results; in Tanzania 38% (Ngowi et al., 2004), Kenya 14% (Githigia et al., 2002), Burundi 39 and 44% (Nsengiyumva et al., 2002; Prado Jean et al., 2007), Democratic Republic of Congo, (2.6%) (Praet et al., 2010). In Uganda, information on human cysticercosis is still scanty. The diagnosis of human cysticercosis remains a challenge in most hospitals and other health facilities due to lack of skilled health professionals and specialized medical equipment such as Computed Tomography (CT) scans and Magnetic Resonance Imaging (MRI). In addition, there are no studies that have been done on immune response to *T. solium* cysticerci among rural communities in Northern Uganda.

The objective of the present study was to determine the proportion of patients with epilepsy who are positive for metacestodes of *T. solium* antigens and anticysticercal IgG antibodies in three districts of Northern Uganda.

MATERIALS AND METHODS

The study was conducted in the three rural districts of Northern Uganda from April 2012 to June 2013. This includes the districts of Adjumani, Moyo and Gulu. The three districts cover a total area of over 6,500 km² with an estimated population of 916,000 inhabitants (https://en.wikipedia.org/wiki/Adjumani_District Wikipedia), (<http://www.ugandatourguide.com/guludistrict.html>), (https://en.wikipedia.org/w/index.php?title=Moyo_District&action=info).

These areas have high level of poverty, lack sanitation infrastructure and the communities practiced free range pig farming

which provide suitable environment for survival of *T. solium* in the study population. The pigs are usually kept at night in pens. In addition, these areas have shortage of toilet/pit latrine as well as safe sources of water. The main sources of water in these areas are: spring /wells/, bore holes, tap water and protected wells (Nsadha et al., 2011).

Study design

A cross sectional study was done on epileptic patients in the three districts of northern Uganda. The patients were selected on the basis of the chronicity of epilepsy starting with those with most recent onset of seizures using simple random sampling. Serodiagnosis of anticysticercal IgG and circulating antigens was done using indirect antibody ELISA (Ab-ELISA) and Monoclonal antibody ELISA (MoAb-ELISA) respectively. Anticysticercal IgG was confirmed using immunoblot. Standardized laboratory questionnaires were designed and used to get information from patients in relation to their demographics, eating behaviors and ingestion of raw or under cooked pig meat. These were to relate social risk factors for *T. solium* cysticercosis to seropositivity.

The research protocol was approved by Gulu University, Faculty of Medicine Ethic Committee and Uganda National Council of Science and Technology (Ref: HS 987).

Community based sampling and patient selection

This study conducted community based surveys in the districts of Gulu, Adjumani and Moyo to identify people with epileptic seizures among the rural communities. This was preceded by initial visits to sensitize the local communities in the study areas. The health authorities, the local leaders and the veterinarians were involved in this exercise. They also helped in identifying areas in each district with high cases of patients with epilepsy and areas where pig farms are practiced. Forty two thousand nine hundred three people were randomly selected using multistage cluster sampling and screened for epileptic seizures through interviews. Interviews of household members were conducted after obtaining oral consents from the heads of households.

The sample size for this population was calculated based on formula by Kish Leslie (1965). Using the prevalence study conducted in a neighboring country, Tanzania with a prevalence of epilepsy at 12/1000 in an area with 45,000 participants and at a 95% confidence interval, the sample size was calculated. Households with suspected positive cases were identified and later visited by the researcher who administered in-depth questionnaires for confirmation of epileptic seizures. People suspected of epileptic seizures at household level were referred to hospitals or health facilities for further examination by the neurologists. Patients presenting to the hospitals or health facilities were recorded in the register using the patients' identification number. Simple random sampling was then conducted on the six hundred patients confirmed for epileptic seizures.

Briefly, random number was generated from a sampling frame of 1383. These were then aligned to the patients' register. Each patient assigned random numbers was included in the study. Three hundred patients were randomly selected using random numbers. This was done to ensure that each patient constituting the sampling frame had equal chance of being included in the study. Patients who were not selected for further examinations were recommended for treatment for their epileptic seizures. Therefore, the three hundred patients randomly selected were sent for further investigations. The patients were bled through vein puncture of the arm and blood collected into plain vacutainer tubes. These were allowed to clot at ambient temperature, later centrifuged and the

sera were extracted and stored at -20°C for subsequent use. The sera samples were used in antibody ELISA assays done at Faculty of Medicine, Gulu University.

Antibody ELISA assay in detection of *T. solium* anticysticercal IgG

The seroprevalence of IgG from patient sera was screened using indirect antibody ELISA as described by Sloan et al. (1995). Briefly, 1.0 µg/ml of antigens was coated on to the 96 polystyrene microplate (Nunc® Maxisorp) wells and incubated overnight. 10 µl of antisera were then added and incubated. The rabbit anti human horseradish-peroxidase polyclonal IgG (Invitrogen, Germany) specific for the first antibody in the sera was added to form antigen-antibody complex. The substrate o-phenyldiamine (OPD) (Invitrogen) was added. The reaction was stopped by adding 0.5 M H₂SO₄ (BDH UK). The colored substance formed was measured photometrically at 492 nm using ELISA reader (Tecan Austria GmbH, Sunrise).

The amount of antibody present was then calculated. The cut-off points were calculated as the mean of the optical density (OD) values obtained with 8 negative serum samples plus two standard deviations (SD). In the present study, a sample was considered positive if the OD value was greater than 0.35 positive and negative controls were included in all the ELISA reactions for validation. Incubations were conducted for 1 h at 37°C for the subsequent ELISA reactions except for the dark incubation (30 min). Antigens preparations were done in the Department of infectious Diseases and Tropical Medicine (DITM), Munich, Germany at Professor Gisela Bretzel, Laboratory.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Preparation of the immunoblot strips for serodiagnosis was done as described by Parija et al. (2011) but with some modifications. These were required in order to shorten the time of separation of the glycoprotein in Tris-Tris buffers using high voltage and current. Briefly, 750 µg/ml of crude antigens from naturally infected pigs were run on a sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) in a 16.4% resolving and 4.0% stacking gel (in Tris-Tricine buffers) at 100 V 150 mA 10 W for 5 h. The antigens were separated under non reducing conditions by SDS-PAGE in a 10 × 8 cm Mini gel (Austria). The gels were stained with bromophenol blue (BDH UK) and the molecular weight marker (ladder) (Invitrogen, Germany) placed at both ends.

Electroimmunotransfer blotting (Western blot)

The separated antigenic proteins on the gels were electronically transferred in wet 0.22 µm pores size nitrocellulose membranes in mini Transblot cells (USA) at 150 V 100 mA 30 W for 1 h. The two small portions on the nitrocellulose membrane branded with the molecular weight markers were cut and air dried. The larger portion of the nitrocellulose membrane was placed on 5% BSA for 30 min to block non-specific binding sites and thereafter air dried. The immunoblot strips of 4 mm were cut from the larger portion of the dried nitrocellulose membrane for serodiagnosis.

Electroimmunotransfer blot analysis

Serodiagnosis with the immunoblot was done on all patients samples which were positive with antibody ELISA. The dry immunoblot strips were fully submerged in Mini incubator Trays and incubated on 5% Bovine Serum Albumen (BSA) for 30 min for

subsequent procedures. The patient sera were added and incubated for 70 min. Horse radish peroxidase labeled Rabbit anti human IgG antibody (Invitrogen) was used as secondary IgG in a dilution of 1/10000 and diaminobenzidine (Invitrogen) used as substrate. All incubation was followed by subsequent washing with PBS/T20. The presence of the antibody in the sera due to *T. solium* was confirmed when at least two specific bands of 8 or 10 kDa were observed on the diagnostic region (Figure 1). The molecular weight of *T. Solium* characteristic antigenic peptides were determined by comparing the bands in the diagnostic region with the standard molecular weight markers placed alongside the strips (Figure 1). Major antigenic peptides were: 8, 10, 18, 32, 40, 50 and 76 kDa. The bands outside the diagnostic regions were not considered as specific for *T. solium* because these were shared by other helminths.

Monoclonal antibody ELISA assays in detection of circulating antigens

The MoAb-ELISA was done as described previously by Brandt et al. (1992) in the laboratory of Dr. Chummy Sikasunge in the University of Zambia. Briefly, the polystyrene ELISA plate (Nunc® Maxisorp) was coated with 1.1 µl/ml concentrations of Moab. Two Moab (Institute of Tropical Medicine (ITM), Antwerp, Belgium) were used. The first MoAb B158C11A10 was diluted at 1.24 µg/ml in carbonate buffer (0.06M, pH 9.6) and used for coating the ELISA plate while the second biotinylated MoAb B60H8A4 diluted to (3.6 µl/ml) in (Phosphate Buffered Saline-Tween 20 PBS-T20) + 1% New Born Calf Serum (NBCS) and used as detector antibody. The coating of the first MoAb was carried out at 37°C on a shaker for 30 min and all other subsequent steps incubated for 15 min. The blocking was done by adding PBS-T20 + 1% NBCS per well.

Pre-treated sera at of 1/4 concentrations were added to the wells. These were followed by addition of a second biotinylated MoAb B60H8A4 also diluted to 3.6 µg/ml in PBS-T20/1% NBCS. Streptavidin horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10000 in PBS-T20/1% NBCS was added to act as conjugate. This was followed by addition of the OPD solution and 30% H₂O₂. This was incubated without shaking in the dark at room temperature for 30 min. All procedures involved washing the plate in each step five times with PBS-T20.

Fifty microliters of 4 N H₂SO₄ was added to each well to stop the reaction. The plates were read using an ELISA reader at 492 nm. To determine the cut-off, the OD of each serum sample was compared with a series of 8 reference negative serum samples at a probability level of ($P = 0.001$). Thus, was calculated as the mean of the OD values obtained with known negative samples plus two SD. A sample was considered positive if the OD value was greater than the estimated cut-off point.

In the present study, the OD cut-off value was 0.233. Monoclonal antibody ELISA assays were done at Dr. Chummy Sikasunge's laboratory in University of Zambia.

Statistical analyses

The categorical data are presented as mean, standard deviation, median and interquartile range or percent frequency. Seroprevalence to anticysticercal IgG and circulating antigens was calculated by dividing number of Ab-ELISA and antigen positive samples by the total number of people screened. Data were analyzed using univariate and Multivariate logistic regression to test for statistical level of significance between variables. Student-t test was used to test for differences between the means of proportions for levels of significance. Comparisons between groups were made using Fisher exact test. The probability value of ($P < 0.05$) were considered to be statistically significant. Statistical analysis of data

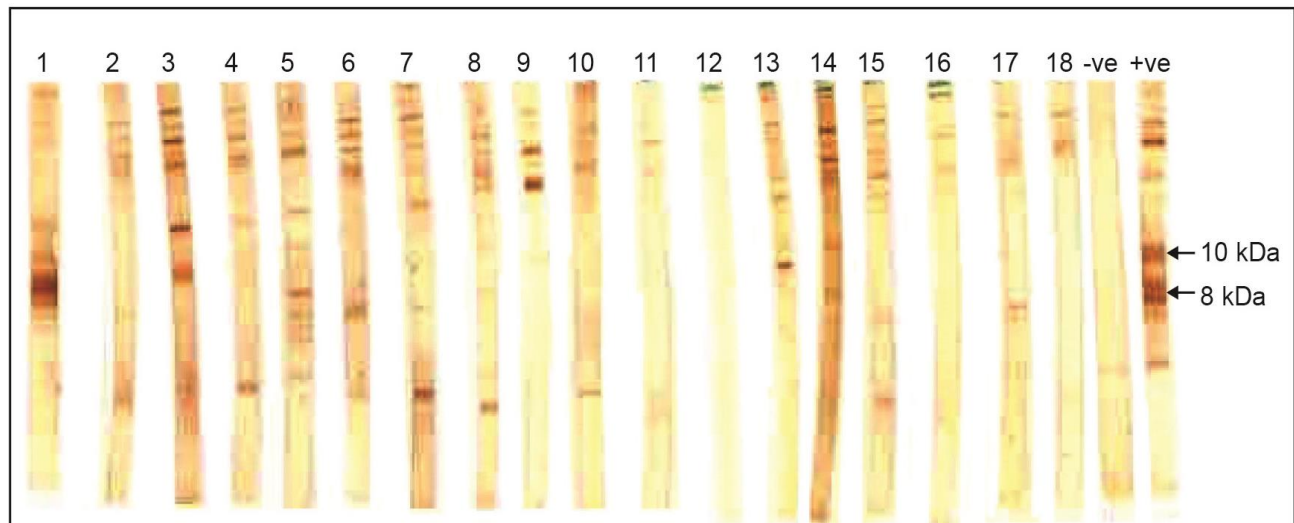


Figure 1. Immunoblot results for patients positive for 8 and 10kDa glycoproteins of *Taenia Solium*. Lane (+ve) (right); immunoblot containing known NCC sera (positive control). Lane (-ve); negative control; Lane 1, 3, 5, 6, 7, 14 and 17 are positive for 8 and 10 kDa glycoproteins.

Table 1. Age and sex distribution of the study subjects.

Age group	Sex	Percentage
	Male/female	%
10-19 ^a	75/57	44.2
20-29	37/31	26.2
30-39	24/16	13.4
40-49	13/7	6.7
50>	21/9	10.0
Total	170/130	100

^aMost males and females are in the age group of 10 to 19.

was performed using the SATA 11.0

RESULTS

Study population

This cross sectional study included 170 (56.7%) males and 130 (43.3%) females with the overall mean age 25 ± 13.2 . The age range was from 12 to 77.

Age and sex distribution of study subjects

Our findings indicate there was a higher proportion of males than females among patients of group aged 10-19, 75 (56.8%), 30-39, 24(60%), 40-49, 13 (65%), >50, 21(70%) (Table 1). Conversely, there was a higher number of females than males among aged groups 20-

29, 37(47.4%). However, the difference was not statistically significant, ($P = 0.058$).

This finding indicates age distribution was predominant among younger age group (10-19). Therefore, the younger people with epilepsy were more represented than older people.

Seroprevalence of anticysticercal IgG antibody using indirect antibody ELISA

Our findings from 300 serum samples screened among people with epilepsy for the presence of anticysticercal IgG indicate 15.0% (45/300) (CI 95%: 14.5-15.5) of epileptic patients positive for *T. solium* anticysticercal IgG in the three districts studied (Table 2). The districts of Gulu, Adjumani and Moyo had positivity to anticysticercal IgG of 17, 18 and 10 with a corresponding seroprevalence: 5.7(95% CI: 4.23-7.18), 6.0 (95%CI:

Table 2. Seroprevalence of anticysticercal IgG antibody using antibody ELISA.

Districts	Number of patients screened	Antibody ELISA positive	Seroprevalence, (N=300) n (95% CI)
Gulu	111	17	5.7(4.23-7.17)
Adjumani	92	18	6.0(4.53-7.47)
Moyo	97	10	3.3(1.83-4.77)
Total	300	45	15(14.5-15.5)

N; Total number of patients screened, n; number of samples positive for anticysticercal IgG; CI, confident interval.

Table 3. Seroprevalence of anticysticercal IgG antibody using immunoblot assays.

Districts	Immunoblot positive sample ^a (n=39)	Seroprevalence, (N= 300) n (95% CI) ^b
Gulu	12	40 (-1.4-1.51)
Adjumani	17	5.7(4.23-7.17)
Moyo	10	3.3(2.8-5.2)
Total	39	13(12.5-13.5)

N, total number of patients screened; n, number of sample positive for 8 and 10 kDa glycoproteins.; CI, confident interval; ^aNumber of immunoblot samples positive for 8 and 10 kDa glycoproteins, ^bSeroprevalence of anticysticercal IgG on immunoblot at 95% confidence interval.

Table 4. Immunoblot blot kDa 8 and 10 seropositivity in relation to age.

Districts	Number of patients per age group ^a					
	10<19	Number(%) positive	20<39	Number(%) positive	40+	Number(%) positive
Gulu	58	3(7.7)	44	6(15.4)	9	3(7.7)
Adjumani	39	4(10.2)	33	7(17.9)	20	8(20.7)
Moyo	36	2(5.2)	41	3(7.7)	20	3(7.7)
Total	133	9(23.1)	118	16(41)	49	14(35.0)

^aFrequency of seropositivity to IgG positive for 8 and 10 kDa glycoproteins in different age groups increased in the different age

4.53-7.47) and 3.3(95% CI: 1.88-7.17) respectively.

Seroprevalence of anticysticercal IgG antibody using immunoblot assays

45 antibody positive sera were screened using immunoblot for specific *T. solium* 8 and 10 kDa glycoprotein (Table 3). Our findings indicate that 86.7% (39/45) were positive on the diagnostic bands 8 and 10 kDa proteins (Figure 1). Twenty one patients, 53.8% (21/39) sera strongly reacted with higher molecular weight of 14, 21, 24, 38-42 and 50 kDa proteins while twenty five, 64.1% (25/39) patients sera showed reactions below the diagnostic bands (below 8 to 10 kDa). The frequency of reactivity of patients sera to higher molecular weight glycoproteins and the bands below the diagnostic region (8-10 kDa) was not

statistically significant ($P = 0.26$). The seroprevalence of anticysticercal IgG among the patient using immunoblot was 13%, (95% CI: 12.5-13.5).

Immunoblot blot kDa 8 and 10 seropositivity in relation to age

39 immunoblot positive samples were screened for specific *T. solium* 8 and 10 kDa glycoproteins in relation to age (Table 4). The frequency of seropositivity was 23.1, 41 and 35% among the age groups 10-19, 20-39 and ≥ 40 years old respectively. The highest seropositivity was in the age group 20-39. The influence of age on seropositivity significantly differed among the age groups ($P = 0.001$).

Our finding indicates that there was a statistically significant difference in the frequency of seropositivity to

Table 5. Immunoblot blot kDa 8 and kDa10 for seropositivity in relation to gender.

Districts	Number of patients (N=300)	Number of seropositivity (n) ^a	Male/Female ^b	Seroprevalence
Gulu	111	12	6/6	2.0/2.0
Adjumani	92	16	9/7	3.0/2.3
Moyo	97	11	6/5	2.0/1.7
Total	300	39	21/18	7.0/6.0

N; Total Number of patients screened, n; number of seropositivity, ^a Number of patients positive for 8 and 10 kDa glycoprotein, ^b data indicate more males positive for 8 and 10 kDa than females but this was not statistical significance ($P = 0.38$).

Table 6. Multivariate logistic regressions for some risk factors for *T. solium* cysticercosis

Risk factors for <i>T. solium</i>	No. of seropositive%	OR	95% CI	P value
Male ^a	21	1.8	(0.33-3.35)	0.01
Pork consumption ^b	170(57.0)	1.1	(0.2-6.9)	0.62
Free range pigs ^c	24(8.0)	1.7	(1.1-2.5)	0.01
No hand washing ^d	11(3.6)	1.4	(0.8-2.0)	0.04
Absence of toilet	6(2.0)	1.2	0.8-1.6)	0.001
Drinking of un boiled	3(1.0)	0.2	0.1-1.6	0.02

^a Being male; ^b pork consumption, ^c free range rearing of pigs and ^d no hand washing were statistically significant ($P = 0.001$).

IgG positive for 8 and 10 kDa glycoproteins among the age group ($P = 0.003$). Thus older people are more likely to be affected with *T. solium* cysticercosis than the young ones.

Immunoblot blot kDa 8 and 10 for seropositivity in relation to gender

Of the 39 patients analyzed for kDa 8 and 10 seropositivity, 56.4% (22/39) were males (Table 5). Gulu, Adjumani and Moyo had 12, 16 and 11 patients positive with immunoblot respectively. Of the 12 patients (Gulu), 50% (6 /12) were males and 50% (6 /12) were females. Adjumani had 56.2% (9/16) males and 43.8% (7/16) females positive with immunoblot. While, Moyo had 54.5% (6/11) males and 45.5% (5/11) females positive on immunoblot. The seroprevalence of patients in relation to gender was 7% (95% CI: 6.5-7.5) and 6% (95% CI: 5.5-6.5) respectively. The seroprevalence in each district was; Moyo 2.0/1.7, Gulu, 2.0/2.0, Adjumani, (3.0/2.3) respectively. There was no statistical difference between males and females for risk of infection with cysticercosis. (OR= 1.10; CI= 0.17-6.90 ($P = 0.62$)).

Some risk factors to *T. solium*

The aim was to find which of the risk factors had significant impact in the proliferation of *T. solium* parasites in the community. Our results indicate that of the 207 patients who consumed pork, 24 (11.6%) were

positive for anticysticercal IgG on antibody ELISA assays (Table 6). The analysis with multivariate logistic regression model (Table 6) show significant positive association ($P = 0.01$) with pork consumption. The OR = 1.7 (1.1-2.5); ($P = 0.01$). Similarly, out of the 86 households who reared pigs, 11 (12.6%) had patients' positive for anticysticercal IgG. The risks of infections with *T. solium* was significantly higher among those 11 patients; OR = 1.4 (0.8-2.0), ($P = 0.04$). Additionally, the seropositivity to anticysticercal IgG was significantly higher in patients who do not wash their hands after visiting toilet/pit latrine ($P = 0.001$). As of groups with or without toilets, the risk of infections was significantly higher among patients without toilets. Three, 3 (42.9%) patients without toilets were positive for anticysticercal IgG. The OR = 1.2 (0.8-1.6); ($P = 0.04$). There was no statistical significance ($P = 0.25$) in seropositivity to IgG between those patients who drank and those who did not drink unboiled water. Risk of being male was highly significant ($P = 0.001$).

Seroprevalence of circulating antigens using monoclonal antibody ELISA

When three hundred sera samples from epileptic patients in three districts of Adjumani Gulu and Moyo were screened for the presence of *T. solium* metacestodes (Table 7), Gulu, Adjumani and Moyo had 5 (4.5%), 9 (9.8%) and 13 (13.4%) patient samples positive for metacestodes of *T. solium* with the seroprevalence of 1.7, 3.0 and 4.3 respectively. The overall seroprevalence

Table 7. Monoclonal antigen ELISA assay for *T. solium* cysticercosis.

Districts	Number of patients tested (N=300)	antigen ELISA positive samples n (%) (n=27)	Seroprevalence (95% CI) (n=9)
Gulu	111	5(1.66)	1.7 (0.23-3.17)
Adjumani	92	9(3.0)	3.0 (1.53-4.47)
Moyo	97	13(4.0)	4.3 (2.83-5.77)
Total	300	27(8.99%)	9.0% (8.5-9.5)

N; total number of patients screened, n; number of samples positive for metacestodes of *Taenia solium* antigen, CI; confidence interval.

was 9% (27/300) and 95% (CI: 8.5-9.5).

DISCUSSION

Seroprevalence studies on anticysticercal IgG indicate high rates of exposure to cysticercal antigen of *T. solium* parasites in several countries worldwide. While many of these studies are in other developing countries, there is still limited information on this subject in Uganda. Recent studies conducted by Nsadha et al. (2011) revealed increase in porcine cysticercosis in Northern Uganda. This is an indication of human exposure to *T. solium* in the population. This study investigated the proportions of people with *T. solium* cysticercosis among epileptic patients in Northern Uganda. Three hundred patients were sampled and their sera analyzed using ELISA, MoAb-ELISA and immunoblot. The seroprevalence of *T. solium* anti-cysticercal IgG on Ab-ELISA and western blot were 15 and 13.0% respectively while the seroprevalence of circulating antigens was 9.0%. This indicates a possible exposure to this parasite and occurrence of active infections. In addition, the result reflects porcine cysticercosis reported earlier by Nsadha et al. (2011). While this is the first report on prevalence of human cysticercosis in the three districts of Northern Uganda, limited studies in these areas hamper comparison of the present data. However, several seroprevalence studies have been reported in other countries with varying results. Jin-Mei et al. (2010) conducted a study among rural village communities in Lyte, in the Philippines and reported a seroprevalence of anti-cysticercal IgG of 24.6%. A similar study done among epileptic patients in a rural village in the West and North West Province of Cameroon using Antibody ELISA showed a seroprevalence of *T. solium* specific antibody at 44.6% (Zoli et al., 2003). In addition, Parija et al. (2011) conducted a study to assess the immuno response among patients with epileptic seizures in India and they reported a seroprevalence of anti-cysticercal IgG of 16.3%. Similarly, in Malaysia a study conducted to determine exposure to cysticerci among rural population revealed a seroprevalence of 2.2% (Azian et al., 2006). Garcia et al. (2003) on the other hand carried out a study on prevalence of *T. solium* cysticercosis in endemic areas of Peruvian highlands and reported a

seroprevalence of anti-cysticercal IgG ranging from 7.1 to 26.9% (mean = 13.9%). While the results in the current study among the epileptic patients is nearly comparable to the previous studies reported by Garcia et al. 2003 (13.9%); Githigia et al. (2002) (14%) and Parija et al. (2006) (16.3%), the current seroprevalence of anti-cysticercal IgG is higher than those reported by Basem et al. (2010) (6.5%); Prado et al. (2007) (3.6%); Azian et al. (2006) (2.2%); Ito et al. (2003) (8.6%); Chung et al. (2005) (2.97%) and Sutisna et al. (1999) (1.6%). On the other hand the current seroprevalence of anti-cysticercal IgG is lower than those reported by Jin-Mei et al. (2010) (24.6%); Zoli et al. (200) (44.6%), Wandra et al. (2003) (47.9%). A much higher seroprevalence of anti-cysticercal IgG of 79.0% was reported by Ferrer et al. (2003) in rural village in Mexico. There are many reasons for high seroprevalence to *T. solium* cysticercosis. These may be due to low socioeconomic status of the populations in endemic areas where pork is consumed (Parija et al., 2011). Infected persons may also act as a source of infection by contaminating the food and water through defecation (Parija et al., 2011; Waiswa et al., 2009). In addition, consumption of undercooked infected pork, absence of toilet facilities and allowing pigs to feed on human fecal matter may result in high seroprevalence of anti-cysticercal IgG.

Relations between seroprevalence of anti-cysticercal IgG, gender and age

The present study found more males (7%) affected by *T. solium* cysticercosis than the females (6%). A higher seropositivity may be due to the male traditional roles of providing food and out sourcing meat for family demands. Furthermore, with two decades of war which occurred in Northern Uganda, all livestock were depleted leaving pigs as the only animals for consumption. This resulted in increased pork sales as more males got involved in pork business. Several authors have reported higher seroprevalence of anti-cysticercal IgG among males than females in many seroprevalence studies. Parija et al. (2011) reported higher seroprevalence of anti-cysticercal IgG, among males 2.0% (4/202) than females 0.99% (2/202) in South India. This was attributed to males being more involved in outside activities and consumption of

improperly cooked food than females in South India. In addition, Garcia et al. (2003) reported that more males are likely to be infected because of their adventurous occupations. In contrast, Basem et al. (2010) reported a higher seroprevalence of anti-cysticercal IgG in women (8.5%) than men (3.0%). Similar results were reported by Oliveira et al. (2006) (15 and 11.3%), Moore et al. (1995) (1.8 and 0.71%) in both women and men.

The present study has found the seroprevalence of anti-cysticercal IgG higher among older patients aged 20-39 and 40-49 years old, respectively. However, the anti-cysticercal IgG antibody titers did not differ significantly among these age groups ($P = 0.33$). This may be because older patients have similar immunogenic challenges to *T. solium* cysticerci variants as compared to the young ones. In addition, antibody titre may be influenced by the status of the cysticerci (active or inactive). Previous studies implicate inactive cysticerci or calcifications for late onsets of seizures among older epileptic patients. For instance, patients with inactive cysticerci are known to have reduced antibody titres compared to those with active cysticerci. In the present study, the difference in antibody titres between inactive or active cysticerci is not statistically significant ($P = 0.24$). The seroprevalence of anti-cysticercal IgG of 13.0% in this study indicates a high rate of *T. solium* cysticercosis among epileptic patients in the three districts of Northern Uganda. While this shows the extent of exposures of patients to *T. solium* infections the seroprevalence of anti-cysticercal IgG should be interpreted with caution as previous studies have reported possibility of overestimation (Garcia et al., 2001). However, many studies have reported the influence of age on seropositivity to *T. solium* cysticercosis among the population. Willingham et al. (2003), while conducting a hospital-based survey in Vietnam found a higher prevalence of cysticercosis in older age group. A similar finding was reported by Wandura et al. (2003) in Irian Jaya, Indonesia. Garcia et al. (2003) reported that the peak incidence of cysticercosis is between 30 and 50 years of age while antibodies disappear within 1 to 3 years in 30 to 40% of the seropositive people in endemic countries, reflecting a transient anti-cysticercal IgG antibody reaction after exposure or self-cure. In the present study the high seroprevalence of anti-cysticercal IgG reflects the current levels of endemicity of *T. solium* cysticercosis which is distributed among the patients.

Immunoblot assays for 8 and 10 kD proteins of *T. solium*

In this study 45 antibody positive serum samples were analyzed by immunoblot for specific *T. solium* glycoproteins. Of the samples analyzed, 86.7% (39/45) were positive for the diagnostic bands of 8 and 10 kD proteins. This result indicates that the 86.7% (39/45)

serum samples analyzed contain the glycoproteins components of *T. solium* parasites, a manifestation of the presence of *T. solium* cysticercosis among the epileptic patients. Additionally, the presence of 8 and 10 kD proteins of the anti-cysticercal IgG show evidence of exposure to *T. solium* parasites. The present finding is in agreement with the results reported by Minozzo et al. (2008) who found that the bands between 9 and 12 kDa had 38% sensitivity and 79% specificity. In this study, 53.8% (21/39) sera strongly reacted with higher molecular weights of 14, 21, 24, 38-42 and 50 kD proteins.

This is in contrast with the results of sensitivity (97%) and specificity (98%) reported by Chung et al. (1999). Sixty four, 64% (25/39) patient's sera showed reactions below the diagnostic bands (below 8 to 10 kDa). While Handcock et al. (2004) showed that the band 50 kDa had a higher reactivity. In the present study, the diagnostic bands 8 and 10 kDa have high sensitivity and specificity. Higher molecular weights bands are known to present strong cross reactions with other helminths hence are not allowed for use in diagnosis (Chung et al., 1999; Handcock et al., 2004). In this study it appears that antibodies from other helminths recognized many interacting sites particularly those with high molecular weights. The high frequency of these reactions may be due to the presence of other parasites in the patient's sera. Molinari et al. (2002) reported that samples from patients with viable cysts had higher absorbance than samples from calcified or transitional stages. In addition, cross reacting helminths such as *T. saginata*, *T. hydatigena*, *Echinococcus granulosus*, *Ascaris suum* have been implicated in provoking the immune systems leading to production of antibodies which can interact with the surface protein on the epitopes. While this may not be directly linked with consumption of infected pork, it may explain the diverse views on the presence of other parasites in the study area. In addition, the bands which were recognized in the lower side of the diagnostic bands equally require more investigations.

In conclusion, this study showed that *T. solium* infections occur among communities in the three rural districts of Gulu, Adjumani and Moyo in Northern Uganda. Therefore, there is a potential for proliferation of pork tape worm infections among the communities in this region. This is the first report on human cysticercosis in Northern Uganda and can be used as a baseline data for future management of the disease. However, further studies need to investigate for presence of other parasites which induce the antibodies which interact with the lower kDa glycoproteins below the diagnostic regions. In addition, there is a need to investigate the incidence of human cysticercosis among communities in the three districts of Gulu Adjumani and Moyo. Finally, more highly purified antigens need to be used in the Ab-ELISA format for better understanding the seroprevalence of human cysticercosis in the area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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