Seroepidemiologic Survey for Human Sparganosis in Mto wa Mbu Division, Monduli District, Tanzania

Kavana, N.J; Kasanga, C. J and Kassuku, A.A
Department of Veterinary Microbiology and Parasitology,
Faculty of Veterinary Medicine, Sokoine University of Agriculture,
P.O. Box 3019, Morogoro, Tanzania
E-mail: nkavana@yahoo.com

Abstract
Sparganum is a plerocercoid of pseudophyllidean tapeworm of Spirometra species. Human sparganosis has been reported in Northern Tanzania. A seroepidemiologic survey was undertaken to detect anti-sparganum specific IgG antibodies in serum of normal inhabitants of Mto wa Mbu, Monduli District, Tanzania. Sera were tested by enzyme-linked immunosorbent assay (ELISA) for the antisparganum antibodies. Positive rate for antisparganum antibody in 185 subjects was 116 (62.7%). Out of these 17 (9.2) were adult males, 80 (43.2%) were adult females and 19 (10.3%) were children (<18 years). Data for the questionnaire for all 116 ELISA positive inhabitants revealed that had history of eating game meat and drinking water from running springs. The data revealed that ELISA would be useful to find infected cases among normal inhabitants at sparganosis endemic areas.

Key words: Sparganosis, seroepidemiology, ELISA

INTRODUCTION

Sparganosis is caused by the sparganum, the migrating plerocercoid larva of the tapeworm of the genus *Spirometra* (Sparks, 1976). Human sparganosis has been reported worldwide but is most common in China, Japan and Southeast Asia (Cho, 1975; Sparks, 1976). Its first intermediate host is the copepod, and the second intermediate host include a wide range of vertebrates such as amphibians, reptiles, birds and mammals. Humans are also the second intermediate hosts and can be infected through eating the first or the second intermediate hosts.

Spargana can migrate widely in the human visceral organs and the resulting symptoms are different depending on the particular tissue or organ involved (Thomas, 1995). Frequently, spargana are discovered accidentally during unrelated surgical procedures. Cerebral sparganosis has been reported with increasing frequency in humans and is often characterized by convulsions.

Usually, the final diagnosis depends on the surgical recovery of the larva from lesions. However, enzyme linked immunosorbent assay (ELISA) is a sensitive and specific serodiagnostic tool for subcutaneous or cerebral sparganosis (Cho, 1990; Morakote, 1993). Specific (IgG) antibody can be detected making it possible to determine whether a patient is infected or not (Kim, 1984). Serological survey appears to be the most reasonable approach for epidemiological survey in a susceptible population. In

Tanzania, there are very few reports available on sparganosis. Cases of sparganosis have been reported from the pastoralists (the Masai) in Loliondo district in northern Tanzania (Schmid, 1972). This study was conducted to determine the status of sparganosis infection in normal inhabitants in Mto wa Mbu Sub-district, Monduli District, Tanzania.

Materials and Methods

Study area

This study was carried out in Mto wa Mbu sub-district, Monduli District in northern Tanzania. The wards involved were Esilalei, Losirwa and Mto wa Mbu. The main economic activity of the residents include livestock keeping and subsistance agriculture. Mto wa Mbu Division was purposely selected for this study because it is within the ecosystem with Tarangire National Park and Lake Manyara where there is plenty of game animals and is a hunting block. Data collection was carried out over a period of three months, from March to May 2012.

Sample collection

Ethical consideration

The protocol of this study was approved by the National Institute for Medical Research, Tanzania (Reference: No. NIMR/HQ/R.8a/Vol.IX/1285). During fieldwork, the objectives and procedures were explained to the participants. They were informed that their participation was totally voluntary and that they were free to participate or not to participate to the study without giving any reason whatsoever. Written and signed or thumb-printed consents were obtained from all participants before starting the survey.

Blood collection

A total of 185 serum samples were collected from randomly selected participants attending Mto wa Mbu Health Centre, Monduli District, Tanzania. About 4 ml of blood was collected aseptically from each participant using vacutainer. Blood was left at room temperature for 3 hours before serum was collected. Serum was pipetted in cryotubes stored at -20ºC.

Protein extraction from adult *Spirometra* worm

Adult worm of *Spirometra* was collected from the small intestine of infected dog from Minjingu village near Tarangire National Park. The species of the worm was confirmed by Polymerase chain reaction (PCR).

Pieces of adult worm about 1cm were placed in a falcon tube washed with PBS several times. Transferred into 4 tubes of 1.5 ml where was meshed, 1.5 ml of PBS was added. The tubes were transferred in a box with liquid Nitrogen at -180ºC for 1 min. then transferred in a water bath at 37ºC for 2 min. It was repeated several times until the tissue lysed. The samples were sonicated, and centrifuged for 15 min. at 3000g. The supernatant was transferred into new tubes stored at -20ºC until used.

Determination of protein concentration

Protein determination was done by using Bradford method (The Quick Start Bradford Assay Kit, USA). The procedure was performed in a 250 µl microplate assay. Two wells were pipetted with 500 µl of 1x dye reagent and labeled as blank. The next seven wells in duplicate were labeled as sample, transferred to each well 250 µl of a mixture (10µl of extracted protein in 500µl of 1x dye reagent). The microplate was transferred to spectrophotometer to measure absorbance of the standards, blanks and samples after 5 minutes. Standard curve was plotted with the 595 nm values (y-axis) versus their concentration in µg/ml (x-axis).

Evaluation of recombinant pkMSP-1_{33} in ELISA

A checkerboard ELISA was performed to determine the working concentrations of the coating antigen (pkMSP-1_{33}) and dilutions of patient sera. Recombinant pkMSP-1_{33} was serially diluted starting from the initial protein concentration of 20 µg/ml using 0.05 M sodium carbonate buffer, pH 9.6. The diluted antigen was coated on 96-well microtiter plates (TPP, Trasadingen, Switzerland) and incubated overnight at 4ºC. The wells were washed three times with Phosphate Buffered Saline containing 0.1% Tween-20 (PBS-T). Blocking buffer 1% BSA in PBS (1% BSA/PBS) was added into each well and incubated for two hours at 37ºC. The wells were then washed three times. Known positive and negative patient sera diluted to 1: 50, 1: 80, 1: 100, and 1: 200 in 1% BSA/PBS were added respectively into each well and incubated for one hour at 37ºC. After five time washes, 1:2500 diluted peroxidase-labelled goat anti-human IgG (KPL Inc., USA) was added and followed with one hour incubation at 37ºC. The wells were washed five times with PBS-T and incubated with 3, 3', 5, 5'-Tetramethyl Benzidine, TMB (Amresco, USA) for 30 minutes in dark. The reaction was stopped by adding 2 N H_2SO_4. OD was read at 450 nm. Samples were run in duplicates. The OD of a sample was determined by subtracting the OD of the blank TMB from the mean OD of the sample. The cut-off value was calculated as the $M_N + 2\sigma$ of the healthy donor sera group, where M_N and σ are the mean OD and the standard deviation respectively. Samples with OD values higher than $M_N + 2\sigma$ were considered positive. Finally, the best concentrations of antigens and dilutions of patient sera were determined and the designed ELISA with chosen antigen concentration and patient sera dilution was applied to the same sera sample which used for Western Blot assays.

Results

The antigenicity of crude extracts of adult *Spirometra erinaceieuropaei* was used for serodiagnosis of human sparganosis. Antisparganum specific antibody (IgG) levels in sera was measured by using the crude extract of adult *S. erinaceieuropaei* as antigen. Cysticercosis serum was used as a positive control. Out of 185 sera samples of inhabitants tested, 116 (62.5%) sera showed positive reactions to the adult *S. erinaceieuropaei* antigen. There were 33 males (18-81 years) of these 17 (9.18%) were positive, 125 females (18-80 years) of these 80 (43.2%) were positive and 27 Children (<18 years) of these 19 (10.27%) were positive as shown in Table 1 and Table 2. Boiling of drinking water, 151 (81.6%) of the participants responded not to be boiling water (Table 3). Source of water for drinking, 172 (93.0%) of the participants use water

from running springs (Table 4). The family members eating game meat was 100(54.1%) participants, 185 (100%) of the participants responded the meat was not being inspected. Preparation of game meat, 175 (94.6%) of the participants responded to be boiling (Table 5).

Table 1 Result of ELISA

	Total	**Positive & %**	**Negative**
Mto wa Mbu Sub-district			
Male (18-81)	33	17 (9.18%)	16
Female (18-80)	125	80 (43.2%)	45
Children (<18)	27	19 (10.27%)	8
Total	**185**	**116 (62.7%)**	**69**

Table 2 Number of positive participants according to their sex and age range.

		Age range (years)						
Sex	Total positive	Below 18	18-29	30-39	40-49	50-59	60-69	Above 70
Male	17	6	4	1	6	1	3	2
Female	80	13	41	25	6	4	3	1

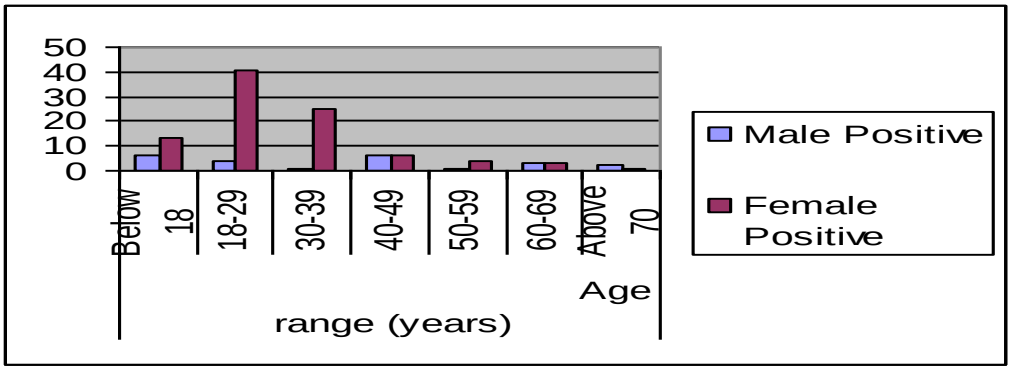

Figure 1: Sex with age range of positive participants from Mto wa Mbu

Table 3: Distribution of participants in relation to the habit of boiling water for drinking

Boiling of water for drinking	**No.of respondents**	**Percent**
Boil all the time	15	8.1%
Boil not always	19	10.3%
Not boiling	151	81.6%
Total	185	100.0%

Table 4: Types of water supply sources and number of participants using

Type of water sources	No. of participants using	Percent (%)
Local Shallow wells	3	1.6
Deep Wells	0	100
River	2	1.1
Running springs	172	93.0

Table 5: Habit of participants in eating game meat

	No.of participants	Percentage (%)
Family members eating game meat		
Yes	100	54.1
No	85	45.9
Inspection of game meat		
Yes	0	0
No	185	100
Preparation of game meat		
Boiling	175	94.6
Roasting	10	5.4

Discussion

The present study shows the prevalence rate of sparganosis infection of participants in Mto wa Mbu to be higher in females than males. These rates are higher than those reported from South Korea 1.6% to 3% (Kong, 1994; Park, 2001; Lee, 2002; Lee, 2003) and lower than that reported in Malaysia 63.6% (Mastura, 1997).

The age range of 18-29 years in females had highest infection rate than other age ranges. The result agrees with that reported previously in Malaysia (Mastura, 1997). But in Kangwon-do and Chollanam-do, Korea, the positive rate reported was 10 times higher in males than females (Hyun, 2001). The high positive rate in females in our study may be because the number of participants was bigger than males.

Human sparganosis in the age < 18 years has been reported by previous workers (Kim, 1984; Mastura, 1997). In our study, children < 18 years were found to be seropositive for human sparganosis. The results are similar to the previous workers. This indicates that human sparganosis can occur even in children.

Parallel to this study a questionnaire survey was carried out to the participants on water sources and habit of eating game meat. It was found that the inhabitants of Mto wa Mbu are using water from rivers, canals and ponds the water is not treated at any stage. Lake Manyara National Park is very close to this town and there are many streams and rivers near the town. The inhabitants share water sources with the animals from the National

Park as a result there is high contamination of water sources. In Tanzania, preliminary studies have shown that game animals in the national parks are highly infected with *Spirometra* (Opuni, 1974; Müller-Graf, 1995). Mto wa Mbu is near to the hunting block where legal and illegal hunters sell game meat without involving meat inspectors. The high rate of *Spirometra* infection in the study area may be due to drinking untreated water that contains infected Cyclops and eating game meat partially cooked which may be infected with spargana. In our study, crude extract from adult worm of *Spirometra erinaceieuropaei* was used for serodiagnosis of sparganosis. This is similar to the study carried out for the serodiagnosis of sparganosis of *Spirometra erinacei* in South Korea (Hyun, 1998).

CONCLUSION

The results have shown that sparganosis is a public health problem in Mto wa Mbu Division, Monduli District. This is the first time in Tanzania to report seroprevalence of human sparganosis. Therefore, we recommend the Ministry of Health and Social Welfare to carry out a study on sparganosis countrywide.

ACKNOWLEDGEMENTS

We are grateful to the Vice Chancellors of Mzumbe University, Sokoine University of Agriculture, Morogoro, Tanzania, District Medical Officer, Monduli for giving permission and staffs of Mto wa Mbu Health Centre for their help and cooperation. The work forms part of PhD studies programme of Nicholas Kavana. This work was financed by Mzumbe University Postgraduate Studies Funds.

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