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Seroprevalence of Human *Taenia solium* cysticercosis and its associated factors in villages of Kongwa and Songwe Districts of Tanzania

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Abstract

Background: *Taenia solium* taeniasis/cysticercosis is a serious public health and economic problem in many low—and middle-income countries. Tanzania is among the sub-Saharan African countries endemic for porcine cysticercosis, which increases the risk of human taeniasis and, eventually, human cysticercosis. This study was performed to estimate the seroprevalence of human *T. solium* cysticercosis and its associated factors in villages of Kongwa and Songwe Districts of Tanzania.

Methods: This cross-sectional study was conducted between June and September 2019 in 42 villages of Kongwa and Songwe Districts. It involved 872 participants, of which 593 and 279 were from Kongwa and Songwe Districts, respectively. All participants were randomly selected from participating villages. Blood samples were collected from all selected participants and tested for human *T. solium* cysticercosis using Ag-ELISA and Wb-Ab assay. Structured questionnaires were administered, followed by direct observations of the study population to investigate factors associated with parasite transmission. Univariate logistic regression model was used to estimate factors associated with seroprevalence of human cysticercosis.

Results: Of the 872 human sera examined by Ag-ELISA, 12 (1.4%) participants were detected with active *T. solium* cysticercosis. Among the actively infected cases, 7(1.2%) and 5(1.8%) of the detected cases were from Kongwa and Songwe Districts, respectively. Furthermore, the results obtained also indicated the considerable variation of T. solium cysticercosis seropositivity across various factors. However, participants who were 45 years of age and above were more likely to be infected with *T. solium* cysticercosis than other age groups (OR=5.9, 95% C. I. 1.37-5.49, $p = 0.001$).

Conclusions: Human *T. solium* cysticercosis is still a public health problem in Kongwa and Songwe District. Being above 45 years of age was a significant determinant of acquiring the infection. However, more work is required to understand other factors that contribute to the transmission dynamics of T. solium in endemic rural areas. Therefore, appropriate interventions, including health education for the entire population at risk, should be implemented for sustainable control.

Keywords: Human *Taenia solium*; Cysticercosis; seroprevalence; Tanzania

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Introduction

Human cysticercosis (HCC) is a zoonotic disease and infection caused by larval stage (cysticercus) of the parasite *Taenia solium*. The disease and infection has serious public health and economic impact in endemic countries (Praet et al. 2010a). The larval stage is also found in pig as intermediate host where it causes porcine cysticercosis (PCC). Human acquire HCC when accidentally ingest eggs of the parasite (Flisser, 2006). When cysticerci lodge in the central nervous tissues, this results into a condition referred to as neurocysticercosis (NCC) (Gonzales et al. 2016; WHO, 2016). Humans also harbor the final (adult) stage of the parasite in the intestines, a condition called taeniasis acquired through consuming raw or partially cooked cystic pork (Arriola et al. 2014). *T. solium* cysticercosis represents a substantial burden because of its impact on human health, including NCC, animal health and welfare as well as economic losses due to disability and lost pig productivity (Phiri et al., 2003; Ngowi et al., 2004; Carabin et al., 2006; Trevisan et al., 2017).

In sub-Saharan Africa, the presence of PCC is well established (Mafojane et al. 2003; Sikasunge et al. 2008), however limited information on HCC/NCC is existing (Winkler, 2012). The prevalence of HCC ranges from 7.4% in South Africa to 20.5% in Mozambique (based on specific antibody detection) and was reported to be 21.6% in the Democratic Republic of Congo (based on circulating antigen detection) (Afonso et al., 2011). A recent meta-analysis on the prevalence of NCC in people with epilepsy in endemic areas, found that NCC was the cause of epilepsy in almost 30% of people with epilepsy (Ndimubanzi et al. 2010). Furthermore, NCC has been reported to be the main cause of epilepsy in humans in pig-keeping communities of many low- and middle income countries (Winkler et al. 2009; Trevisan et al. 2017).

Tanzania is known to be among countries endemic for *T.solium* infection, a situation strongly associated with lack of knowledge about the parasite and its zoonotic potentials, poverty, poor hygiene and sanitation, free-range pig management and lack of meat inspection (Ngowi et al., 2004, 2010; Komba, 2008; Mwanjali et al., 2013; Mwang'onde et al., 2014). Also, lack of or inadequate diagnostic capacity (e.g., serological and neuroimaging tests) for detecting HCC and NCC in Tanzania is a limiting factor for effective management and control of the infections. Moreover, the high cost and limited availability of neuroimaging tests (CT scan or MRI) is prohibitive for the right diagnosis for most people (WHO, 2005; Mwanjali et al., 2013).

In Tanzania, HCC prevalence of 16–17% has been estimated in the general population based on Ag-ELISA or IgG western blot methods (Mwanjali et al., 2013; Mwang'onde et al., 2014). These data emphasize the need for more studies in humans to gather information on the persistence of the parasite and factors associated with its transmission despite various ongoing efforts to control it. Narrowing down to specific socio-demographic factors that influence HCC transmission could provide evidence for setting up effective intervention programmes and allocate appropriate resources (Sohrabi et al. 2021).

This cross-sectional study was conducted to estimate seroprevalence of HCC and associated predictors before a large village level health education randomized trial in 42 villages of Kongwa and Songwe Districts of Tanzania.

Materials and Methods

Study Area

This study was carried out between June and September 2019, in Kongwa and Songwe Districts located in central zone and southern highlands of Tanzania, respectively. The districts were selected based on the fact of free range pig production and confirmed cases of *T. solium* infections in human and pigs (Eom et al., 2011; Mwanjali et al., 2013; Maganira et al., 2019). Kongwa District (**Fig. 1**) occupies an area of 4,041 square kilometres and has 22 wards and 87 villages. The human population was 365,952 made up of 61,914 households (NBS, 2016). The key economic activities of Kongwa District are agriculture, livestock production and other informal

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sectors. The mean temperature is about 26.5°C and main rain season is from November to April with an average annual rainfall of 500 - 800 mm (NBS, 2012).

Songwe District (**Fig. 2)** had a total population of 157,089 people living in 28,282 households. Inhabitants mainly engage in agriculture, livestock activities, mining, fishing and other informal sectors as sources of income. The district experiences a hot season from early September to late April and a cool season from May to late August. The mean temperature is about 26.5°C. The district has one long rainy season, usually from November to Mid- May, which records between 750 mm and 2000 mm per annum (Songwe-Profile, 2015).

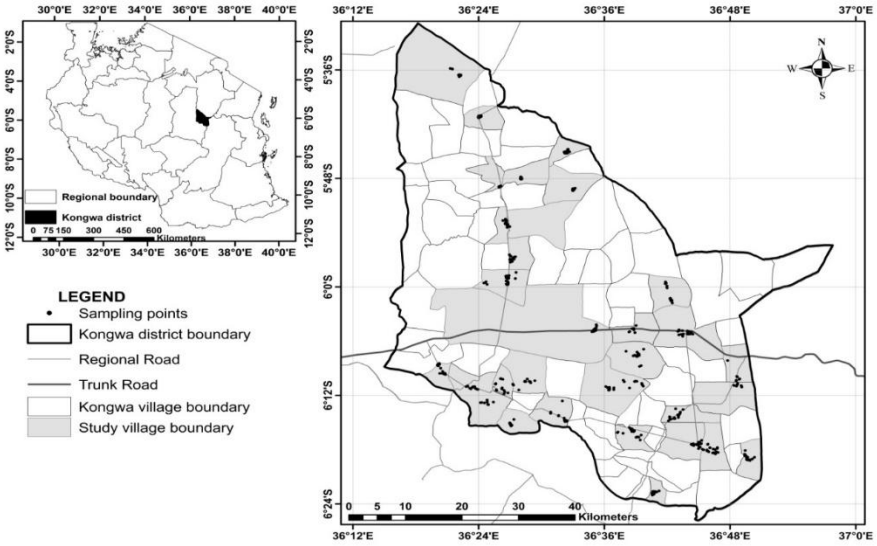

Figure 2.1: Map of Kongwa District showing distribution of the sampled households in study villages to estimate seroprevalence of HCC. The sampling points refer to households.

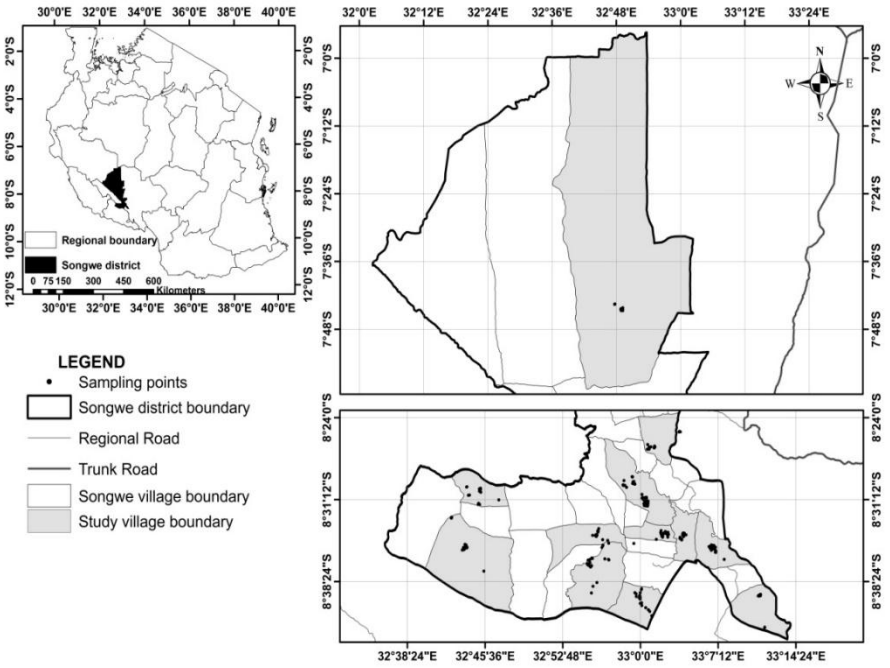

Figure 2. 2: Map of Songwe District showing distribution of the sampled households in study village to estimate seroprevalence of HCC. The sampling points refer to households.

Study design and sample size estimation

This was a cross-sectional study. Seroprevalence of 16.7% HCC based on Ag-ELISA in the general population in Mbozi District which is nearby Songwe District has been considered in this study (Mwanjali et al., 2013). Sample size estimation was calculated using the formula $n=Z^2P(1-P)/d^2$, in which n =required sample size, Z is a Z statistic value of 1.96 at confidence level of 95%, P =16%, estimated prevalence of HCC near by the study area and d = 0.05, relative precision. Thus, $n = (1.962)^2 \times 0.16 \times (1-0.16)/0.052 = 382$ households (one person per household). This number was more than doubled to adjust for multi-stage sampling design effect, thus, adjusted to = 872 for two districts. Participants were randomly selected from 42 villages of the two districts and the randomization was done at the villages and household level.

Recruitment of participants and data collection

The survey was conducted in 42 randomly selected villages in the study districts. In order to recruit required participants, sensitization and mobilization meetings involving researchers and the community were conducted to explain purpose of the study. Households were selected randomly using excel sheet from an existing list of all households in the selected villages and selected participants were asked for their consent to participate in the study. The selected households were visited for selection of eligible household member (aged between 15–60 years) to participate in the study. The study recruited equal number of households (20 households) per village for all 42 villages.

A structured questionnaire was developed and consisted of three parts: (i) general information of the household characteristics, such as age, sex, location, education level (ii) Behavioral information on pig rearing systems, presence and use of toilets, hand washing after defecation, washing of fruits and vegetables before consumption (iii) Clinical history on self-deworming and regular intestinal worm diagnosis. The questionnaires were administered to each of the 872 participants. After questionnaire administration, 5 ml venous blood was drawn from all participants at the cephalic or median cubital vein (median basilic vein) by a medical laboratory technician, the blood was collected in a specific plain blood vacutainer tube, well labelled and allowed to clot at room temperature overnight. At nearby village health centres, the clotted samples were centrifuged at 3500 rpm for serum extraction, ali-quoted and stored in cryogenic vials in freezers at -4^{o}C before analysis.

The laboratory analysis involved the use of immunological methods for the detection specific antibodies and for circulating parasite antigen in serum. Western blot IgG kit (WB-IgG) (LDBIO Diagnostics 69009 Lyon-France) was used for detection of specific antibodies (exposure) against HCC and the Cysticercosis Ag ELISA kit (Ag-Elisa) (apDia, Belgium) was used to detect circulating antigens (current infection) of *T. solium* cysticerci. The sensitivity and specificity of the Ag-ELISA for active infection with HCC has only been reported from a preliminary study conducted in Vietnam. The study indicated a sensitivity of 94.4% and a specificity of 100% for the diagnosis of current infection with cysticercosis (Dorny et al., 2003, 2004).

For the Western blot IgG kit, reaction to one or all of the seven glycoprotein bands was considered a positive indication of cysticercosis or at least of exposure to *T. solium* among the 7 bands. These diagnostic bands are GP50, GP42-39, GP24, GP21, GP1S, GPI4, and GP13. The letters GP signify the glycoprotein nature of these antigens, and the numbers denote their respective molecular weights in thousands (Tsang, Brand and Boyer, 1989). Presence of *T. solium* cysticercus antigens in human serum was measured using a commercially available kit (apDia, Belgium). The Cysticercosis Western blot IgG kit and apDia cysticercosis Ag- ELISA were used according to the manufacturer's protocol supplied with the kit.

Diagnostic Definition of HCC cases

Clinical features for HCC are not specific. Therefore, definition of HCC cases is often based on immunodiagnostic methods (Flisser et al., 2002). In this study, the definitions of HCC case have been considered as the active infection with viable cysticerci, therefore prevalence of infection is the proportion of respondents actively infected with viable cysticerci rather than prior exposure to *T. solium* in past, without active infection with living cysticerci. Also, the two testing methods were used for further indication of 'hot spots' in the study area where preventive and control measures should be applied.

Ethical Considerations

This study protocol was revised and approved by the Tanzania National Institute for Medical Research (NIMR) (NIMR/HQ/R.8a/Vol.1X/2802). The study also received approval from the ethics committee of the Klinikum rechts der Isar, Technical University of Munich, Germany, under the number of 537/18 S-KK. District authorities were asked for their consent to conduct the study. Consent for participation of the selected participants in a household was obtained from the selected individuals as well as the head of the household. Before sampling, each selected participant was approached individually to obtain written informed consent. For minors (<18 years) informed assents were obtained orally from them and thereafter a written informed consents were signed by their parents or guardians.

Statistical Analyses

Data entry and validation was carried out using Microsoft Excel 2010 (Ms Corp., Redmond, WA, USA). Then, descriptive analyses on the study population were conducted, followed by assessing the association between each potential risk factor and the prevalence of active HCC at the individual-level. Household characteristics measured through questionnaires were attributed to everyone since only one individual was sampled per household. All descriptive analyses were conducted using the SPSS version 24. To control confounding bias, Univariate logistic regression model was used to estimate association between active HCC seropositivity and other important risk factors using crude and adjusted odds ratios and their 95% confidence intervals. Statistical association was considered significant at $p < 0.05$.

Results

Socio demographic, behavioral and clinical factors

The socio-demographic, behavioral and clinical factors related to participants in the study villages are presented in Table 1. The total number of participants was 872, among them 593 (68.0%) and 279 (32.0%) were from Kongwa and Songwe District respectively. The majority 587(67.3%) of participants were males and based on education levels, majority 624 (71.6%) had primary level of education. Based on pig rearing practices, most 857(98.2%) of the participants agreed to always observe free-roaming pigs in their community.

Table 3. 1: Socio demographic, behavioral and clinical factors related to participants (N= 872)

Factor	n (%)	
Social-demographic		
Districts		
Kongwa	593	(68.0)
Songwe	279 (32.0)	

Sex		
Male	587	(67.3)
Female	285 (32.7)	
Age		
15-25	72 (8.3)	
26-45	444 (50.9)	
>45 years	356 (40.8)	
Educational level		
Informal	152 (17.4)	
Primary	624 (71.6)	
Secondary	77 (8.8)	
Post-Secondary	19 (2.2)	
Behavioural		
Water source		
Untreated	673 (77.2)	
Treated	199 (22.8)	
Pig rearing system		
Closed system	15 (1.7)	
Free range system	857 (98.2)	
Washing peeled fruits and vegetables		
No	80 (9.2)	
Yes	792 (90.8)	
Presence of toilets		
No	355 (40.7)	
Yes	517 (59.3)	
Every family member using toilet (n=517)		
No	34 (6.6)	
Yes	483 (93.4)	
Wash hands with soap after defecating		
No	293 (33.6)	
Yes	579 (66.4)	
Clinical		
Self-deworming		
No	404	(46.3)
Yes	468 (53.7)	
History family member with Epilepsy		
No	340 (39.4)	
Yes	522 (60.6)	
Antibody presence		
No	851 (97.6)	
Yes	21 (2.4)	

Seroprevalence of *T. solium* cysticercosis

Seroprevalence of HCC was determined and related to selected demographic, behavioural and clinical factors as shown in Table. 2. Results indicated that out of 872 human sera examined, the Ag-ELISA detected 12 (1.4%) participants with active HCC. Also 21(2.4%) of participants were detected with antibodies indicating exposure to the *T. solium* cysticerci. The seropositivity varied depending on participants' listed factors, whereby majority 10 (2.8%) of participants with the age of above 45 years were detected with active HCC. Further findings indicated that, there was no significant difference in active HCC seropositivity between individual who had toilets at their household and those who hadn't.

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Table 3. 2: Seroprevalence of human Cysticercosis across associated factors(N=872)

Factor		Antigen		P value	Antibody		P-value
		Yes (%)	No (%)		Yes (%)	No (%)	
Social-demographic							
Districts							
	Kongwa	7 (1.2)	586 (98.8)	0.47	12 (2.0)	581 (98.0)	0.28
	Songwe	5 (1.8)	274 (98.2)		9 (3.2)	270 (96.8)	
SexMale							
	Female	11 (1.9)	576 (98.1)	0.117	20 (3.4)	567 (96.6)	0.004
		1 (0.3)	284 (99.7)		1 (0.4)	284 (99.6)	
Age							
	15-25	0 (0.0)	72 (100)	0.015*	0 (0.0)	72 (100)	0.049*
	26-45	2 (0.5)	442 (99.5)		7 (1.6)	437 (98.4)	
	>45 years	10 (2.8)	346 (97.2)		14 (3.9)	342 (96.1)	
Educational level							
	Informal	1 (0.7)	151(99.3)	0.648	1 (0.7)	151 (99.3)	0.160
	Primary	11 (1.8)	613 (98.2)		20 (3.2)	604 (96.8)	
	Secondary	0 (0.0)	77 (100.0)		0 (0.0)	77 (100.0)	
	Post-Secondary	0 (0.0)	19 (100.0)		0 (0.0)	19 (100.0)	
Behavioural							
Water source							
	Untreated	10 (1.5)	663 (98.5)	1.000	18 (2.7)	655 (97.3)	0.439
	Treated	2 (1.0)	197 (99.0)		3 (1.5)	196 (98.5)	
Pig rearing system		1 (6.7)	14 (93.3)	0.189	1(6.7)	14 (93.3)	0.341
	Closed	11 (1.3)	846 (98.7)		20 (2.3)	837 (97.7)	
	Free range						
Presence of toilets		3 (0.9)	352 (99.1)	0.378	5 (1.4)	350 (98.6)	0.121
	No	9 (1.7)	508 (98.3)		16 (3.1)	501 (96.9)	
	Yes						
Family member using toilet		1 (2.9)	33 (97.1)		3 (4.0)	71(96.0)	0.413
	No	8 (1.7)	475 (98.3)	0.271	18 (2.3)	780 (97.7)	
	Yes						
Wash hands with soap after defecating		2 (0.7)	291(99.3)	0.356	5 (1.7)	288 (98.3)	0.483
	No	10 (1.7)	569 (98.3)		16 (2.8)		
	Yes						

					563 (97.2)	
Washing /peel fruits and vegetable						
	2 (2.5)	78 (97.5)		3 (3.8)	77	
No	10 (1.3)	782 (98.7)	0.303	18 (2.3)	(96.2) 774 (97.7)	0.431
Yes						
Clinical						
Self deworming						
No	7 (1.7)	397 (98.3)	0.562	10 (2.5)	394 (97.5)	1.000
Yes	5 (1.1)	463 (98.9)		11 (2.4)	457 (97.6)	
History family member with Epilepsy						
	4 (1.2)	336	0.773	6(1.8)	334	0.302
No	8 (1.5)	(98.8)		15(2.9)	(98.2)	
Yes		514 (98.5)			507 (97.1)	
Antibody presence	2 (0.24)	849 (99.8)	<0.001*	-	-	-
No	10 (47.6)	11(52.4)				
Yes						

*Chi square p-value < 0.05 from fisher`s exact test

Univariate analysis

Table 3.2 shows the univariate analysis results, whereby demographic, behavioural and clinical factors were associated with Ag-ELISA seropositivity, findings indicated that older age was a factor associated with active HCC, as the Ag-ELISA was higher in the age group of 45years and above (OR= 6.7) compared to other age groups. In addition, those who clinically were confirmed to have been exposed to the *T. solium* cysticerci in the past were more likely to be Ag-ELISA seropositive.

Table 3. 3: Associations between Ag-ELISA seropositivity and associated factors (N=872)

Characteristics	CrOR (95%CI)	*p*-value
Socio demographic		
Districts		
Kongwa	1	0.657
Songwe	1.52 (0.38,5.65)	
Sex		
Male	1	
Female	0.18 (0.00,1.28)	0.115
Age		
15-25		
26-45	1	
>45 years	6.71 (1.55,61.07)	0.005*
Educational level		
Informal	1	
Formal	0.89 (0.28,2.48)	1.000
Behavioral		

Water source			
	Untreated	1	
	Treated	0.67	0.920
Pig rearing system			
	Closed	1	
	Free range	0.18 (0.02,8.39)	0.378
Presence of toilets			
	No	1	
	Yes	2.08 (0.51,12.01)	0.418
Every family member using toilet		1	
	No	0.56 (0.07,25.4)	0.542
	Yes		
Wash hands with soap after defecating		1	
	No	2.55 (0.54,24.13)	0.349
	Yes		
Washing peeled fruits and vegetables		1	
	No	0.50 (0.10,4.77)	0.606
	Yes		
Clinical			
Self deworming			
	No	1	0.582
	Yes	0.61(0.15,2.26)	
History family member with Epilepsy		1	
	No	1.31 (0.35,5.98)	0.907
	Yes		
Antibody presence			
	No	1	
	Yes	361.71 (66.95,3766.02)	<0.001*

OR estimated from exact logistic regression
*Chi square p-value< 0.05 from fisher`s exact test

Discussion

The objective of this study was to estimate the seroprevalence of active HCC and assess factors associated with its transmission in villages of Kongwa and Songwe Districts. Previous studies indicated Kongwa and Songwe to be among the districts where PCC is endemic (Ngowi et al., 2008; Komba et al., 2013 and Maganira et al.,2019). The sero-Ag ELISA assay detected HCC seroprevalence of 1.4% (n=12) indicating the presence of viable cysts and as such active infections in these individuals. Detection of this infection implies environmental contamination with *T. solium* eggs from human feces and fecal–oral infections due to the lack of clean and safe water or consumption of contaminated food. Furthermore, the study found participants aged between 45 year and above and those detected with IgG antibodies against HCC to be significantly associated with the active HCC.

Seroprevalence of HCC in this study is lower than those found by similar studies in Mbozi and Mbulu Districts of Tanzania whereby prevalence of HCC was 16% and 17% based on Ag-ELISA and Western blot IgG antibody detection assay respectively (Mwang'onde, Nkwengulila and Chacha 2012; Mwanjali et al. 2013). The low prevalence of HCC estimated in the present study may be contributed by the improved hygienic conditions as a result of established and ongoing national public health interventions such as Water Sanitation and Hygiene (WASH) and Mass Drug Administration (MDA), the latter targeting soil transmitted helminths and schistosomiasis (MoH, 2012; Thomas et al., 2013).

A similar scenario has been reported in India (Vora et al. 2008). It was further established that age groups of the study population and presence of IgG antibodies for HCC were associated with HCC seropositivity, with higher prevalence in the age of 45 years and above, compared to the younger ones. Similar findings have been reported by previous studies in Tanzania and India (Vora et al. 2008; Mwanjali et al. 2013).

The study also associated the presence of IgG antibodies with presence of viable *T. solium* antigen and therefore presence of IgG antibodies does not confer protection against viable *T. solium* antigens. The differences in HCC seropositivity in age groups possibly reflect the effect of other interventions such as deworming program which is implemented mostly to school children excluding other age group (MoHSW, 2012), also levels of exposure to tapeworm eggs as age increases so as the likelihood of exposure status also increases. In village settings, elderly people spend more time in local beer bars located outside of their homes. This increases chances of eating undercooked and unhygienic food.

However, such area has limited sources of safe and clean water, exposing them to acquiring infections including *T. solium* infections (Maridadi et al.,2011 and Mwanjali et al.,2012). Therefore, further studies need to be done to determine potential risk factors associated with these categories of population to find better solutions.

The use of immunodiagnostic methods (Ag- ELISA and Western Blot IgG) for estimation of the seroprevalence is pointed as a limitation in this study. Until now only a few of the current serological techniques have been standardized and fully validated. The validation of the tests is hindered by the lack of a gold standard for the diagnosis of HCC. Efforts should be made to make cheap, reliable and standardized immunodiagnostic tools more widely available, especially in developing countries.

Conclusion

Findings of this study indicate that HCC is prevalent in villages of Kongwa and Songwe districts of Tanzania, which are among the main pork suppliers in the country. Further, studies are needed in rural pig keeping communities for mapping of human *T. solium* infections and guide development of sustainable control measures using one health approach with particular focus on elderly age groups and post exposure group as found to be a high risk groups while other measures are underway.

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