STUDIES ON *SPIROMETRA* SPECIES ISOLATE FROM MINJINGU AND SEROPREVALENCE OF HUMAN SPARGANOSIS IN SELECTED DISTRICTS OF MANYARA AND ARUSHA REGIONS, TANZANIA

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

The genus *Spirometra* belongs to the order Pseudophyllidea and family Diphyllobothridea. The aim of the present study was to determine the complete life cycle of the tapeworm *Spirometra* and characterize the parasite using traditional parasitological methods and molecular biology techniques, and determine seroprevalence of spargana antibodies, knowledge, attitudes and practices on sparganosis infection among humans in Babati and Monduli districts, Tanzania. The investigation was conducted through targeted experimental life cycle, molecular biology, serology and questionnaire survey. *Spirometra* eggs were collected from faeces of lions and dogs from Tarangire National Park and Minjingu village. Eggs were cultured by modified Harada-Mori method. Hatched coracidia were experimentally fed to *Cyclops* to develop to procercoid. Infected *Cyclops* were orally fed to mice, rat, guinea pigs, New Zealand rabbit, pig and goat to develop to plerocercoids. The adult worm recovered from naturally infected dog from Minjingu village was used for morphological description after staining with Carmine. Polymerase Chain Reaction (PCR), DNA sequencing and phylogeny were used for identification and genetic characterization of the worm. A total of 216 serum samples obtained from normal inhabitants of Babati and Monduli districts were tested by enzyme-linked immunosorbent assay (ELISA) for anti *Spirometra* plerocercoid IgG. Questionnaire was used to collect information on knowledge, attitude and practices for sparganosis among inhabitants from the two selected districts. Coracidia hatched out of *Spirometra* eggs developed to procercoid in *Cyclops*. Plerocercoid was not obtained in both naturally and experimentally infected animals. Identification of Tanzanian *Spirometr*a spp. using morphological studies and molecular techniques confirmed to be *Spirometra erinaceieuropaei*. Serum samples revealed 62.5% (n =135) positive anti *Spirometra* plerocercoid IgG. All (100%) participants (n = 345) had no knowledge about sparganosis and mode of transmission of the disease. This study revealed inadequate knowledge, attitudes and practices on

sparganosis among inhabitants in the two districts. The health significance of *Spirometra* infection needs further investigation to establish relevance in health programmes in Tanzania.

DECLARATION

I NICHOLAS J. KAVANA, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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TABLE OF CONTENTS

ABSTRACT ..... **ii**

DECLARATION ..... **iv**

COPYRIGHT ..... **v**

ACKNOWLEDGEMENTS ..... **vi**

TABLE OF CONTENTS ..... **vii**

LIST OF TABLES ..... **xv**

LIST OF FIGURES ..... **xvii**

LIST OF APPENDICES ..... **xix**

ABBREVIATIONS AND SYMBOLS ..... **xx**

CHAPTER ONE **1**

1.0 INTRODUCTION **1**

1.1 Background Information 1

1.2 Objectives 5

1.2.1 General objective 5

1.2.2 Specific objectives 5

1.2.3 Research questions 6

1.2.4 Hypothesis 6

CHAPTER TWO **7**

2.0 LITERATURE REVIEW **7**

2.1 Parasite Classification 7

2.2 The biology of *Spirometra* 8

2.3 The Life Cycle of *Spirometra* 9

2.3.1 Egg 12
2.3.2 Hatching of the egg 12
2.3.3 Coracidia 13
2.3.4 Procercoid 13
2.3.5 Plerocercoid (sparganum) 13
2.3.6 Adult 14
2.4 The Intermediate Hosts 15
2.4.1 First intermediate host 15
2.4.2 Second intermediate host 15
2.4.3 Definitive host 16
2.5 Morphology 16
2.6 *Spirometra* Species 18
2.6.1 *Spirometra mansonoides* (*Spirometra* Muller 1937) 18
2.6.2 *Spirometra erinacei* 19
2.6.3 *Spirometra theileri* 19
2.7 Ecology 20
2.8 Pathogenesis of sparganosis 21
2.9 Clinical disease 22
2.9.1 Sparganosis 22
2.9.2 Sparganosis in man 23
2.9.3 Sparganosis in non-human primates 23
2.10 Source of Infection and Mode of Transmission 24
2.11 Etiology of sparganosis 25
2.12 Epidemiology **25**
2.13 Diagnosis **25**
2.14 Treatment **27**

2.15 Public Health and Prevention....28

2.16 The Current Study....28

CHAPTER THREE....29

3.0 MATERIALS AND METHODS....29

3.1 Study Areas....29

3.1.1 Babati District....29

3.1.2 Monduli District....31

3.2 Tarangire National Park....33

3.3 Minjingu Ward....33

3.4 Determination of the Life Cycle of Spirometra....35

3.4.1 Ethical Clearance for Use of Animals in the Experiments....35

3.4.2 Cultivation of *Cyclops*, the first intermediate host of *Spirometra*....35

3.4.3 Culture of *Paramecium* in Laboratory....35

3.4.4 Culture of *Cyclops* in Laboratory....36

3.4.5 Collection of *Spirometra* eggs....37

3.5 Faecal Sampling from Lions....37

3.6 Collection of *Spirometra* Eggs from Faeces of Lion....38

3.6.1 Floatation method....38

3.6.2 Sedimentation method....39

3.7 Collection of *Spirometra* Eggs from Faeces of Naturally Infected Dogs....39

3.8 Morphological study of *Spirometra* Eggs....40

3.9 Measurement of *Spirometra* Eggs....40

3.10 Hatching of Eggs....41

3.10.1 Preparation of aquarium....41

3.10.2 Incubation of *Spirometra* eggs by using modified Harada-Mori method at laboratory temperature of 26-29ºC 41
3.10.3 Incubation of *Spirometra* eggs in relation to light and darkness 43
3.10.4 Incubation of *Spirometra* eggs at laboratory temperature (26-29ºC) with light 43
3.10.5 Incubation of *Spirometra* eggs at room temperature (27-30ºC) in darkness 43
3.10.6 Studies on viability of eggs 43
3.11 Studies on Hatched Coracidia 44
3.11.1 Preparation of coracidia for morphological studies 44
3.11.2 Studying survival of coracidia 44
3.11.3 Experimental infection of *Cyclops* with coracidia 45
3.11.4 Experimental infection of Guinea pigs, rats, New Zealand rabbits, mice, pig and goat with *Cyclops* infected with procercoids 45
3.12 A survey of Cyclops (first intermediate host) for natural infection with procercoids 46
3.13 A Survey of Second Intermediate Host (Birds, Rats and Wild Animals) for Natural Infection with Spargana 47
3.14 Recovery of Spargana from Experimentally Infected Laboratory and Domestic Animals (pig ang goat) 47
3.15 Recovery of Adult Worm from Naturally Infected Dog 48
3.16 Staining of the Adult Worm of *Spirometra* 48
3.16.1 Preparation of Carmine HCl 48
3.16.2 Procedure of staining with carmine HCl 49
3.17 Identification of *Spirometra* Using Molecular Techniques 51
3.17.1 DNA Extraction from *Spirometra* adult worm 51

3.17.2 Polymerase Chain Reaction (PCR) 52
3.17.3 Agarose gel electrophoresis 53
3.17.4 Preparation of 2% Agarose gel 53
3.18 Cloning of Post PCR DNAs into a Cloning Vector 54
3.18.1 Ligation of DNA into the pGEM-T plasmid 54
3.18.2 Transformation of the ligated pGEM-T plasmid 54
3.18.3 Preparation of competent cells (Top10 E. coli) by PEG method 54
3.19 Colony PCR 55
3.20 Purification of cloned DNA 56
3.21 Analysis of DNA Sequence Data 57
3.22 Ethical Clearance in Relation To Taking Sera for Serological Tests for Sparganosis 57
3.23 Blood Collection 57
3.24 Protein Extraction from Adult *Spirometra* Worm 58
3.25 Protein Determination 58
3.26 Data Analysis 58
3.27 Antigen-ELISA 59
3.28 Knowledge, Attitude, Practices and Risk Factors of Sparganosis among Inhabitants in Northern Tanzania 60
3.28.1 Study area 60
3.28.2 Selection of study participants 61
3.28.3 Sample Size Calculation 61
3.28.4 Data collection 62
3.28.5 Socio-economic and demographic characteristics 62
3.29 Statistics 63

CHAPTER FOUR **64**

4.0 RESULTS **64**

4.1 Culture of *Paramecium* and *Cyclops* 64

4.2 A survey of *Spirometra* eggs from Faeces of Naturally Infected Lions 66

4.3 A Survey of *Spirometra* Eggs from Faeces of Naturally Infected Domestic Animals (Dogs and Cats) 67

4.4 Studies of the Morphology of *Spirometra* Egg 67

4.5 Studies of Embryonic Development of *Spirometra* Eggs 67

4.6 The Importance of Optimum Temperature, Light and Duration of Storage of 70

4.7 Studies of Viability of *Spirometra* Eggs Hatched After Incubation at Temperature of 26-29ºC with Light to Assess the Hatching Percentage (%) Eggs Hatched 71

4.8 Studies of *Spirometra* Eggs from Dog Faeces 71

4.9 Studies of Survival of Coracidia Observed Under Laboratory Condition (Temp. 26-29ºC) 72

4.10 Studies on the Development and Infectivity of Coracidia to *Cyclops* 73

4.11 Measurements of Coracidia 75

4.12 Prevalence of Procercoids in Naturally Infected *Cyclops* 75

4.13 Establishment of Infection Rate of Procercoids in Experimentally Infected 76

4.14 Experimental Infection of Laboratory and Domestic Animals with Procercoids 76

4.15 A Survey of Birds for Natural Infection with Spargana 76

4.16 A Survey of Rats for Natural Infection with Spargana 77

4.17 A Survey of Spargana in Naturally Infectected Wild Animals from Around 77

4.18 Recovery of Adult Worm from Naturally Infected Dog 77

4.19 Identification of Tanzanian *Spirometra* spp. Using Morphological Characteristics 79

4.20 Species Determination of *Spirometra* 81

4.21 Seroprevalence Studies of Sparganosis 83

4.22 Knowledge, Atittude, Practices and Risk Factors Studies 84

Single Table Analysis $X^2 = 20.54$; df =12; P = 0.05 87

CHAPTER FIVE 89

5.0 DISCUSSION 89

5.1 Studies on Culture of *Paramecium* and *Cyclops* 89

5.2 Collection of *Spirometra* Eggs from Lion and Dog Faeces 90

5.3 Studies on the Morphology of *Spirometra* Egg 92

5.4 Measurements of *Spirometra* Eggs 92

5.5 Incubation of *Spirometra* Eggs 93

5.6 Studies on Sedimentation of *Spirometra* Eggs 94

5.7 Studies on Effect of Temperature on Hatching of *Spirometra* Eggs 94

5.8 Studies of the Viability of the Eggs 95

5.9 Studies of the Survival of Coracidia 95

5.10 Measurements of Coracidia 96

5.11 Studies on the Role of *Cyclops* as First Intermediate Host 96

5.12 Survey of Spargana in Natural Infection of Wild Animals, Rats and Birds As 97

5.13 Studies of Dogs as Definitive Hosts 97

5.14 Studies of Adult Worm of *Spirometra* 98

5.15 Molecular Characterization of *Spirometra* 99

5.16 Determination of Seroprevalence of Sparganosis 100

5.17 Determination of Knowledge, Atittudes, Practices and Risk Factors of sparganosis **102**

CHAPTER SIX **106**

6.0 CONCLUSIONS AND RECOMMENDATIONS **106**

6.1 Conclusions 106

6.2 Recommendations 108

REFERENCES **109**

APPENDICES **138**

LIST OF TABLES

Table 1: Range of names which *Spirometra* has been classified in the past.....3

Table 2: Known *Spirometra* species and Characteristics of the two groups of *bresslauei* and *okumurai* within the genus *Spirometra* 4

Table 3: Sampling of faeces of lion (*Panthera leo*) at Tarangire National Park, Manyara, Tanzania.....38

Table 4: Comparison of efficiency of Floatation and Sedimentation methods to recover *Spirometra* eggs 66

Table 5: Faecal sample of dogs and cats examined for natural infection of *Spirometra*.....67

Table 6: Observation on the effect of optimum temperature and light on the hatching of *Spirometra* eggs from lion faeces 70

Table 7: Observation on the effect of optimum temperature and darkness on the hatching of *Spirometra* eggs from lion faeces 70

Table 8: Studies on the Viability of 1½ Years Old Eggs at Incubation of Laboratory Temperature (26-29ºc) With Light.....71

Table 9: Survival of coracidia observed under laboratory conditions (temperature 26-29^{0}C) 73

Table 10: Natural infection of *Cyclops* with procercoids from Tarangire National Park.....75

Table 11: Experimental infection of laboratory and domestic animals with procercoids.....76

Table 12: Rats examined for natural infection with spargana from Minjingu village near Tarangire National Park 77

Table 13: Morphological characteristics of the different developmental stages of Tanzanian *Spirometra* spp., *S. erinacei* and *S. mansonoides.* 80

Table 14: PCR screening results and confirmatory sequencing results of *Spirometra erinaceieuropaei* isolates from Tanzania 81

Table 15: Summary of ELISA results showing positive and negative sera for sparganosis from inhabitants of Babati and Monduli Districts 84

Table 16: Socio-Economic and Demographic Characteristics of respondents 85

Table 17: Practices of participants on economic activities 86

Table 18: Aim of participants on keeping dogs and cats 86

Table 19: Knowledge on how animals are kept 87

Table 20: Type of water which participants drink 87

Table 21: Types of Water supply sources 88

Table 22: Frequency distribution of respondents with different habits in preparing game meat before consumption 88

LIST OF FIGURES

Figure 1: Life cycle of *Spirometra* spp. 11

Figure 2: Map of Babati District showing wards of study area. Map of Tanzania on top left shows the location of Babati district. 30

Figure 3: Map of Monduli District showing wards of study area. Map of Tanzania on top left shows the location of Monduli district. 32

Figure 4: Map of Tarangire National Park showing sites where *Cyclops,* faecal sample of lions were collected and Minjingu village. Map of Tanzania on top right shows the location of Tarangire National Park. 34

Figure 5: Water pond in Tarangire National Park where egg sacked *Cyclops* were collected. (Source: Present study 2012). 36

Figure 6: Incubation of *Spirometra* eggs by modified Harada-Mori method. 42

Figure 7: Procedures for experimental studies undertaken in this study 46

Figure 8: Various studies done on adult *Spirometra* recovered from infected dog. 50

Figure 9: Flow chart of life cycle showing hosts and potential hosts in the environment identifying possible future investigations. 51

Figure 10: Laboratory cultured *Paramecium t*hat was used as food of *Cyclops i*n this study. 64

Figure 11: Culture of *Cyclops* 65

Figure 12: Developmental stages of *Spirometra* egg on incubation. 69

Figure 13: Normal and joined eggs of *Spirometra* 72

Figure 14: Hatched coracidia and developmental stages in the *Cyclops.* 74

Figure 15: *Cyclops* naturally infected with procercoid. Arrow showing procercoid with calcareous corpuscle 75

Figure 16: Recovery of an adult *Spirometra* from an infected dog 78

Figure 17: Appearance of *Spirometra* uterus after staining with carmine 79

Figure 18: (a) Gel electrophoresis of PCR products for *Spirometra* spp. suspected samples from Tanzania and (b) Gel electrophoresis of colony PCR 82

Figure 19: Phylogenetic tree of *Spirometra erinaceieuropaei* (Tanzania isolate) reconstructed using 11 representative nucleotide sequences by NJ method employing the Kimura – 2 – parameter option. 83

LIST OF APPENDICES

Appendix 1: Informed Consent Form 138

Appendix 2: Questionnaire Survey 141

Appendix 3: Ethical Clearance 145

Appendix 4: A pride of lion resting in Tarangire National Park. 146

Appendix 5: Sampling of lion faeces deposited on the ground 146

Appendix 6: Preparation for faecal sampling from the rectum of immobilized lion 147

Appendix 7: Sampling of lion faeces from the rectum after immobilization. 147

Appendix 8: Sampling of faeces from the rectum of a carcase of spotted hyena (*Cocruta cocruta*) at Makuyuni. 148

Appendix 9: Collection of *Spirometra* eggs from faeces of lion by Sedimentation method in the laboratory 148

Appendix 10: Collection of dog faeces per rectum. 149

Appendix 11: Dissecting water duck to search for natural infection of spargana. 149

Appendix 12: Carcase of warthog to be dissected to search for spargana. 150

Appendix 13: Carcase of wildebeest dissected to search spargana 150

Appendix 14: A researcher teaching a Clinical Officer how to fill questionnaires at Mto wa Mbu Health Centre, Monduli District 151

Appendix 15: Researcher describing the disease sparganosis to villagers at Mamire Ward, Babati District 151

Appendix 16: Water pond near houses bordering with Tarangire National Park. 152

Appendix 17: Masai woman carrying a bucket of water from a pond bordering Tarangire National Park 152

ABBREVIATIONS AND SYMBOLS

ARU	Animal Reasearch Unit
ATL	A tissue lysis buffer
BLAST	Basic Local Alignment Seaarch Tool
bp	base pair
BSA	bovine serum albumin
CO_2	carbon dioxide
CT	Computerized Tomography
DNA	Deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
ELISA	enzyme-linked immunosorbent assay
Fig	figure
g	gram
HCl	Hydrochloric acid
KAP	Knowledge, Attitudes and Practice
MEGA	Molecular Evolutionary Genetics Analysis
Min	minute
ml	milliliter
MRI	Magnetic Resonance Imaging
NIMR	National Institute for Medical Research
O_2	Oxygen
ºC	degree Celsius
PBS	Phosphate buffered Saline
PCR	Polymerase Chain Reaction
pH	hydrogen ion concentration

TAE	Tris acetic acid buffer
TANAPA	Tanzania National Parks
TMB	tetramethylbenzidine
USA	United States of America
VIC	Veterinary Investigation Centre
µm.	Micrometer
µ	micro-10^{-6}

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Spirometra is a pseudophyllidean cestode of dogs and cats (Mueller, 1974). Its life cycle requires two different intermediate hosts, the fresh water *Cyclops* as first intermediate host and some vertebrates such as amphibians, birds, reptiles and mammals as second intermediate host (Mueller, 1974; Lee *et al*., 1990; Garcia and Bruckner, 2007). The larval stages are procercoid and plerocercoid (sparganum), these are infective stages. Human can be infected by three possible routes: First route is ingestion of raw flesh of snakes, frogs or other animals that harbour the spargana (Lee *et al*., 1975). Second route is ingestion of infected *Cyclops* with procercoid in drinking water (Lee *et al*., 1975). Third route is the application of the flesh of infected frogs to a wound or eye sores (Kittiponghansa *et al.,* 1988). The spargana develop to adult worm in the small intestine of dog, cat or lion (definitive hosts). Sparganosis is an infection caused by sparganum of the *Spirometra.* Human sparganosis has been reported worldwide. In East Africa, human sparganosis has been reported in Tanzania (Schmid and Watchinger, 1972) and Uganda (Kiremerwa, *et al.,* 1956). In Tanzania, Schmid and Watchinger (1972) reported sparganosis from the Masai of Serengeti. However, Nobrega-Lee *et al.* (2007) reported sparganosis in baboons while Müller-Graf *et al*. (1999) reported infection of *Spirometra* in two populations of lions in the Serengeti National Park and Ngorongoro Crater.

Members in the genus *Spirometra*, have three parasitic stages in their life cycle (the procercoid, plerocercoid and adult) and one non-parasitic stage (the coracidium) which requires fresh water environment. They utilize a wide range of hosts the first intermediate host is a copepod, the second intermediate and paratenic hosts which are

cold and warm blooded vertebrates and the definitive host is carnivore and felid (Garcia and Bruckner, 2007).

Rudolphi (1819) reported *Spirometra*-like parasite for the first time. Thereafter, followed numerous parasites which were described under a wide range of generic and specific names (Table 1). The validity of many of these names is not clear. Stunkard (1965) indicated that for most diphyllobothriids variation within the genus results from differences among individuals in age and degree of maturity, physiological states owing to nutritional dissimilarities, crowding, type and condition of the host. Baer (1925) first recognized a cestode with general feature of *Diphyllobothrium* but with an internal seminal vesicle, male and female genital pores opening separately. The identification based on a single worm lacking a scolex he named the new genus and species *Lüheella pretoriences* and placed it in a new family, *Lüheellidae*. The genus and the family names were never accepted by subsequent workers most of whom retained *Diphyllobothrium.* Baer used the name *Diphyllobothrium pretoriensis* as late as 1955 (Baer and Fain, 1955). Joyeux and Houdemer (1928) synonymised *Lüheella* with *Diphyllobothrium* and *Lüheellidae* with Diphyllobothriidae. Schmidt (1974) named it *Lueheella,* Wardle and McLeod (1952) named *Lüheellidae*, but Yamaguti (1959) never used the names *Lüheella* and *Lüheellidae* as synonyms in his volumes on cestodes. Faust *et al.* (1929) divided *Diphyllobothrium* into subgenera. They named subgenus *Spirometra* for species with a spiral uterus rather than rosette-shaped one and with pointed rather than round eggs. Mueller (1937) raised *Spirometra* to generic rank, basing on the following characters: presence of an internal rather than external seminal vesicle, separation of male and female genital pores, spiral shape of uterus, pointed eggs and behaviour of larval stages. Wardle and McLeod (1952) listed 16 species of *Spirometra* with two groups: *bresslauei* and *okumurai* (Table 2).

Table 1: Range of names which *Spirometra* has been classified in the past

Name	Recognized by	Year
Dubium erinacei/erinaceieuropaei	Rudolphi	1819
Bothriocephalus felix	Creplin	1825
Bothriocephalus maculatus	Leuckart	1848
Dibothrium decipiens	Diesing	1850
Dibothrium serratum	Diesing	1850
Ligula reptans	Diesing	1850
Sparganum affine	Diesing	1854
Ligula ranarum	Gastaldi	1854
Bothriocephalus sulcatus	Molin	1858
Sparganum ellipticum	Molin	1859
Sparganum lanceolatum	Molin	1859
Ligula pancerii	Polonio	1860
Ligula mansoni	Cobbold	1882
Bothriocephalus liguloides	Leuckart	1886
Sparganum proliferum	Ijima	1905
Sparganum baxteri	Sambon	1907
Sparganum rallieti	Ratz	1912
Bothriocephalus longicollis	Parodi and Widakowich	1917
Diphyllobothrium tangalongi	MacCallum	1921
Sparganum phillipinensis	Tubangui	1924
Lüheella pretoriensis/pretoriensis	Baer	1924
Diphyllibothrium theileri	Baer	1924
Diphyllibothrium bresslaui/bresslauei	Baer	1927
Diphyllobothrium gracila/gracilis	Baer	1927
Diphyllobothrium erinacei	Faust, Campbell and Kellog	1929
Diphyllobothrium houghtoni	Faust, Campbell and Kellog	1929
Diphyllobothrium okumurai	Faust, Campbell and Kellog	1929
Diphyllobothrium fausti	Vialli	1931
Diphyllobothrium mansonoides	Mueller	1935
Diphyllobothrium serpentis	Yamaguti	1935
Sparganum canis	Fernandez and Vogelaang	1935
Diphyllobothrium trinitatis	Cameron	1936
Diphyllobothrium urichi	Cameron	1936
Diphyllobothrium giljacica	Ruckevich	1937
Sparganum okapica	Fain	1948
Sparganum cmeivai	Vogelsang and Gallo	1949
Sparganum fermandezi	Vogelsang and Gallo	1949
Spirometra janiskii	Furmaga	1953
Diphyllobothrium medium/media	Fahmi	1954

Table 2: Known *Spirometra* species and Characteristics of the two groups of *bresslauei* and *okumurai* within the genus *Spirometra*

Spirometra	Species	Host species (intermediate and final hosts)	Localities
Group 1	*S. bresslauei*	*Didelphys aurita*	South America
Bresslauei	*S.decipiens*	Frog (*Microhyla sowerbyi, R. nigromaculata)*	
Characteristics			
Vitellaria meet in front of the genital aperture,		domestic cat (*Felis padu)*; Leopard.(*Felis pardus villosa)*	Asia
	S. erinacei	Hedgehog (*Erinaceus europaeus)*	
		Frog (*Rana* spp.)	Europe
		Domestic cat (*Felis domestica*)	Asia
	S.felis	Domestic cat (*Felis domestica*)	Europe
	S. houghtoni	Human	Asia
	S.gracile	Wild cat (*Felis macrura*)	South America
	S.mansoni	Human; Frogs (*R. nigromaculata*, *R. esculenta*)	Asia
	S. reptans	Snakes	Asia
	S. mansonoides	Cats, dogs, snakes	USA
	S. serpentis	Frogs	Asia
	S. urichis	Wild cats (*Felis pardalis*)	Canada
Group 2			
Okumurai	*S. okumurai*	Frogs, snakes	Asia
	S. pretoriensis	*Otocyon megalotis*	
Characteristics		*Lyacon pictus*	Africa
Vitellaria do not meet in front of the genital aperture.	*S. raillieti*	hogs	Europe
	S. ranarum	Frogs: *R. esculenta*	
		R. tigrina	Asia
		Tripidonotus natrix	
		Canis procyonides	
	S. tangalongi	*Viverra tangalonga* (spotted civet)	Asia

Myers and Kuntz (1967) reported plerocercoid infection in baboons in Tanzania. Opuni (1974) recovered spargana from the warthog. He reported that *Spirometra* species present in Tanzania was *Spirometra theileri*. Muller-Graf *et al.* (1995; 1999) reported *Spirometra* infection .in lions of Serengeti National Park and Ngorongoro Crater after recovering

eggs in their faeces. Nobrega-Lee *et al*. (2006) reported *Sparganum proliferum* infection in baboons. The first case of human sparganosis in the country was reported by Schmid (1972) who recovered spargana from a Maasai in the Masailand. In the present study *Spirometra* eggs were recovered from faeces of lions of Tarangire National Park, eggs and adult worm from naturally infected dog from Minjingu village bordering with Tarangire National Park.

1.2 Objectives

1.2.1 General objective

To study *Spirometra* species, determine seroprevalence, knowledge, attitudes and practices on spargana infection among human population in Tanzania.

1.2.2 Specific objectives

i. To isolate plerocercoids from naturally infected second intermediate hosts in the field.
ii. To establish a complete life cycle of *Spirometra* spp.in the laboratory.
iii. To determine the morphological characteristics of the parasite using traditional methods of Parasitology.
iv. To determine the molecular characteristics of the parasite using molecular techniques
v. To assess antispargana antibodies in human sera collected from selected areas of Tanzania
vi. To determine Knowledge, Attitudes and Paractice among inhabitants of Babati and Monduli Districts regarding sparganosis infection.

1.2.3 Research questions

1. Can the plerocercoid of Tanzanian *Spirometra* species be isolated from naturally infected second intermediate hosts?
2. Can *Spirometra* species be established by using traditional and molecular methods?
3. Can the life cycle of Tanzanian *Spirometra* species be carried out in the laboratory?

1.2.4 Hypothesis

Spirometra exist in Tanzania but the species are not clearly known.

CHAPTER TWO

2.0 LITERATURE REVIEW

Cestode of the genus *Spirometra* was first reported by Rudolphi (1819) in Europe based on spargana he collected from the hedgehog, *Erinaceus europaeus.* Manson (1882) reported first case of human infection of spargana from the kidney fat of a Chinese corpse in Amoy, China. Faust *et al*. (1929) recovered spargana from the muscle fascia of the hedgehog, *Erinaceus dealbatus* in Peking, China, which was named as *erinacei* of Faust, *et al*. (1929). He propagated the spargana in cats and dogs where they developed to adult worms.

2.1 Parasite Classification

Spirometra is an intestinal cestode of cats and dogs (Mueller, 1974) which belong to the phylum platyhelminthes, class cestoidea and genus *Spirometra*. They are hermaphrodites, possessing both male and female reproductive organs.

The classification of *Spirometra* species according to Miyazaki (1991) is as follows:

Kingdom:	Animalia
Phylum:	Platyhelminthes
Class:	Cestoidea
Order:	Pseudophyllidea
Family:	Diphyllobothriidae
Genus:	*Spirometra*
Species:	*S .erinnacei, S. mansonoides*, *S. proliferum* and *S. theileri*.

2.2 The biology of *Spirometra*

The biology of *Spirometra* species has been well studied in *S. mansonoides* from USA (Mueller, 1974), *S. theileri* (Opuni, 1974) and *S. erinacei* from Asia (Lee *et al.,* 1990). *Spirometra* spp. are hermaphrodites, possessing both male and female reproductive organs. They are oviparous, laying eggs in the intestine of the definitive host, which get mixed with stool of the host, then excreted out to embryonate and hatch in water into coracidia. A single coracidium develops and hatches from each fertile egg which is shed (unembryonated) in the host´s faeces. When the fully developed coracidium is hatched in water, swims by using cilia which are on the body surface. Both species can survive in water for 2 days if they are not eaten by the *Cyclops* (first intermediate host). The *Spirometra* species use *Cyclops* as first intermediate host. Once the coracidium is eaten by *Cyclops* it develops into procercoid in the body cavity of the *Cyclops*. The procercoid develops to infective stage in 7 days for *S. theileri* (Opuni, 1974), 10 to 14 days for *S. mansonoides* (Mueller, 1938) and 10 to 20 days *S. erinacei* (Lee *et al.,* 1990). When infected *Cyclops* is eaten by tadpole, bird or mammals which act as second intermediate host, procercoid develops to plerocercoid (spargana). When infected second intermediate host is eaten by the definitive host the plerocercoid develops to adult worm in the small intestine of the host.

The adult worm starts to liberate eggs through the uterine pore into the small intestine which get mixed with the faeces of the host. The adult has a scolex with two bothria and a strobila comprising numerous proglottids with the genital openings: cirrus, vagina, and uterine pore which are all aligned along the midline. The adult produces and liberates large numbers of eggs which are voided with the host´s faeces in the environment. The pre-patent period for *S. erinacei* (Lee *et al.,* 1990) in dogs is 20 days, *S. mansonoides* (Mueller, 1938) in cats is 10 days, and *S. theileri* (Opuni, 1974) in dogs is 19 days.

2.3 The Life Cycle of *Spirometra*

The life cycle of all *Spirometra* spp. is the same, each species has two intermediate hosts. Adult *Spirometra* species are found in the small intestine of the definitive host and do not shed egg-laden proglottids in the host´s faeces, unlike cyclophylidian tapeworms, in their definitive hosts. But, the eggs are shed in the faeces after being discharged from the uterine pore of gravid proglottids of adult worms. The life cycle of *Spirometra* spp. starts from the stage of an egg as illustrated (Fig.1). The gravid uterus discharge eggs via uterine pore and large number of eggs appear in the faeces of the definitive host. When eggs are discharged out they are not embryonated (undeveloped). They start embryonation after reaching water with temperature not more than 39ºC or less than 16ºC. The operculated egg hatches in water, releasing a free swimming coracidium. The coracidium is covered with a ciliated outer envelope, active swimming is achieved by cilia. The coracidium is ingested by the first intermediate host, copepod of the genus *Cyclops*, and develops into a procercoid. The coracidium contains little food reserves and dies if it is not ingested by *Cyclops* within about forty eight hours (Arme and Pappas, 1983). Sharp, *et al.* (1990) observed coracidia of *Diphyllobothrium dendriticum* die after twelve hours if they were not ingested by the copepod.

When a second intermediate host (any vertebrate other than a fish) ingests the infected *Cyclops*, the procercoid develops into a plerocercoid in the muscle or connective tissue of this host (Mueller, 1937; Corkum, 1966; Moreira, *et al.,* 1997). If flesh of the second intermediate host is ingested by definitive hosts such as cats, dogs, wild felids, and raccoons the plerocercoid develops to adult worm (Mueller, 1938; Nelson, *et al.,* 1965; Lee, *et al.,* 1990; Müller-Graf, *et al.,* 1995, 1999; Engh, *et al.,* 2003). The plerocercoid attaches to the small intestine and develops into an adult tapeworm, which can reach up to 1.5 metres long. It starts to release eggs from the uterine pores of the gravid proglottids in

the host´s faeces approximately 10 to 30 days after infection (Mueller, 1974; Bowman, 1999). If the second intermediate host is eaten by another vertebrate not a definitive host, the plerocercoid migrate through the tissue and remain as plerocercoid in the new paratenic (transport) host, thus the larva of *Spirometra* can infect and survive in a series of paratenic hosts until the paratenic host is finally ingested by a suitable definitive host (carnivore). This adaptation not only increases the likelihood that the parasite will eventually find an appropriate definitive host but also results in large numbers of plerocercoids in omnivorus animals that regularly eat infected second intermediate hosts (Mueller, 1974; Bowman, 1999). The plerocercoid may be harboured by different vertebrates such as reptiles, amphibians, birds, mammals, man, and non-human primates. Fish are resistant to both procercoid and plerocercoid, therefore, are unsuitable intermediate hosts for *Spirometra* spp. (Mueller, 1960). The different stages in the life cycle are used for identification of the parasite.

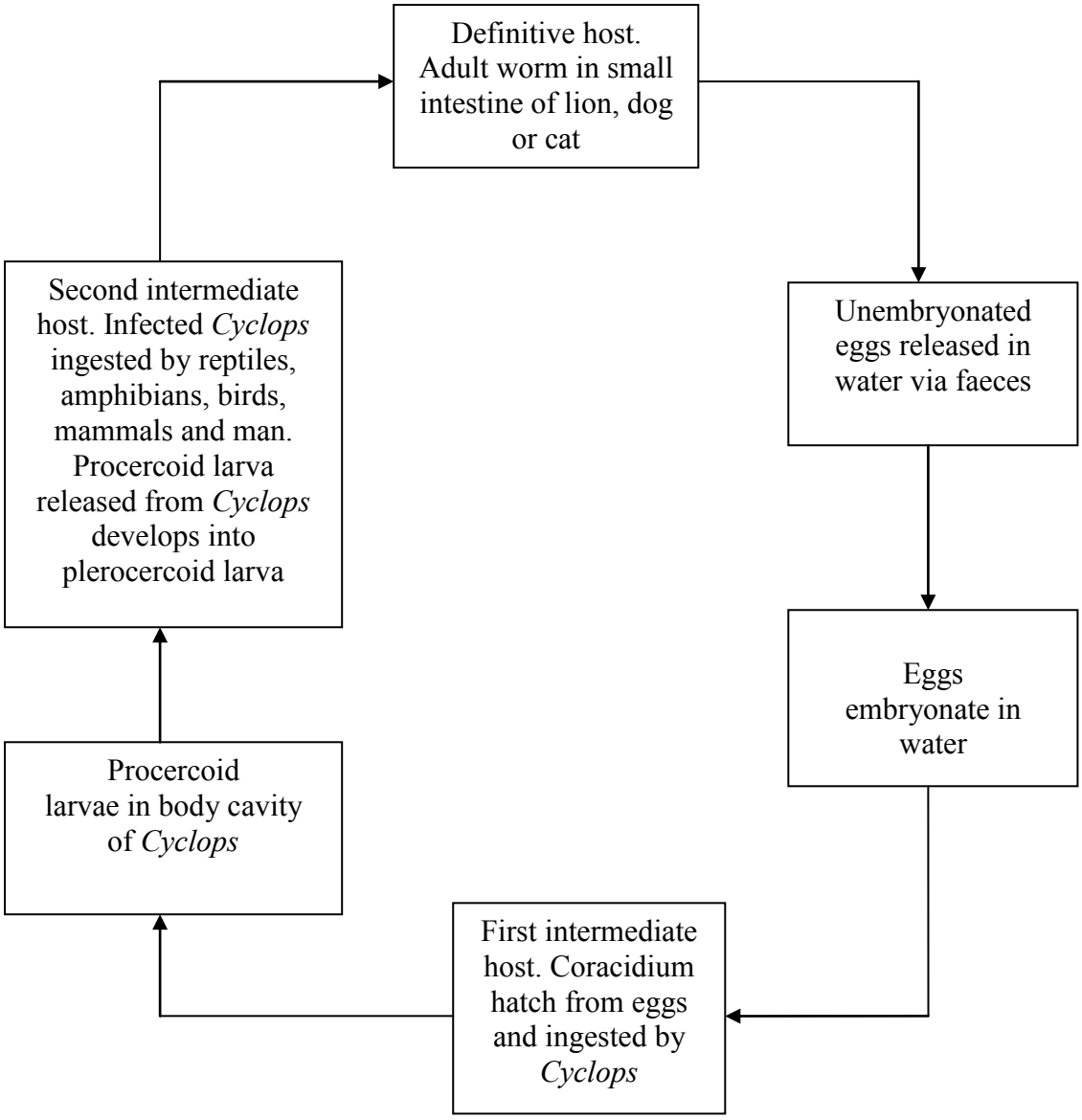


Figure 1: Life cycle of *Spirometra* spp.

2.3.1 Egg

Eggs are voided out of definitive host through the faeces, are unembryonated and possess an operculum on one end. The coracidium hatches out of egg in water. The coracidium swims in water and attracts potential first intermediate hosts (*Cyclops*). The *Spirometra* egg has been identified by using the characters of colour, shape, length, width, operculum, operculum suture and pitted surface (Mueller, 1938). *Spirometra* egg falls into a group of those produced by cestodes with extensive vitellaria. The eggs produced by *Spirometra* have thick operculate capsule, which mature outside the uterus. Since the eggs require water for embryonation and hatching, they die of desiccation within a short time. However, they can be stored for a considerable period in a refrigerator at about 4ºC without losing viability. This is a convenient method of storing eggs for experimental work (Mueller, 1959).

2.3.2 Hatching of the egg

When the egg is excreted out from the definitive host, the embryo is not developed. It starts developing when it comes in contact with water. When it is fully developed, the egg hatches to coracidium. There are many physicochemical factors involved in the embryonation of the egg. In order to hatch there must be water, optimum temperature and light (Kobayashi, 1931; Mueller, 1959; Lee *et al*., 1990). Li (1929) observed that eggs hatch in darkness. In the life cycle of *Spirometra*, the temperature plays a great role. When the temperature increases the time of embryonation decreases, and the percentage of hatching tends to fall at higher temperatures. Embryonation of *Spirometra* eggs has been reported to take place at different temperatures and time taken. *Spirometra theileri* eggs require 21 days to embryonate at 22-25ºC (Opuni, 1973), *S. erinacei* egg requires 8-14 days to embryonate at 29ºC (Lee, *et al*., 1990) and eggs of *S. mansonoides* embryonate in 10 days at 25-27 ºC (Mueller, 1959). When eggs of this species are incubated at body

temperature of 37ºC several abnormalities are observed including the development of minature coracidia, coracidia lacking hooks and coracidia with inverted primary axes (Mueller, 1961). Eggs for studies should be stored in water, in a stoppered bottle, and kept in a refrigerator at 4ºC, in this condition they remain for months without deterioration (Mueller, 1959). Eggs are susceptible to drying and get destroyed if kept for a long time (Mueller, 1959). Stored eggs are frequently attacked by fungus, to prevent this, a few drops of iodine solution should occasionally be added to the water in which eggs are stored (Mueller, 1959).

2.3.3 Coracidia

The coracidia are the second stage in the life cycle of *Spirometra* spp. It hatches from the egg, actively swim in the water by using the cilia which covers the whole surface of the body. The coracidia can be identified by using its active swimming, shape, cilia, and the three pairs of hooks on the posterior part of the body (Mueller, 1959).

2.3.4 Procercoid

The procercoid is an infective stage in the life cycle of *Spirometra* spp. The procercoid is infective if it has been in the abdominal cavity of the *Cyclops* for 10 days or more. When the second intermediate host eats the infected *Cyclops*, the procercoid develops to plerocercoid or spargana. Human get infected through drinking water containing *Cyclops* infected with procercoid (Mueller, 1959).

2.3.5 Plerocercoid (sparganum)

Sparganum is a plerocercoid larva of *Spirometra* spp. The sparganum has no segments but has surface ridges the sexual organs are not mature. Whereas, the adult worm is segmented and the sexual organs are mature (Mueller, 1959).

Studies have shown that the plerocercoid can be maintained in laboratory mice for the life time of the infected animal (Mueller, 1974). It can be identified by using morphological and histological characteristics. The plerocercoid is long and slender. It is ribbon-like, white in colour with a scolex and strobila, the neck is not differentiated (Mueller, 1974; Pampiglione, *et al.,* 2003). The scolex is thicker than the strobila. The anterior part of the scolex is invaginated. The larva can grow only if the scolex is intact, the growing point is in the anteriormost portion of the scolex within 0.5 mm from anterior end (Sohn and Chai, 1993). Histological studies on sparganum have shown to consist of tegument, parenchymal cells, loose connective tissue, muscles, excretory canals and calcium corpuscles. Tegument has various functions as site of metabolism, absorption of nutrient, secretion and protection. Calcium corpuscles contain several protein components and possess binding activity (Chowdhury *et al.,* 1955; von Brand *et al.,* 1960; Yang, 2000; Chung *et al.,* 2003). Spargana can survive and remain infective in decaying flesh and storage in a refrigerator at (5ºC) for 2 weeks, but freezing at (-12ºC) for not less than five days or thorough cooking render the plerocercoids noninfectious to humans (Mueller, 1974; Macdonald, 1998). The sparganum can survive in human for 20 years (Chang et *al.,* 1987).

2.3.6 Adult

The definitive hosts for adult *Spirometra* are wild and domestic canids and felids. The animals get infected by eating amphibians, reptiles and mammals infected by plerocercoids. When the plerocercoid reach the intestine of the definitive host, it attaches to the mucosa of the small intestine, within 10 to 30 days it matures into an adult worm, starts discharging eggs in faeces of the definitive host, thus completing the life cycle. The adult worm can survive for 19 months in the intestine of the definitive host (Mueller, 1974).

2.4 The Intermediate Hosts

2.4.1 First intermediate host

A number of copepods can act as intermediate hosts of *Spirometra* species. Susceptible copepods which have been reported to save as intermediate hosts are: *Cyclops strenuous*, *Cyclops affinis, C. phaleratus, C. magnus, C. vicinus, C. bicuspidatus, C. serrulatus, C. albidus, C. oithonoides*, *C. leuckarti, C. diaphanus, C. sigatus, C. phlexpedum, C. soli, C. fimbriatus, C. viridans, C. viridus*, *C. vicuspididatus* (Li, 1929; Kobayashi, 1931; Mueller, 1938; Lee *et al.,* 1990). The species of *Diaptomus* such as *Diaptomus gracilis*, *D. graciloides, D. vulgaris* are susceptible to coracidia infection but they are very difficulty to culture (Kuhlow 1953). The copepodid stages are more susceptible than the mature copepods which are often resistant to infection. When coracidia is ingested by copepod, the embryophore is shed and the contained hexacanth bores its way rapidly through the intestine wall into the haemocoele. The coracidia develops into procercoid, which lacks the differentiated anterior end with attachment organs (scolex) but possesses a posterior appendage (cercomer) that contains six embryonic hooks, reaches an infective stage when the hooks become separated into a constricted posterior region the cercomer.

2.4.2 Second intermediate host

Second intermediate hosts include amphibians, reptiles, birds, mammals, man and non-human primates (Mueller, 1959). Through the ingestion of infected *Cyclops*, the procercoid enters their tissues and develops into the plerocercoid stage. The sites of development may differ, with the larvae being localized in almost any organ. Plerocercoids usually lie unencapsulated in the host tissue.

2.4.3 Definitive host

Definitive hosts include canines and felids. Through ingestion of a prey infected with plerocercoids the plerocercoids enter their intestine and attaches to the intestinal mucosa, develop rapidly into adult worms discharging their first eggs 10 to 30 days later (Mueller, 1959).

2.5 Morphology

The external morphology of *Spirometra* spp. is divided into three parts. Scolex is an anterior end organ which has dorsal and ventral depressions called bothria, the scolex serve as holdfast organ. This is different from other orders, the Tetraphyllidea has bothridia which are broad, leaf-like structures with thin, flexible margins and the Cyclophyllidea have acetabula (suckers) which are true sucking organs and may be armed with hooks (Smyth and Clegg, 1959). The neck is between the scolex and the strobila, it is thin, long and unsegmented, contains germinal cells that have the potential for budding off the segments, a process called strobilization. Strobila is a distal part of the adult *Spirometra*, it is a chain of proglottids. The proglottides are broader than long. Inner longitudinal musculature usually weak. The proglottid has three genital openings: the vagina, cirrus and uterine pore (Mueller, 1959). The vagina and cirrus open independently. The vagina runs posteriorly from its opening and joins the oviduct before entering an ootype which is surrounded by the cells of Mehlis gland. The ovary is bilobed lead by an oviduct into the ootype (Wardle and McLeod, 1952). Uterus is simple spiral. The vitellaria are scattered throughout the lateral fields of the proglottids, their ducts join to form a short median duct which swells to form a vitelline reservoir before entering the ootype. The taxonomy of cestodes is based primarily upon the anatomy of the organ system, therefore, an understanding of these systems is essential.

(a) Nervous system

It is a longitudinal cord near each lateral margin and transverse commissures in each segment (Rees, 1941). The two lateral cords are united in the scolex in a complex arrangement of ganglia. The nervous system is rarely used as a taxonomic character.

(b) Osmoregulatory system

The organ of osmoregulation is the protonephridium or flame cell.

Body wall (tegument) consists of an outer layer formed from cytoplasmic extensions which come from an inner nucleated layer containing tegumental cells. Ultrastructure studies of tegument have been done by (Threadgold and Robinson, 1984; Smyth and McManus, 1989).

Microtriches these are spine-like which assist in maintaining the position of the worm in the gut. Also amplify the surface area for absoption (Threadgold, 1984; Smyth and McManus, 1989).

Parenchyma this fills the spaces between the internal organs, it is filled with parenchymal fluid rich in carbohydrate reserves in the form of glycogen (Smyth and McManus, 1989).

Nervous system consists a pair of longitudinal trunks running the length of the strobila (Rees, 1941).

Excretory system there are dorsal and ventral vessels on each side of the strobila.

(c) Reproductive system

The *Spirometra* spp. are monoecious, each proglottid contains one complete set of male and female reproductive organ. As the proglottides differentiate from anterior to posterior end of the strobila the reproductive organs mature and unembryonated eggs are formed. The male organs mature first and produce sperm, which are stored until maturation of the

ovary. The male reproductive system consists of many testes, each has a fine vas efferens. The vasa efferentia unite into a common vas deferens which drains the sperm toward the genital pore (Smyth, 1962). The vas deferens dilate into external seminal vesicle which function in sperm storage. Eventually, the vas deferens leads into a cirrus pouch, which is a muscular sheath containing the terminal portion of the male system. Distally, the duct is modified into a muscular cirrus, the male copulatory organ which opens on the surface of the proglottid. Female reproductive system consists of a single ovary located within the proglottid. Associated with the ovary are vitelline cells or vitellaria, which contribute to egg-shell formation and nutrition for the developing embryo. These are scattered as follicles in various patterns. As ova mature they leave the ovary through a single oviduct. Fertilization usually occurs in the proximal oviduct (Smyth, 1962). Cells from the vitelline glands pass through a common vitelline duct. Together they pass into a zone of the oviduct surrounded by unicellular gland called Mehli´s gland. The lumen of this zone is known as the ootype. The Mehli´s glands secrete a very thin membrane around the zygote and associated vitelline cells. Egg shell formation is completed from within by the vitelline cells (Smyth, 1962). The form of the uterus is convoluted or spiral which opens to the surface through the uterine pore. The eggs are released from the worm through the uterine pore. The vaginal pore usually opens near the cirrus pore. Near the proximal end there is a dilation called the seminal receptacle that stores sperm received in copulation. From the seminal receptacle a duct continues into the ootype.

2.6 *Spirometra* Species

2.6.1 *Spirometra mansonoides* (*Spirometra* Muller 1937)

The parasite was first reported in North America by Mueller (1935). When the parasite was discovered it was not known if it was separate from *S. erinacei*, Polymerase Chain Reaction (PCR) analysis of the two worms has shown the two to be separate but closely

related organisms (Lee *et al.,* 1997). It was first documented in North America (Min, 1990). *S mansonides* produces spargana growth factor (SGF), it possesses all the characteristics of mammalian growth hormone (GH) and does not cross-react with immunologic assays for GH (Kudesia *et al.,* 1988). The worm is small to medium-sized, scolex elongate, spoon shaped scolex, with bothrial lips. Bothrial slit broad and shallow. Neck is long and slander. Proglottids broader than long. Inner longitudinal musculature is weak. Testes numerous, medullary, in two lateral fields uniting in front. Cirrus and vagina open independently. Ovary is bilobed and vitellaria surrounding whole medulla. Uterus is simple spiral, not rosettelike, uterine pore ventral, and median. Adults are found in mammals, procercoid in *Cyclops*, plerocercoid in amphibians, reptiles and mammals. The life cycle involves *Cyclops* as first intermediate host and amphibian, reptile, bird and mammal second intermediate host and final host are carnivores.

2.6.2 *Spirometra erinacei*

Okumura (1919), provided the first complete life cycle description of *Spirometra erinacei*, copepod as first intermediate host, amphibians, reptiles and mammals as second intermediate hosts, and carnivorous animals such as dogs and cats as final hosts. Morphology and life cycle is the same as other species.

2.6.3 *Spirometra theileri*

Morphology, the life cycle is like other species, the only difference is that it does not develop in amphibians, reptiles and poorly develops in cats (Opuni, 1974). It does not produce growth hormones as in other species.

2.6.4 *Spirometra proliferum*

It is an aberrant tapeworm larva which is also known from man (Mueller, 1974). The plerocercoids of some *Spirometra* species replicate asexually in the intermediate or paratenic host, resulting in a severe pathogenic condition known as proliferative sparganosis (Buergelt *et al*. 1984). It is characterized by continuous branching and budding, the resulting progeny invading the entire region involved (Tashiro, 1924; Mueller, 1938). The biological feature of *S .proliferum* including taxonomic status is still unknown. Molecular studies have demonstrated that *S. proliferum* is a distinct species from *S. erinacei* and that *S. proliferum* belongs to the order Pseudophyllidea. The clinical manifestation appears under the skin as acneform pustular nodules in which living worms are contained and may be expressed. The histological components of the buds are of normal spargana, but have disturbed organization and symmetry, when fed to definitive hosts such as dog and cat they do not mature to adult worm (Mueller, 1938).

Sparganum proliferum in humans has been reported in Venezuela, Taiwan, Japan, North and South America and Asia (Moulinier *et al*., 1982; Lo *et al.,* 1987; Nakamura *et al.,* 1990; Noya *et al.,* 1992). Also has been reported in non-human primates such as the vervet (*Cercopithecus aethiops*), Sykes monkey (*Cercopithecus mitis*) and baboon (*Papio* spp.) (Chai *et al.,* 1997; Kim and Lee, 2001; Nobrega-Lee *et al.,* 2006).

2.7 Ecology

It is important to know the environmental conditions of the different habitats of the plerocercoid and the adult worm. The plerocercoids can be found in the amphibians, reptiles, birds and mammals. The important adaptability of the sparganum is its tolerance to a wide range of pH and its ability to thrive in cold-blooded hosts. In contrast to the selectivity of the adults, the sparganum shows lack of specificity. It grows in any class of

vertebrates, warm or cold-blooded, except fish. It is highly paratenic and re-establishes itself in the tissues of the body wall or under the skin (Mueller, 1938). Sparganum has high resistance to various kinds of stress (Mueller, 1974).

The larvae have different locations to their hosts comparing to adults. Larval stages have two intermediate hosts. Procercoid is found in the haemcoel of *Cyclops* (first intermediate host) while the plerocercoid is found in the muscles and tissues of amphibians, reptiles, birds and mammals. The location of adults is in the lumen of the small intestine of cats, dogs and other carnivorous mammals at the level of jejunum. The location of larvae and adult worms in different habitats of their hosts is due to the physiological factors (Mueller, 1938). *Spirometra sp.* have heteroxenous life cycle (involving more than one host), environmental conditions play part in controlling the development of the parasite. The physical-chemical conditions of a habitat-pH, pCO_2, O_2, viscosity and temperature are important for the survival of *Spirometra* spp.

2.8 Pathogenesis of sparganosis

Studies on experimental animals (monkeys and mice) and clinical findings of infected humans have shown the pathology and pathogenesis of this zoonotic disease (sparganosis). The pathology is associated with the early migratory phase of infection (Opuni, 1974). The pathological lesions are haemorrhagic skin lesions, oedema of the joints, ascites, peritonitis, infarcts in the liver, lungs and spleen. Penetration activity of the sparganum is by enzymes which contain acid thiol proteinase, which acts against azocoll, actin and myosin (Nakamura *et al.,* 1984). The scolex of sparganum has no proteolytic gland although a proteolytic enzyme is present (Kwa, 1972a, b).

2.9 Clinical disease

2.9.1 Sparganosis

Sparganosis is an infection of humans and animals caused by the, plerocercoid larvae (spargana) of various diphyllobothroid tapeworms belonging to the genus *Spirometra* (Sparks *et al.,* 1976). Sparganosis is a worldwide disease (Sun, 1999; Gray *et al.,* 1999; Pampiglione *et al.,* 2003; Wiwanitkit, 2005; John and Petri, 2006). In China sparganosis has been reported as an important foodborne parasitic disease (Zhou *et al.,* 2008; Li *et al.,* 2011). Sparganosis is most prevalent in Eastern Asia. In Thailand it has been reported in all parts of the country (Kittiponghansa *et al.,* 1988; Tesjaroen, 1991; Chamadol, *et al.,* 1992; Ausayakhun *et al.,* 1993; Jirawattanasomkul *et al.,* 2000; Phumanee *et al.,* 2001). Sparganosis has been reported in humans, mammals, and non-human primates (Huang and Kirk, 1962; Round, 1968; Tansurat, 1966; Morton, 1969; Kittiponghansa *et al.,* 1988; Noya *et al.,* 1992). There are four species of *Spirometra* which have been described to be of medical importance: *Spirometra mansonoides, S. erinacei, S. theileri* and *S. proliferum* (Miyazaki, 1991). These species are the most accepted at the present time, but, it should be noted that to differentiate them is a difficult task. The definitive hosts are domestic and wild canids and felids. Clinical presentation of sparganosis occurs when the plerocercoid reach the site of destination. Initially, the plerocercoids cause painful inflammatory reaction of the surrounding tissue. Later, migratory subcutaneous nodules develop. Other symptoms are eosinophilia in blood (John and Petri, 2006). Seizures, hemiparesis, and headaches are common symptoms in cerebral sparganosis. Location of spargana in the lymph channels result to elephantiasis, peritonitis due to perforation of the intestines, brain abscess due to location of the sparganum in the brain. Genital sparganosis may present as tumor-like in the groin, labia or scrotum. Ocular sparganosis presents with eye pain, excessive watering of the eye, droping of the upper lids, periorbital oedema, lacrimation, orbital cellulitis, exophalmos (protrution of the eye ball) and exposed cornea

ulcer (Walker and Zunt, 2005; Garcia and Bruckner, 2007). Also may present as a mass lesion in the eye. Ocular sparganosis may lead to blindness if untreated (Yang *et al.,* 2007).

2.9.2 Sparganosis in man

Sparganosis in human has an incubation period of 20 days to 14 months. The common sites of localization of spargana reported are: subcutaneous tissues (Tsou and Huang, 1993; Grifin *et al.,* 1996; Herzberg *et al.,* 1995; Garin *et al.,* 1997), ocular tissue (Zhong *et al.,* 1983; Kittiponghansa *et al.,* 1988; Sen *et al.,* 1989; Ausayakhun *et al.,* 1993; Yoon *et al.,* 2004), pulmonary tissue (Lin *et al.,* 1978; Phunmanee *et al.,* 2001; Iwatani *et al.,* 2006), cerebral tissue (Liao *et al.,* 1984; Lo *et al.,* 1987; Chamadol *et al.,* 1992; Chang *et al.,* 1992; Tsai *et al.,* 1993; Tanaka *et al.,* 1997; Kim *et al.,* 1997; Jeong *et al.,* 1998; Sundaram *et al.,* 2003), lower extremity (Ha and Oh, 2011), intramuscular (Cho *et al.,* 1987; Kim *et al.,* 2001), breast tissue (Moreira *et al.,* 1997; Sim *et al.,* 2002), testicular sparganosis (Sakamoto *et al.,* 2003), bladder (Oh *et al.,* 1993), kidney (Chung *et al.,* 1990) and spinal cord (Cho *et al.,* 1992). The symptoms of the disease vary with the site of localization. Sparganosis of the subcutaneous tissues present as a nodular lesion, pruritus and occasional migration of the larva. Ocular sparganosis may present with painful oedema of the eyelids, lacrimation and pruritus. Blood examination may show eosinophilia. The most serious form of the disease is proliferative sparganosis which is caused by *Sparganum proliferum.*

2.9.3 Sparganosis in non-human primates

Proliferum sparganosis in non-human primates has been reported in East Africa (Kuntz *et al.,* 1970; Nobrega-Lee *et al.,* 2006). It presents as nodules under the skin. The nodules are cystic, located on the inner thighs, abdominal cavity, and skeletal muscles. The

disease in animals can manifest in two different stages. Canids and felids are definitive hosts, the adult worm is localized in the small intestines, in general does not affect the health of the animal. It may cause weight loss, emaciation, irritability and abnormal or exaggeration of appetite of the animal. Other animals may be infected with the plerocercoids which are localized in the subcutaneous tissues and other organs. The presentation of the disease depends on the site of localization. The disease is asymptomatic when the number of spargana is small.

2.10 Source of Infection and Mode of Transmission

Natural and artificial water bodies (lagoons, lakes, water ponds, marshes and others) get contaminated with faeces of canids and felids mixed with eggs of *Spirometra* spp. The eggs develop to coracidia which then hatch to coracidia which is ingested by *Cyclops*. The infected *Cyclops* are ingested by the second intermediate host. The procercoid develops to plerocercoid in the body of the second intermediate host. The common route of infection is ingestion, mammals and birds become infected by ingesting parasitized *Cyclops*. Contamination of water bodies by faeces of wild canid that share the habitat makes the cycle persist in that area. The infection rate in human is low compared to animals. Man acquires sparganosis by ingesting not well boiled or cooked meat of second intermediate host infected with spargana. Other modes of infection are drinking of water from water bodies contaminated with infected *Cyclops* and larval transfer by contact (Manson *et al.,* 1921; Hughes and Biggs, 2001; Garcia and Bruckner, 2007). In some countries such as Vietnam and Thailand, raw flesh of frogs and snake are applied on open wounds, lesions and eyes for medicinal or ritualistic reasons (Walker and Zunt, 2005).

2.11 Etiology of sparganosis

Man is an accidental intermediate host and can be infected with sparganum through three possible routes (Tansurat, 1966):

i. eating raw or undercooked snake, frog and other animals that harbor plerocercoid larvae
ii. drinking water containing *Cyclops* infected with procercoids
iii. local application of the infected flesh of the host such as snake or frog as poultice to the eye and skin for treatment of an inflammation.

2.12 Epidemiology

Sparganosis is found throughout the world, human infection is more common in Japan, China, Korea, and Southeast Asia. Other countries in which sparganosis has been reported are USA, Latin America, Caribean, Uruguay, Ecuador, Colombia, Venezuela, Puerto Rico, Africa and Europe. The number of cases reported vary from country to country but the prevalence of the disease is higher in Japan, Korea and Southeast Asia because of their habit of eating raw flesh of frogs and snakes and application of their flesh to wounds (Tansurat, 1966).

2.13 Diagnosis

Human cases of sparganosis are usually recognized by the appearance of an itching and irritating migratory pea-sized nodule under the skin which on incision or removal contains the worm. The clinical presentation of sparganosis varies according to the site of tissue invasion. It is possible for the larva to encyst in any area from subcutaneous tissue of the abdominal wall to the viscera (Tansurat, 1971). Subcutaneous mass is a common presentation of sparganosis. Spargana are commonly found in the subcutaneous tissues and rarely in the viscera and eyes (Tsai, 1970; Chen and Chow, 1971; Sen *et al.,* 1989;

Fung *et al.,* 1989), brain (Mitchell *et al.,* 1990; Yamashita *et al.,* 1990; Ho *et al.,* 1992) and spinal cord (Lo and Chao, 1984). When there is history of migratory subcutaneous swelling with elevated blood eosinophilia it is more suggestive of a parasitic cause rather than a tumour (Campbell and Beals, 1977). The spargana can cause death if the brain is involved (Miyazaki, 1991). The duration of the migratory period is not clear, although it is thought that the sparganum can live up to 20 years in man (Chang, 1987).

(a) Incubation period

When plerocercoid is ingested the disease (sparganosis) can be established between 20 days to 3 years.

(b) History

Initial diagnosis of infection can be made based on the past history of the person eating raw snake or frog meat, drinking spring water containing infected *Cyclops* and use of poultice with flesh of infected snake or frog.

(c) Biopsy or surgery

Definitive diagnosis of sparganosis can be confirmed by biopsy or surgery of the involved tissue.

(d) Radiological diagnosis

It is not easy to differentiate subcutaneous sparganosis from other soft tissue mass such as lipoma with plain X-ray. Granulation of a long track of sparganum is shown as radiolucent lesion (Park *et al.,* 1993).

(e) Computerized tomography (CT) and Magnetic reasonance imaging (MRI) scans

The CT and MRI are diagnostic methods that are useful for the identification of intracranial sparganosis. These methods can be used to compliment each other (Moon *et al.,* 1993).

(f) Serologic diagnosis

The use of serologic tests such as ELISA, with specific Immunogloblin G (IgG) antibodies is mainly carried out in suspected groups for sparganosis. This is especially useful in countries where people eat raw snake or frog meat (Cho *et al*., 1975). It can also be used to screen those who suffer from neurological disease such as epilepsy (Kong *et al.,* 1994). Preoperative diagnosis (Chang *et al.,* 1987) and post operative control after surgical removal of sparganum from cranial cavity or spine (Lee, *et al.,* 1987; Kim *et al.,* 1984). It is also used for epidemiological investigation in endemic areas.

2.14 Treatment

The most successful treatment for sparganosis is removal of the worm or lesion by surgical operation. Adult worm of *Spirometra* is treated by using Praziquantel (Groll, 1980; Andrews *et al.,* 1983; Rim *et al.,* 1981; Eslami and Bazargami, 1986). It has been observed that treatment of *Spirometra* using 5 mg/kg single dose of praziquantel is ineffective but 7.5 mg/kg for 2 consecutive days was effective (Kirkpatrick, 1983; Sakamoto, 1977; Georgi, 1987). The drug induces vascuolization of the tegument around the neck of the adult worm which looses the ability to resist digestion by the host enzyme (Becker *et al*., 1981). Oral administration of praziquantel is a common route for treatment of human infection of adult *Spirometra.* In veterinary practices it can be administered orally or by injectable route (Andrews *et al.,* 1983). Other drugs used are Bithionol, Niclosamide, Dichlorophen, Paromomycin and Buramidin hydrochloride (Yokogawa *et*

al., 1982; Burrows and Lillis, 1966; Poole *et al.,* 1971; Kee *et al.,* 1988). Failure has been reported with Albendazole (25mg/kg twice daily) for 10 days and Niclosamide (333 mg/kg) single dose (Kirkpatrick, 1983). Toxicity problems have not been reported with these praziquantel doses in small animals (Shmidl *et al.,* 1981, 1982). Treatment of proliferative sparganosis with high doses of Praziquantel and Mebendazole has been persued but without results (Torres *et al.,* 1981; Beveridge *et al.,* 1998).

2.15 Public Health and Prevention

To prevent sparganosis infection, public health strategies should focus on providing basic access to safe water. Health education about sparganosis and the importance of food sanitation should be implemented in all rural endemic areas (Song, *et al.,* 2007).

2.16 The Current Study

The current study was undertaken to establish the life cycle of Tanzanian *Spirometra* under laboratory conditions. Natural infection of second intermediate hosts with spargana was assessed by examining animals, birds and rats. The adult worm of *Spirometra* was characterized by using morphological characteristics and molecular biology methods. Seroprevalence of spargana antibodies among the Babati and Monduli districts inhabitants in Tanzania was determined. Knowledge, attitudes and practice among the inhabitants of Babati and Monduli districts regarding sparganosis infection was determined.

CHAPTER THREE

3.0 MATERIALS AND METHODS

To determine the Tanzanian *Spirometra* species, life cycle of the parasite, experiments were carried out in the laboratory. Different stages of the life cycle were used to study the morphological characteristics of the parasite using the traditional methods of Parasitology. Therefore, the adult worm of *Spirometra* recovered from naturally infected dog was characterized by using molecular techniques. Assessment of antispargana antibodies in human sera collected from two districts of Babati and Monduli bordering Tarangire National Park, Manyara, Tanzania were determined. Finally, knowledge, attitudes and practices among the population in the two districts regarding sparganosis infection was determined. Previous study has shown the presence of *Spirometra* eggs in lion faeces from Serengeti National Park, Tanzania. In this study, *Spirometra* eggs were collected from faeces of naturally infected lions from Tarangire National Park and from dogs of Minjingu ward bordering with Tarangire National Park. Adult worm was recovered from a dog that was excreting *Spirometra* eggs.

3.1 Study Areas

3.1.1 Babati District

Babati District is one of the five districts of Manyara Region of Tanzania (Fig.2). The district covers an area of 6069 km^2 (2343 sq mi), a large proportion (640 sq km) of which is covered by the water bodies of Lake Babati, Lake Burunge and Lake Manyara. The district is bordered to the north by Arusha Region, to the south east by Simanjiro District, to the south by Dodoma Region, to the south west by Hanang District, and to the north west by Mbulu District. Babati District is located south of the Equator between latitude 3° and 4° South and longitude 35° and 36° E.

According to the 2002 Tanzania National Census, the population of the Babati District was 303 013. About 90% of the population of Babati District live in the rural areas and depend on agriculture and livestock for their livelihood. They are mostly small-scale farmers or agro-pastoralists practicing a semi-traditional farming system characterized by low use of farm inputs. Mixed crop-livestock, mostly maize-based systems are widely found in the district. Livestock comprise of local breeds of cattle, sheep, goats, chickens and pigs. Only 44% out of the 96 villages have health facilities, such as dispensaries or health centers.

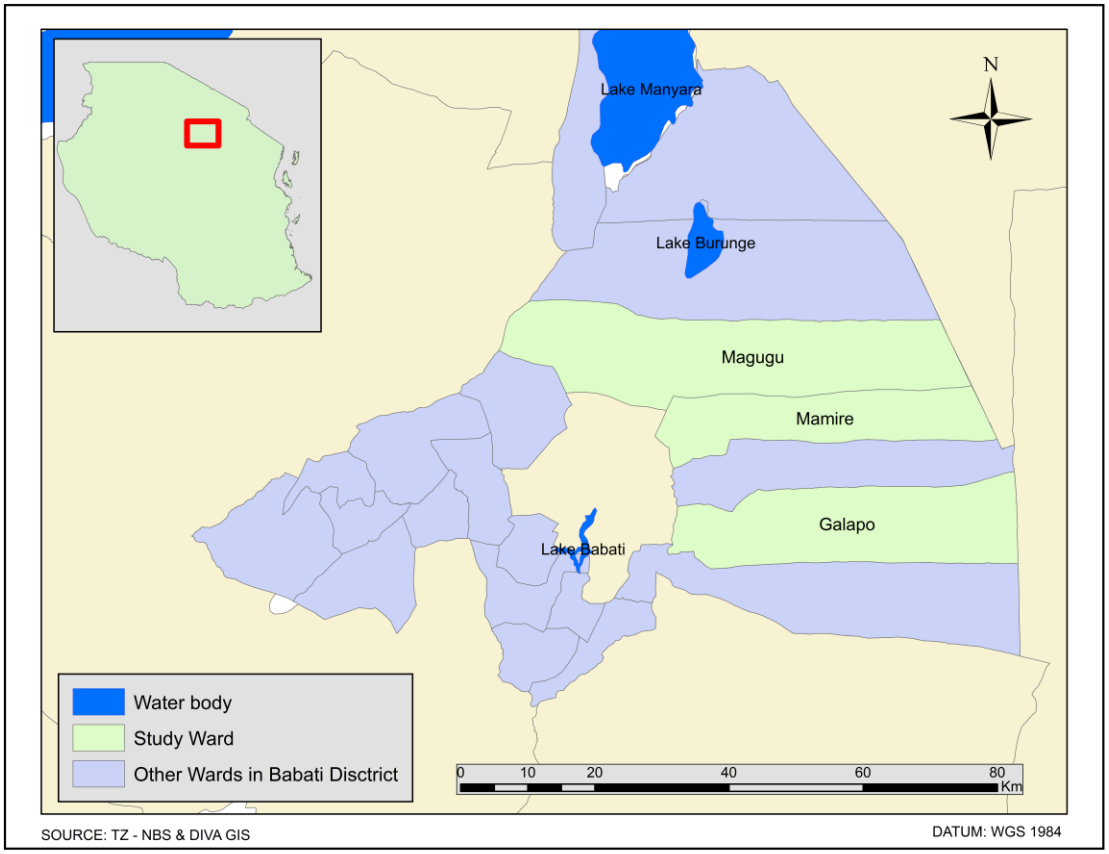


Figure 2: Map of Babati District showing wards of study area. Map of Tanzania on top left shows the location of Babati district.

3.1.2 Monduli District

Monduli District is one of the five districts of the Arusha Region of Tanzania (Fig.3). It is located in the northeastern section of the country. It is bordered to the north by Kenya, to the east by the Kilimanjaro Region and Arumeru District, to the south by the Manyara Region and to the west by Ngorongoro District and Karatu District. According to the 2002 Tanzania National Census, the population of the Monduli District was 185 237. The Monduli Mountains run through the area. The area contains plain land, rainforest, and mountains. The people that reside in the Monduli District are the Maasai people. They are a nomadic people who shepherd sheep and cattle. The women do most of the caring for children and farming, while men and adolescents roam the fields with the livestock during the day.

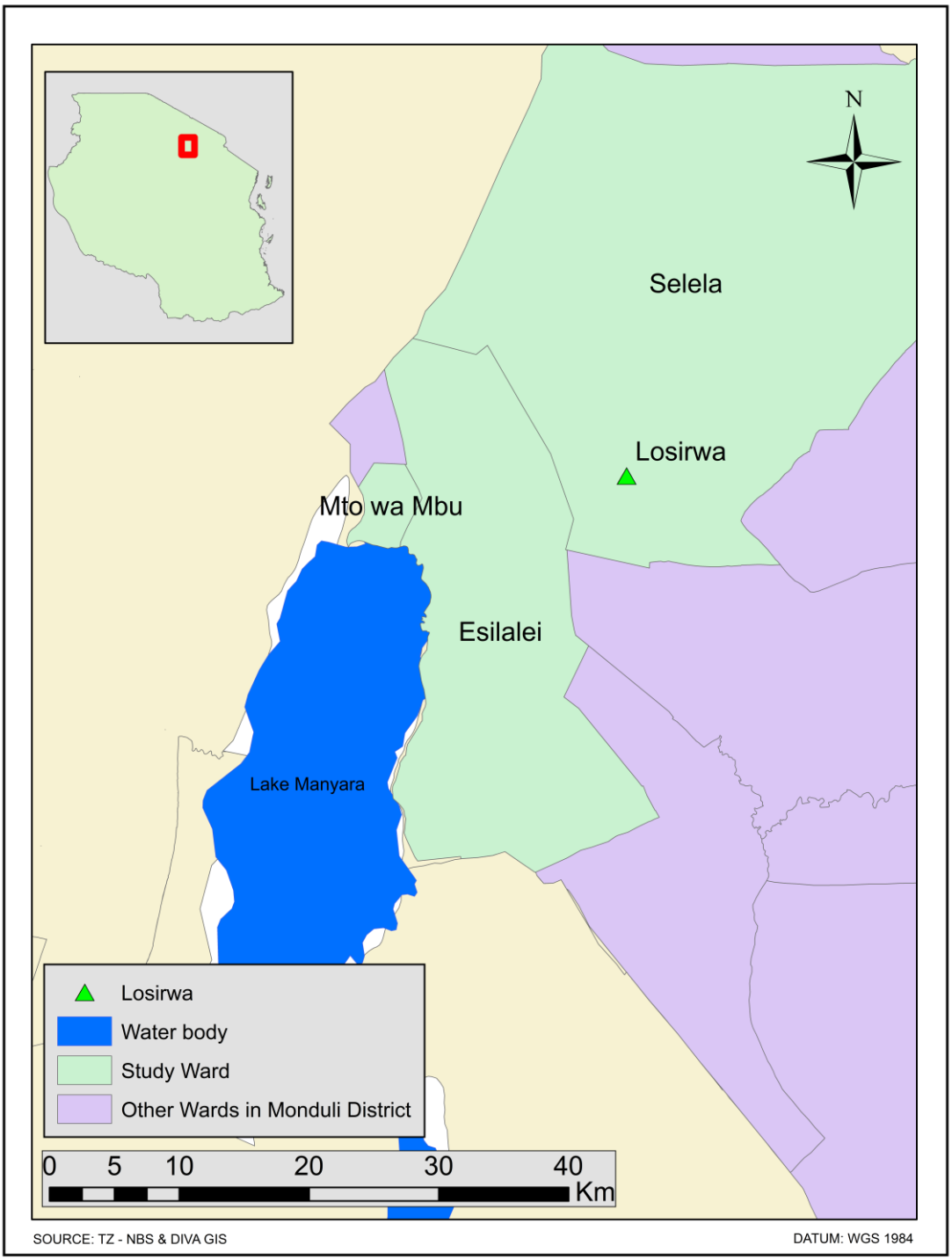


Figure 3: Map of Monduli District showing wards of study area. Map of Tanzania on top left shows the location of Monduli district.

3.2 Tarangire National Park

Tarangire National Park is located between 3º 40' and 5º 35' south and 35º 45' and 37' 00' East at an elevation of between 1200 metres and 1600 metres above sea level. Tarangire National Park occupies an area of 2850 km², making it the 5th largest park in Tanzania (Fig. 4). It is located about 120 km southwest of Arusha.

3.3 Minjingu Ward

Minjingu ward was chosen as a study area in this study. The area is located 120 km Southeast of Arusha. The ward is close toTarangire National Park (Fig. 4). It is bordering with the National Park to the North and part of Lake Manyara to the South. The road from Arusha to Babati divides the ward into North and South.

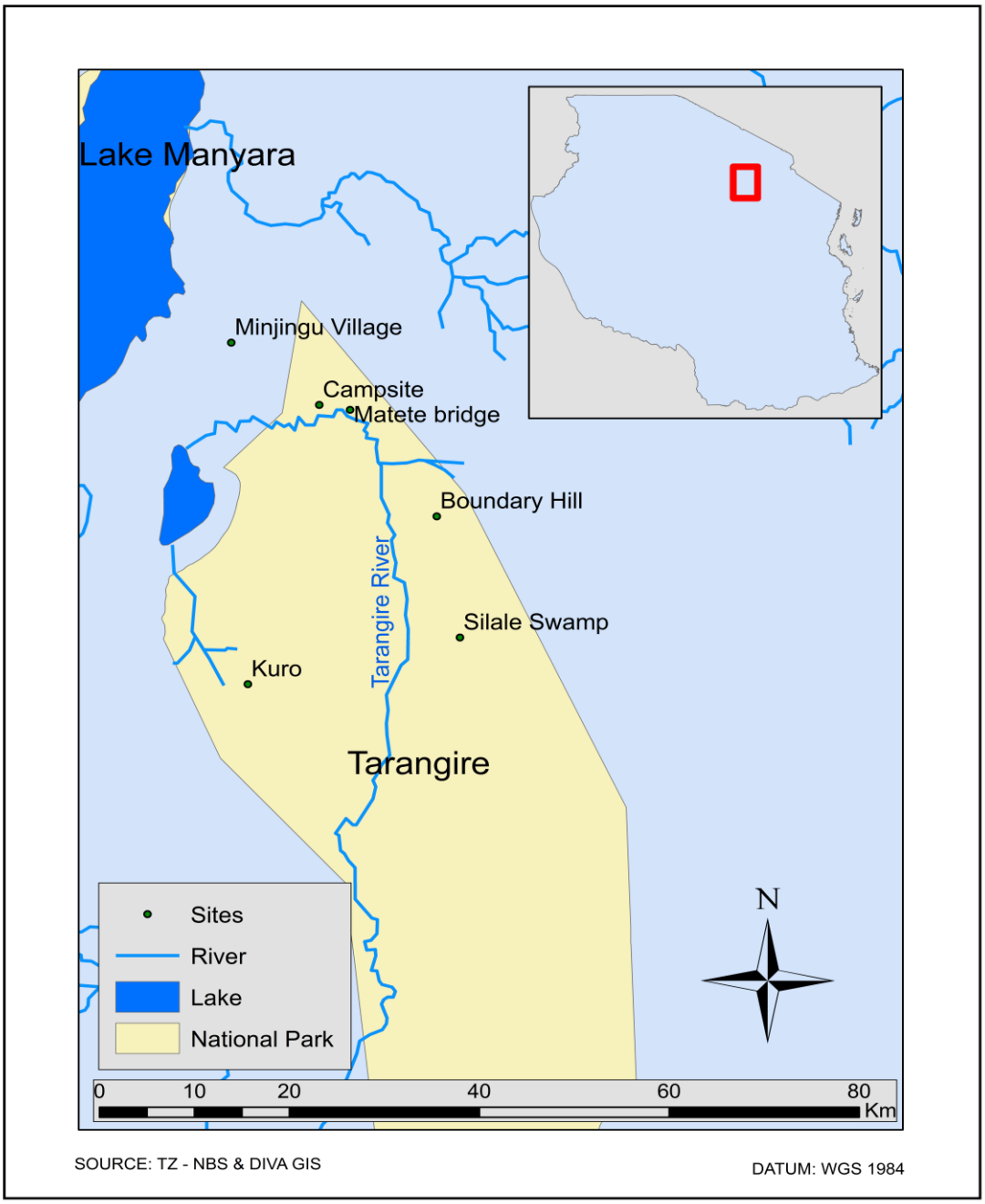


Figure 4: Map of Tarangire National Park showing sites where *Cyclops*, faecal sample of lions were collected and Minjingu village. Map of Tanzania on top right shows the location of Tarangire National Park.

3.4 Determination of the Life Cycle of *Spirometra*

In the present study, experiments were carried out in the laboratory to determine the life cycle of *Spirometra*.

3.4.1 Ethical Clearance for Use of Animals in the Experiments

In Sokoine University of Agriculture there is no committee of ethical clearance for use of animals in the experiments. However, TAWIRI was contacted, responded that they never issue ethical clearance retrospectively.

3.4.2 Cultivation of *Cyclops*, the first intermediate host of *Spirometra*

Cyclops are first intermediate hosts in the life cycle of *Spirometra* species. In this study, copepods of the genus *Cyclops* were cultured in the laboratory to be infected with coracidia of *Spirometra*. The *Cyclops* were isolated from water ponds in Tarangire National Park, Babati. *Paramecium* was cultured to feed the *Cyclops*. The followings are methods used to culture and maintain *Paramecium* and *Cyclops* in the laboratory.

3.4.3 Culture of *Paramecium* in Laboratory

Paramecium were collected from a pond near Roman Catholic Chapel at the main campus of Sokoine University of Agriculture. In this study, *Paramecium* were cultured in order to provide food for copepods that were afterwards infected with coracidia. Two Petri dishes of size 8 cm diameter were used, transferred in each about 50 ml of water collected from a pond. Four pre-boiled wheat grains were added in each Petri dish. The Petri dishes were kept uncovered in the laboratory at temperature 26-29ºC and were observed daily. The bacteria multiplied on the pre-boiled wheat grains which served as food organisms for *Paramecium*. A small portion of population was transferred with a Pasteur pipette to another Petri dish containing tap water and 4 pre-boiled wheat grains where *Paramecium*

proliferated again to form a dense population. A small portion was again transferred to a new Petri dish. This procedure was repeated in order to get pure culture of *Paramecium* which was used as food organism for *Cyclops*. *Paramecium* were cultured before *Cyclops* were collected from water ponds in Tarangire National Park.

3.4.4 Culture of *Cyclops* in Laboratory

Cyclops were obtained from ponds in Tarangire National Park (Fig.5). A locally made plastic container with a wooden handle was used to collect water. Water was emptied into a 1 liter plastic water container and transported to the laboratory. In the laboratory, water was poured into a Petri dish to search for egg sac bearing *Cyclops* which was sucked with a Pasteur pipette and transferred to a cavity block. Finally, the egg sac bearing *Cyclops* was transferred in Petri dish with cultured *Paramecium* for culture according to Adrian and Frost (1993). The egg sac bearing *Cyclops* hatched the naupliar which developed to adult *Cyclops*. *Cyclops* were maintained in a number of flasks containing tap water, pre-boiled wheat grains and *Paramecium* until used in the experiments.

Figure 5: Water pond in Tarangire National Park where egg sacked *Cyclops* were collected. (Source: Present study 2012).

3.4.5 Collection of *Spirometra* eggs

In this study, lion faeces were obtained from Tarangire National Park, Manyara, Tanzania and dog faeces from Minjingu ward bordering with Tarangire National Park. The faeces were screened for *Spirometra* eggs.

3.5 Faecal Sampling from Lions

Sampling of faeces from lions of Tarangire National Park was carried out with the help of staff from Tanzania Wildlife Research Institute (TAWIRI), Arusha and Tarangire Lion Research Project, Tarangire. Lions of Tarangire National Park are in prides which are well known to Tarangire Lion Research Project. In each pride, one lion has a collar which makes it easy to trace them by frequencies from the tracking box and antenna used in the research car. Sites in the National Park where lions were found resting are: Boundary hill, Kuro and Silale swamp. Three prides (Tarangire Hill, Altipiano and Wazi) were identified. Places where lions were resting were observed (Appendix 4). Two methods were used to collect faecal samples (i) opportunistic sampling of faeces from the ground where it has been deposited by using gloved hands (Appendix 5). (ii) Invasive collection of faecal samples from the rectum after the animal was immobilized (Appendix 6) then manual removal of faeces from the rectum (Appendix 7). Immobilization was done by using Ketamin at a dose of 3 ml intramuscularly (Kyron Laboratories, (Pty) Ltd). A total of seven faecal samples were collected. One faecal sample of a lion from Tarangire Hill pride was collected from the rectum after immobilization. Six faecal samples were collected from the ground by using gloved hands from individual lions observed defecating (Table 3). The collected samples were kept in labeled plastic bags, preserved in cool box with ice cubes then transported by bus from Arusha to the laboratory at Sokoine University of Agriculture, Morogoro. On the way back to Arusha, when we

reached Makuyuni Village we were taken by Game Ranger to collect faecal sample from a carcase of a hyena that had been knocked down by a motor vehicle (Appendix 8).

Precautions were taken against risk of contamination with *Echinococcus* eggs during sampling of faeces of lions and hyena. Faeces were sampled by using gloved hands. Hands were washed with water and soap after sampling and before eating food. In addition to this 70% Alcohol was used to clean hands and fingers after sampling faeces.

Table 3: Sampling of faeces of lion (*Panthera leo*) at Tarangire National Park, Manyara, Tanzania

Pride	Lion S/No	Site collected	Collection method
Tarangire Hill	1	From rectum	Immobilization by darting
	2	On the ground	Collected on the ground
Altipiano	3	On the ground	Collected on the ground
	4	On the ground	Collected on the ground
	5	On the ground	Collected on the ground
	6	On the ground	Collected on the ground
Wazi	7	On the ground	Collected on the ground
Hyena	1	From rectum	carcas

3.6 Collection of *Spirometra* Eggs from Faeces of Lion

In the laboratory faecal samples were preserved in the refrigerator at 4ºC. The samples were screened for the presence of *Spirometra* eggs by using floatation and sedimetation methods.

3.6.1 Floatation method

The aim of this procedure was to concentrate *Spirometra* eggs by using solutions of sufficiently high specific gravity. Two different solutions were used and compared their efficacies in detecting *Spirometra* eggs. Amount of 5g of lion faeces was dissolved

separately in sugar solution (Specific Gravity 1.459) and Sodium chloride solution (Specific Gravity 1.210). The mixture was stired well to break the lumps then filtered through tea strainer. Mixture was poured in centrifuge tubes which were placed on a rack and more saturated sugar and Sodium chloride solutions were added up to the brim, cover slide applied on top for 3 minutes. The cover slide in contact with the solution was transfered onto the slide without changing the side that was in contact with the solution and the slide was examined under compound microscope.

3.6.2 Sedimentation method

About 5g of lion faeces was taken and suspended in tap water in a beaker, filtered through tea strainer and sediments collected in a sedimentation flask. Tap water was added in the sedimentation flask up to the level of 1000 ml. It was left to stand for 30 minutes then supernatant poured off, water was added and left to stand for another 30 minutes (Appendix 9). The process was repeated until the supernatant was clear. Sediment was poured into a Petri dish, small amount of water was added to dilute it. The specimen was screened on a dissecting microscope. Identification and confirmation was done by examining the eggs under compound microscope (x10). Small amount of sediment from Petri dish was sucked by using a Pasteur pipette, placed on a slide and a cover slide placed onto it and examined under compound microscope. This method of sedimentation was also used to collect eggs which were used for other experiments such as incubation to hatch coracidia, and morphological study.

3.7 Collection of *Spirometra* Eggs from Faeces of Naturally Infected Dogs

Dog faeces were collected from Minjingu ward, Babati District bordering with Tarangire National Park. The faeces were screened for *Spirometra* eggs to establish natural infection of dogs. Permission from the Ward Executive Officer was sought before starting to carry

out the study. The Ward Executive Officer introduced the researcher to households identified to be keeping dogs. The researcher asked households the number of dogs they kept. They were given labelled plastic bags and gloves same to the number of dogs keeping for collecting faeces, the next day bags with faeces were collected. The plastic bags with faecal samples were preserved in the cool box, transported to the Veterinary Investigation Centre (VIC), Arusha, for processing of the faecal samples. The households of positive faecal samples were again revisited and faecal samples of dogs were taken for the second time. The dog was restrained, applied dog muzzle and faeces were taken per rectum with a gloved hand (Appendix 10). Dogs were labelled, faecal samples were taken to Tarangire National Park Veterinary laboratory, the faecal samples were processed by sedimentation method and examined under compound microscope to confirm the positive faecal samples. Two positive dogs were taken alive, kept in a cage and transported to Sokoine University of Agriculture for post morterm

3.8 Morphological study of *Spirometra* Eggs

Eggs collected from lion and dog faeces were used for morphological study, shape, colour and size were observed by using light microscope.

3.9 Measurement of *Spirometra* Eggs

Eggs collected from lion and dog faeces by sedimentation method were used for measurement of length and width. A drop of sediment with eggs was placed on the slide by using a Pasteur pipette then slide cover applied on top. The slides with eggs were examined under compound microscope mounted with ocular micrometer at a magnification of x10. A total of 100 eggs had their length and width measured.

3.10 Hatching of Eggs

Development of *Spirometra* eggs was carried out in a culture of eggs on a filter paper in falcon tube with aquarium at laboratory temperature (26-29ºC).

3.10.1 Preparation of aquarium

The aim of using aquarium was to give a natural condition for the eggs to hatch. Aquarium was prepared in the following manner:

Sand was collected, washed thoroughly and sterilized in oven at 180ºC for 1 hour and left overnight to cool. Stones were washed thoroughly with hot water and allowed to cool. Water was boiled and left overnight to cool. To disinfect the containers 1% Hydrochloric acid was used for 10 minutes then rinsed with cooled water. Sand and stones were placed in containers and cooled water was added. This preparation was used in the experiment of incubating eggs.

3.10.2 Incubation of *Spirometra* eggs by using modified Harada-Mori method at laboratory temperature of 26-29ºC

This method was designed after trying several times to incubate eggs by methods used by Stephanson (1985), Mueller (1938) and Harada-Mori (Beaver *et al*. 1964) without any success. The experiment consisted of a thin film of washed eggs on a strip of filter paper in an upright 15 ml falcon tubes containing aquarium water prepared in the laboratory, stones and sand prepared by sterilizing in an oven at 180ºC for 1 hour. Two types of *Spirometra* eggs were used, one from faeces of lions which had been in stock for 1½ years and the second one was fresh faeces of dog. The eggs were thoroughly washed by changing water several times. Sediments with clean eggs were smeared on a filter paper at the middle 1/3. Small amount of aquarium sand was added in each of the 6 falcon tubes

up to the level of 1cm. Filter papers were inserted in falcon tubes, aquarium water added up to the level just below the egg smear. Falcon tubes including control were placed on a rack and left at laboratory temperature (26-29ºC). Caps were placed unscrewed at the top of each falcon tube (Fig. 6). Water was added every day, on day 5 temperature shock was applied by placing the falcon tubes in a refrigerator (4ºC) for one hour then transferred to laboratory temperature. After 24 hours (Day 6) water in the falcon tubes was sucked with Pasteur pipette transferred into cavity block examined under dissecting microscope for observation of swimming coracidia. Observation for hatching of eggs continued daily and recorded first and last days of hatching.

Parallel to this experiment, few eggs were pipetted in a cavity block to observe development of eggs under compound microscope. Eggs from cavity block were sucked with a Pasteur pipette and applied on a slide and a slide cover was applied onto it. Pictures for egg development were taken by using a compound microscope Olympus BHT210314.

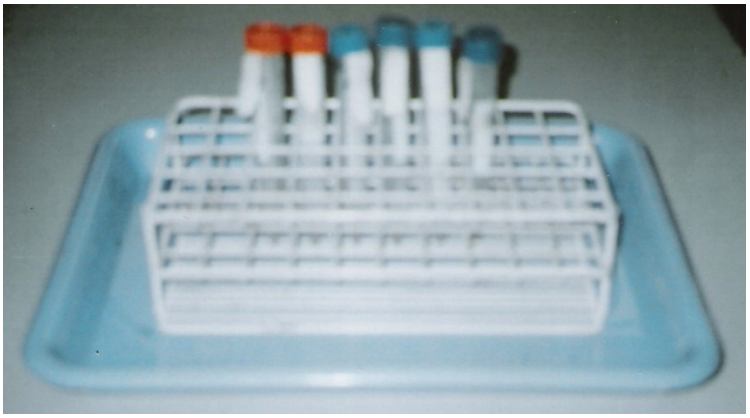


Figure 6: Incubation of *Spirometra* eggs by modified Harada-Mori method (Source: Present study 2012).

3.10.3 Incubation of *Spirometra* eggs in relation to light and darkness

In this study, a comparison of incubation of *Spirometra* eggs in light and darkness was carried out.

3.10.4 Incubation of *Spirometra* eggs at laboratory temperature (26-29ºC) with light

The experiment was conducted by using *Spirometra* eggs from lion faeces which had been in stock for 1½ years. The eggs were collected by sedimentation method. Eggs were sucked with Pasteur pipette one after another being counted and transferred into cavity blocks with aquarium water. A total of 150 eggs were used in the study, 5 cavity blocks were used each with 30 eggs. Eggs were incubated at laboratory temperature (26-29ºC), observed daily and recorded the first and the last days of hatching eggs with light.

3.10.5 Incubation of *Spirometra* eggs at room temperature (27-30ºC) in darkness

The experiment was conducted by using *Spirometra* eggs from lion faeces which had been in stock for 1½ years the eggs were collected by sedimentation method. Eggs were sucked with Pasteur pipette one after another being counted and transferred into cavity blocks with aquarium water. A total of 150 eggs were used in the study, 5 cavity blocks were used each with 30 eggs, placed in a box which was closed to ensure total darkness. The box was placed at room temperature (27-30ºC), observed daily and recorded first and last days of hatching eggs in darkness.

3.10.6 Studies on viability of eggs

In this study, the viability of *Spirometra* eggs collected from lion faeces and preserved in stock for 1½ years was studied. Eggs were sucked with Pasteur pipette one after another being counted and transferred into cavity blocks with small amount of aquarium water. A total of 300 eggs were used in the study, 6 cavity blocks were used each with 50 eggs.

Eggs were incubated at laboratory temperature (26-29ºC), observed daily and recorded eggs which hatched and that did not hatch. The hatched eggs were recorded as viable eggs and that did not hatch were recorded as not viable eggs.

3.11 Studies on Hatched Coracidia

The coracidia hatched from eggs were collected and used for morphological studies and infection of *Cyclops* (first intermediate host) for the life-cycle study.

3.11.1 Preparation of coracidia for morphological studies

Coracidia hatched from eggs were sucked with Pasteur pipette from a Petri dish and transferred to a slide. A drop of 10% Formal saline was applied on a slide to kill the coracidia. Under compound microscope dead coracidia were placed at the middle of a drop of water and Formal saline on a slide by using a fine needle and a slide cover was applied onto the slide. The slide was examined under compound microscope.

3.11.2 Studying survival of coracidia

The aim of the study was to see how long (life span) coracidia can survive in water after hatching if it is not eaten by the first intermediate host (*Cyclops*). A total of 50 coracidia after hatching from 1½ year old eggs were placed in aquarium water in cavity blocks and observed under laboratory conditions. Each cavity block contained 10 coracidia. The temperature of the room was at (26-29ºC). The coracidia were observed twice a day each observation recorded live and dead according to Mariana *et al*. (1998). The experiment was a continuous observation, which started at 9 am in the morning and the first observation was at 3 pm (after 6 hours) of Day 1. The next observation was made the following day at 9 am (after 24 hours), followed by another observation at 3 pm (after 30

hours) Day 2. The following day (Day 3), the observations were made at 9 am (after 48 hours) and at 3 pm (after 54 hours) and the results were recorded.

3.11.3 Experimental infection of *Cyclops* with coracidia

Cyclops obtained from River Tarangire and water ponds in the Tarangire National Park were cultured in the laboratory and used in the study as first intermediate host in the life cycle of Tanzanian *Spirometra* species. Coracidia hatched from *Spirometra* eggs were placed in cavity blocks with tap water then 10 *Cyclops* were transferred in each cavity block by using Pasteur pipette where they fed on coracidia. After two days *Paramecium* and two pre-boiled wheat grains were added to each cavity block.

3.11.4 Experimental infection of Guinea pigs, rats, New Zealand rabbits, mice, pig and goat with *Cyclops* infected with procercoids

In this study, Guinea pigs, rats, New Zealand rabbit, mice, pig and goat were used as second intermediate hosts. Six Guinea pigs, 10 Rats, 2 New Zealand rabbits, one pig and one goat were fed orally with *Cyclops* infected with procercoids and 6 mice were infected subcutaneously. Each experimental animal was given 20 infected *Cyclops*. The following procedure was used used to infect small experimental animals: *Cyclops* were sucked in a Pasteur pipette with about 1ml of water, Guinea pigs, rats, and mice were held skin at the back of the neck with left hand and turned dorso ventrally with the back laying on the palm. The Pasteur pipette was introduced in the mouth of the animal and forced fed the contents. Pig, goat and New Zealand rabbits were held on the neck firmly, opened the mouth then forced fed the contents in the Pasteur pipette. The small animals were cared in the Small Animal Unit fed with green grasses, growers mash and water while the pig and goat were cared at the Animal Research Unit (ARU). Experimental studies undertaken in this study are summarized (Fig.7).

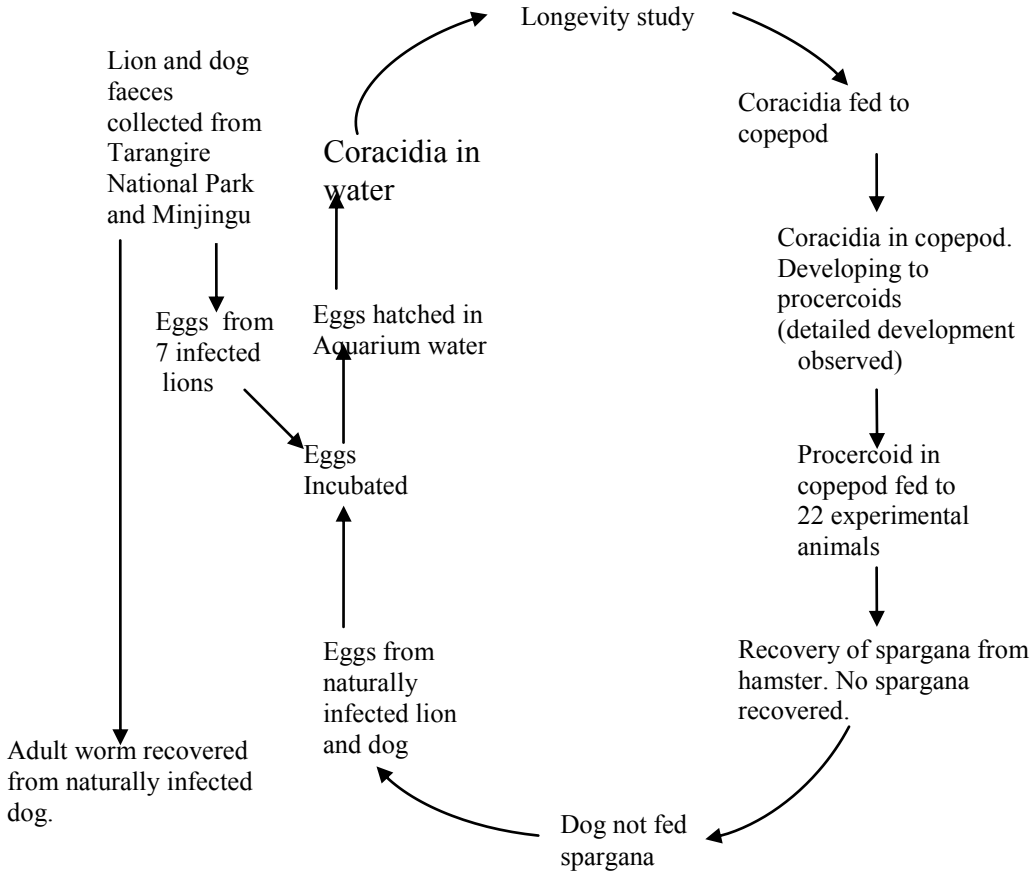


Figure 7: **Procedures for experimental studies undertaken in this study**

3.12 A survey of *Cyclops* (first intermediate host) for natural infection with procercoids

Cyclops collected from ponds in Tarangire National Park were examined for the presence of procercoids in their body cavities. They were examined by using two methods. First, Neiland method (1952), this method was accomplished by sucking *Cyclops* from cavity blocks by using Pasteur pipette, placed on a slide and slide cover applied onto the slide. Sufficient pressure was exerted on the slide cover by using a forcep. The exerted pressure caused the *Cyclops* to burst, thus releasing the contents of the abdominal cavity. Procercoid extruded outside the *Cyclop*s body cavity if it was harbored. Second method,

the *Cyclops* were placed on a slide, a drop of 10% Formal saline was added to kill the cyclop then a slide cover was applied onto the slide. The slide was examined under compound microscope. The copepods found to harbor procercoid, were recorded and pictures taken.

3.13 A Survey of Second Intermediate Host (Birds, Rats and Wild Animals) for Natural Infection with Spargana

A survey of birds, rats and wild animals for natural infection with spargana was carried out around Tarangire National Park in Babati District. Birds and animals were obtained by shooting while rats were trapped. Both birds and animals were dissected and internal organs were thoroughly examined for spargana (Appendix 11, 12, 13).

A survey of rats for natural infection with spargana was carried out at Minjingu ward, Babati District. Households selected for survey of dogs for natural infection with *Spirometra* were used in this part of study. Trapping of rats was carried out near the water sources of these households. Special traps with groundnut butter bait were set in the evening and left overnight. The next morning traps were checked, those found to have trapped rats, the rats were transferred in cages, fed with food and water in cages. Rats were transported to Morogoro for dissection and search for spargana infection.

3.14 Recovery of Spargana from Experimentally Infected Laboratory and Domestic Animals (pig ang goat)

Experimental animals fed with infected *Cyclops* were sacrificed after 30 days to observe for infection. The small animals were killed with chloroform which was applied on a piece of cotton and placed in a dessicator. The dead animal was placed on a dissecting board, incision made along the mid-ventral section of abdomen and loose skin pulled

back to expose the muscle tissue, thorough examination of the subcutaneous tissue and internal organs for spargana was performed. The domestic animals (pig and goat) were first euthanized with (i) Ketamine 50 mg/kg body weight IV (ii) Xylazine 20mg/kg body weight IV, and (iii) Potassium chloride 10% (8ml) Intra cardial. Examination for spargana infection was carried out in the Post moterm room of Pathology Department.

3.15 Recovery of Adult Worm from Naturally Infected Dog

Two positive dogs from Minjingu Ward, Babati District were brought to Sokoine University, Morogoro. After one day the dogs were sacrificed in the post moterm room of the Pathology Department for recovery of the adult worm of *Spirometra*. Drugs used were: (i) Ketamine 50mg/kg body weight IV, (ii) Xylazine 20mg/kg body weight IV and (iii) Potasium chloride 10% (8mls) intracardial. The abdomen of each dog was opened. Small intestine was identified from the duodenum and opened with a scissor until the adult worm was seen. The adult worm was transferred in a plastic dish which was flooded with Normal saline. It was stretched to be taken measurements. The recovered adult worm was used for various studies as shown (Fig.8). In this study, the life cycle of Tanzanian *Spirometra* spp. is represented by the flow chart showing hosts and potential hosts in the environment (Fig. 9).

3.16 Staining of the Adult Worm of *Spirometra*

Staining of the adult worm of *Spirometra* was done with Carmine HCl stain. The aim was to stain the internal organs to make them visible so that they can easily be studied.

3.16.1 Preparation of Carmine HCl

Carmine powder 4 gm was dissolved in distilled water 15 ml in a conical flask, concentrated Hydrochloric acid 3 ml was added, the mixture was boiled to boiling point,

95 ml of 85% Alcohol was added, drops of Ammonia were added to neutralize until Carmine precipitated.

3.16.2 Procedure of staining with carmine HCl

1st Day:

Take preserved specimen, place in 70% Alcohol for 10 minutes. Stain in Carmine HCl overnight (1:10) mix 1ml carmine and 10ml distilled water.

2nd Day:

Add one drop Conc. HCl in 10 ml of 85% Alcohol and leave for 2 minutes. Transfer specimen in 85% Alcohol for 10 minutes. Transfer specimen in 95% Alcohol for 10 minutes. Transfer specimen in Absolute Alcohol for 10 minutes. Then in Clove oil for 5 minutes. Finally, mount in Canada Balsam.

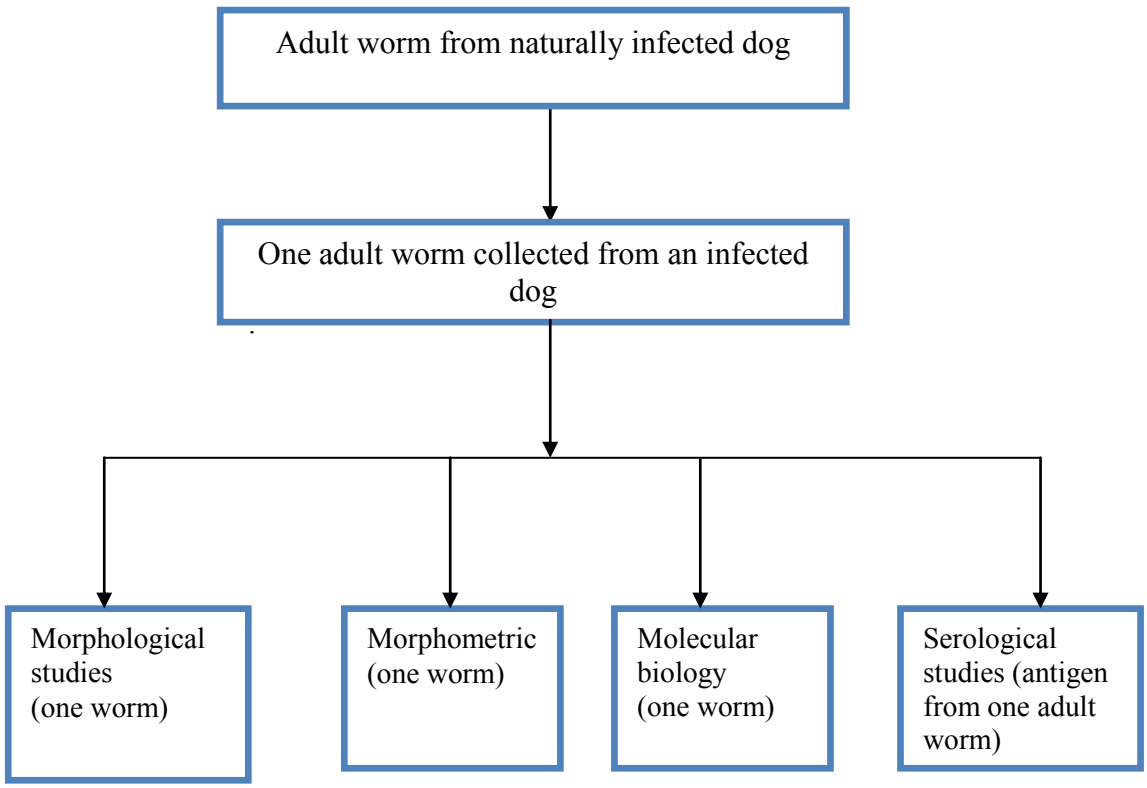


Figure 8: Various studies done on adult *Spirometra* recovered from infected dog.

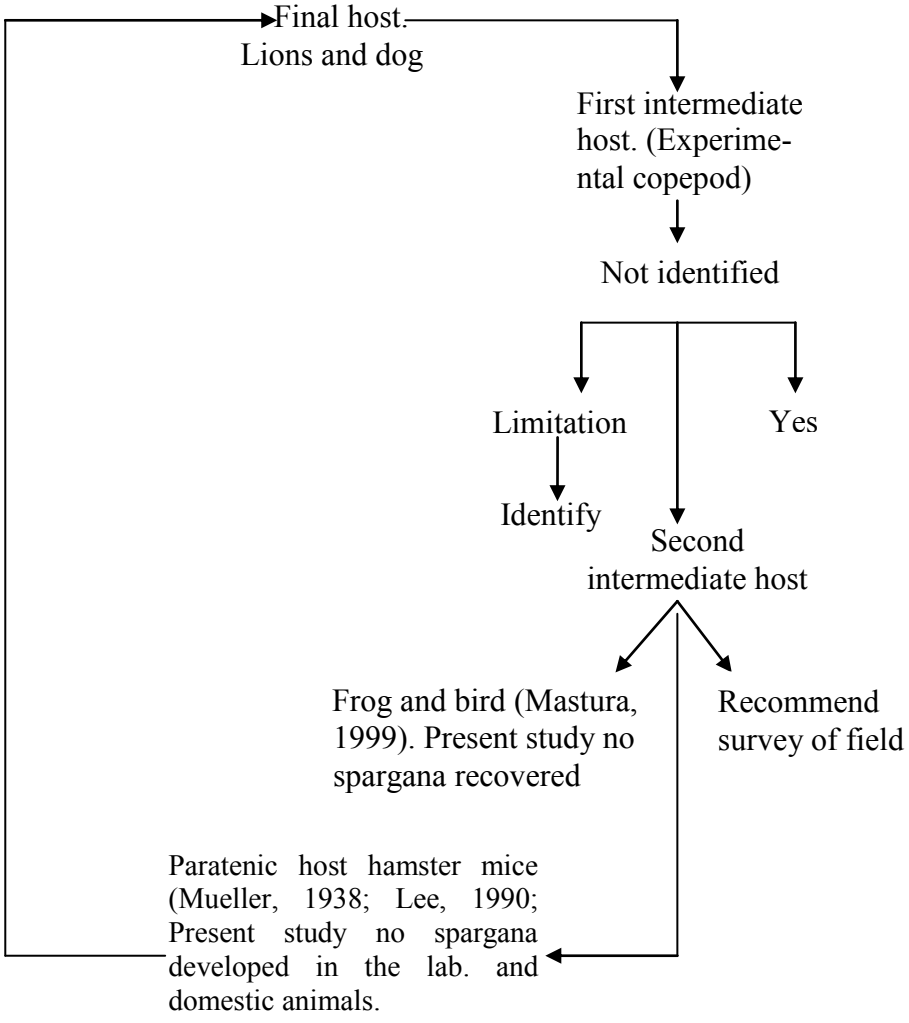


Figure 9: Flow chart of life cycle showing hosts and potential hosts in the environment identifying possible future investigations.

3.17 Identification of *Spirometra* Using Molecular Techniques

Molecular techniques were used to identify Tanzanian *Spirometra* spp.

3.17.1 DNA Extraction from *Spirometra* adult worm

DNA extraction was performed using QIAGEN DNeasy Tissue Kit 250 as per manufacturer‘s instructions. Two pieces of about 25 mg each of adult worm of *Spirometra* were cut into small pieces, and placed in 1.5 ml microcentrifuge tube. A tissue

lysis buffer (ATL) 180 µl was added followed by Proteinase K 20 µl and vortexed for 15 seconds. Incubated at 56ºC until contents completely dissolved after 1 hr. Buffer ATL 200 µl, Ethanol 200 µl was added to the sample and vortexed for 15 seconds. The mixture was pipetted into a DNeasy Mini spin column in a 2 ml collection tube, centrifuged at 8,000 rpm for 1 min. at room temperature (24-26ºC). Discarded flow-through and collected spin column. The spin column was transferred in a new 2 ml collection tube. Then 500 µl Buffer AW1 was added, centrifuged at 8000 rpm for 1 min. at room temperature. Water and collection tube discarded. Spin column placed in a new 2 ml collection tube. Then 500 µl Buffer AW1 was added, centrifuged at 8000 rpm for 1 min. Water and collection tube were discarded. The spin column was placed in a new 2 ml collection tube, added 500 µl Buffer AW2, centrifuged at 14 000 rpm for 3 min., at room temperature. Water and collection tube were discarded. The spin column was transferred to a new 1.5 ml microcentrifuge tube and added 200 µl Buffer AE (elution buffer) to elute DNA bound to the membrane. The tube was spined at 8000 rpm for 1 min, at room temperature. The spin column was discarded, retained the elution solution containing DNA, stored at (-20ºC) until use.

3.17.2 Polymerase Chain Reaction (PCR)

Spirometra DNA was extracted from adult worm using tissue extraction kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was performed in a thermocycler (BIO-RAD MY CYCLER™) under the following conditions: 40 reaction cycles of denaturation at 95ºC for 20 seconds, annealing at 55ºC for 30 seconds and extension at 72ºC for 30 seconds followed by 72ºC for 6 minutes (final extension). The PCR reaction mixture of 25 µl contained 4 µl of DNA template, 5 pmol of forward primer JB10 (5'GATTACCCGCTGAACTTAAGCATA3') and reverse primer JB9 (5' GCTGCATTCACAAACACCCCGACTC 3') targeted at 28S rRNA sequence (Bowles

and McManus, 1994), 1X PCR buffer (35 mM Tris-HCl, pH 9.0, 3.5 mM $MgCl_2$, 25 mM KCl, 0.01% gelatine), 0.25 M dNTP, 1 u *Taq* polymerase and 15.3 µl of nuclease free water. The PCR of mitocondrial Cytochrome Oxidase I (mtCOI) were performed using the above described method, but with different primers. The forward primer was JB3 (5' TTTTTTGGGCATCCTGAGGTTTAT3'), and the reverse primer was JB4.5 (5' TAAAGAAAGAACATAATGAAAATG 3') (Bowles and McManus, 1994). The PCR condition used was the same as that described above, except for that annealing was done at 48ºC for 30 seconds. Each amplicon (5µl) was examined by 2% agarose gel electrophoresis to validate amplification efficiency and photographed using a gel documentation system. PCR products were sent to MYTACG BIOSCIENCE ENTERPRISES (MALAYSIA) for sequencing from both directions by using primers used in PCR amplifications.

3.17.3 Agarose gel electrophoresis

2% Agarose gel was prepared by adding agarose powder 0.6 g, 1XTAE Buffer 30 ml, SYBR Safe DNA gel stain 1 µl, casting gel tray, PCR products, comb and DNA Ladder 1 kbp. The PCR products were loaded onto wells, electrophoresed at 100v, 400 mA, for 30 min. in 1XTAE buffer and photographed by gel Doc XR machine.

3.17.4 Preparation of 2% Agarose gel

2% agarose gel was prepared by adding agarose powder 0.6 g, 1XTAE Buffer 30 ml, SYBR Safe DNA gel stain 1 µl, casting gel tray, comb. The small flask with contents was placed in a microwave boiled for 1 min, left to cool at room temperature, poured in the casting gel tray with a comb and left for 30 min. to solidify. Comb was removed, DNA Ladder (10 µl). Was loaded to one well and other wells were loaded with PCR product each 10 µl.

3.18 Cloning of Post PCR DNAs into a Cloning Vector

Post PCR DNA was cloned into a cloning vector after ligation and transformation.

3.18.1 Ligation of DNA into the pGEM-T plasmid

PCR products were ligated into the pGEM-T vector (QIAGEN). Ligation was performed by adding ligation buffer 5 µl, pGEM-T vector 1 µl, PCR product 3 µl, ligation enzyme 1 µl, in a total of 10µl reaction mix. Ligation was performed at 16ºC for 16 hours.

3.18.2 Transformation of the ligated pGEM-T plasmid

The ligated pGEM-T plasmids were used to transform competent cells. *E. coli* strain Top 10. Briefly, competent cells were thawed in ice for 5 mins. Ligation product was added to 100 µl of competent cells, placed in ice for 30 min. Heat-shock at 42ºC for 45sec. was applied , then placed in ice for 5 min. 900 µl of LB broth was added to the heat-shocked bacteria mixed by inversion and the culture was incubated at 37ºC for 1 hour. At the end of incubation performed sedimentation of bacteria at 5000 rpm for 5 min. at room temperature. The sediment was plated onto LB agar plate containing Ampicillin 100 µg/ml and the plates were incubated at 37ºC for over night. The next day the plates were examined for white colony growth, few colonies were picked and transferred to another plate with divided chambers and to 5 PCR eppendorf which was processed to PCR, the PCR products were used for 2% agarose gel electrophoresis.

3.18.3 Preparation of competent cells (Top10 E. coli) by PEG method

Preparation of competent cells (Top10 *E. coli*) was done by using standard method: Polyethylene glycol (PEG method).

(a) Preparation of a culture

i. Streak stock Top10 *E. coli* cells on LB plate overnight at 37ºC.

ii.Inoculate single colony into LB broth (5 ml), incubate overnight, 250 rpm, at 37ºC.

(b) Preparation of solution A

Mixture of glucose powder, Magnesium sulfate and LB broth in a falcon tube were mixed until dissolved. The mixture was filtered by using a syringe filter. Poured in an autoclaved 200 ml flask, 0.5 ml Top10 cultured overnight was added. Incubated at 37ºC, for 2 hrs. O.D value was 0.6.

(c) Preparation of solution B

Solution B was prepared in the same manner, total volume 50 ml.

Glycerol 18 ml was added in a falcon with Magnesium sulfate. LB broth was top up to 50 ml. The mixture was vortex, filtered and stored at 4ºC until used.

(d) Final preparation

Solution A was transferred in ice for 10 minutes, followed by spin for 10 minutes, 1500 rcf (rotar centrifugation force), at 4ºC. Supernatant poured out, pellets preserved, 3 ml of solution B was added in falcon with pellets. The mixture was transferred to 30 tubes each 100 µl. the tubes were placed in a plastic bottle and stored at -80ºC until used for transformation.

3.19 Colony PCR

The aim of colony PCR was to confirm if pGEM-T vector clones contained the DNA of the sample (inserts). Briefly, Agarose powder, conical flask, measuring cylinder, gel trays and related chemicals/materials to make gel, pipettes and tips (100 µl, 20 µl, 10 µl, 2.5

µl), Green Taq Master mix, primers M13, distilled water, eppendorf tubes and PCR tubes, PCR machine and gel docs.

Ligaton stored at -20ºC was taken about 10 µl, streaked to the agar plate, incubated at 37ºC for16 hrs. Culture was observed for presence of colonies on agar plate. Five colonies were picked one after another with a tip. The tip was touched to a new agar plate where chambers had been made then the tip was smeared into an empty PCR eppendorf labelled the number of colony picked. The agar plate with colonies was stored at 4ºC. Master mix 25µl was added to the PCR eppendorf with colony smear.

Master mix for 25 µl reaction solution was prepared according to Maxime PCR Premix Kit (i-Taq) protocol. It included distilled water (11.5µl), buffer (12.5µl), forward primer (0.5µl), reverse primer (0.5µl), and finally the DNA template (smeared colonies to 5 eppendorf). Negative control (without DNA) was used parallel to the samples. Each component of Master mix was multiplied by 6, total master mix of 25 µl was transferred to each PCR eppendorf (5) and 1 control. Prepared PCR solutions were transferred in a PCR machine with setting of denaturation for 10 min. at 95ºC, then 30 sec. at 95ºC, 30 sec. of annealing at 50ºC, and 1.5 min. of extension at 72ºC, followed by final extension of 5 min. at 72ºC at the end of 30 cycles.

3.20 Purification of cloned DNA

The PCR products were purified with an agarose gel prepared in the laboratory and ligated into a pGEM-T vector (Promega). The clones were generated via the transformation of *E. coli* competent cells (Top10) from a stock of the laboratory. The colonies of recombinant clones were selected and grown in 5 ml of LB Broth in the presence of 50 µg Ampicillin at 37ºC over night. The plasmids were purified by using

spin column (QIAprep spin Plasmid kit, USA) and DNA eluted by using 50 µl Buffer EB. The purified DNA samples were sent to commercial company MYTACG. BIOSCIENCE ENTERPRISES (Malaysia) for sequencing.

3.21 Analysis of DNA Sequence Data

Nucleotide sequences were aligned using CLUSTAL-W, which is available online (http://www.ebi.ac.uk/Tools/msa/clustalw2). Pairwise nucleotide differences and genetic distance (defined as p-distance) between the sequences were calculated using MEGA 6 soft ware (Tamura *et al.*, 2007). Phylogenetic trees were re-constructed using the Neighbour-Joining (NJ) and Maximum Parsimony (MP) methods, also described in MEGA 6.

3.22 Ethical Clearance in Relation To Taking Sera for Serological Tests for Sparganosis

The National Institute for Medical Research (NIMR), Tanzania, gave ethical approval NIMR/HQ/R.8a/Vol.IX/1285 (Appendix 3). Respective health and government authorities at regional and district level received copies of the ethical approval as required for the implementation of the study. At individual level an informed consent was sought after detailed explanation of the purpose and benefits of the study.

3.23 Blood Collection

The serum used in this study was from 216 inhabitants in two districts of Babati and Monduli, Tanzania. About 4 ml of blood was collected from each participant using vacutainers. Blood was left at room temperature for 3 hours, serum was pipetted into cryotubes which were preserved in the refrigerator at 4ºC until transported in cool box

with ice cubes to Sokoine University of Agriculture, Morogoro, where it was stored at temperature -20ºC until use.

3.24 Protein Extraction from Adult *Spirometra* Worm

Protein of adult worm of *Spirometra* collected from the small intestine of infected dog from Minjingu village near Tarangire National Park and preserved in 70% ethanol at room temperature ranging from 26-29ºC was used in this study. 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 and stored at -20ºC until used.

3.25 Protein Determination

Protein determination was done by using Bradford method (The Quick Start Bradford Assay Kit, USA). The procedure was performed in a 250µl microplate assay. Two wells were pipetted with 500µl of 1x dye reagent and labelled as blank. The next seven wells in duplicate were labelled as sample, transferred to each well 250µl 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.

3.26 Data Analysis

The spectrophotometer or microplate reader was used for analysis of data. Average of the blank values was subtracted from the average value from the standard and unknown

sample values. A standard curve was then plotted with the 595 nm values (y-axis) versus their concentration in µg/ml (x-axis).

3.27 Antigen-ELISA

The positive control was redesigned from serum of humans from areas suspected to be infected. It has been reported that human cysticercosis may cause cross-reaction with sparganum antigen (Cho *et al.,* 1986; Kim and Yang, 1988; Choi *et al.,* 1988). In this study, cysticercosis serum was used as positive control following the protocol below (Dennis Tappe, University of Würzburg, Germany).

Day 1

Parasite antigen (adult *Spirometra*) was diluted to a concentration of 10 ng/µl in sterile PBS. Then pippeted 30 µl of this solution into the ELISA wells. ELISA plates were incubated over night at 4ºC in a wet chamber.

Day 2

Serum to be tested was diluted to 1:200 in 2% skim milk (blocking solution). Dried ELISA plate with antigen was incubated overnight by beating it on a tissue to get rid of excess antigen solution, then pippeted 200 µl of skim milk in each well for blocking not needed binding sites and was incubated for 30 min. at room temperature ranging from 23-25 ºC. Washed ELISA plates three times with PBS (200 µl in each well). Serum sample to be tested 30 µl was pippeted in each well. Sample incubated for 60 min. at room temperature. Washed ELISA plate three times with PBS (200 µl in each well). Diluted anti-human-IgG-peroxidase-conjugated secondary antibody (Goat anti-Human IgG H+L, USA) to 1:400 in 2% skim milk then 30 µl pippeted in the wells to block not needed binding sites. The plate incubated for 60 min. at room temperature. Washed ELISA plate

three times with PBS (200 µl in each well). Substrate-TMB (amresco) 30 µl was pippeted in each ELISA well. The plate incubated for 30 min. at room temperature. Then read on an ELISA reader (Nano Quant Infinite M200PRO TECAN). In determining the sensitivity and specificity of the respective antigens, a cut off absorbance of 0.006 was determined by the following formula: sample average = 0.381 and standard deviation = 0.0012. Cut off point: 0.381 + 2 x 0.0012. Any reading above the cut of point was considered as positive and any reading below cut off point was considered as negative.

3.28 Knowledge, Attitude, Practices and Risk Factors of Sparganosis among Inhabitants in Northern Tanzania

Sparganosis is a parasitic infection caused by the plerocercoid larvae of the diphyllobothroid tapeworms belonging to the genus *Spirometra* (John and Petri, 2006). The infection is transmitted by ingestion of contaminated water, ingestion of second intermediate host such as frogs, snake and mammals, and contact between a second intermediate host and an open wound or mucus membrane (Hughes and Biggs, 2001; Manson *et al.,* 1921). Humans are accidental hosts in the life cycle, while dogs, cats, and canines are definitive hosts. *Cyclops* is the first intermediate host. In the present study, knowledge, attitudes, practices (KAP) and behaviour related to sparganosis infection in two districts of Babati and Monduli in Northern Tanzania were described. The study was a health facility based research.

3.28.1 Study area

The study was a cross sectional study conducted in two districts of Babati and Monduli in Manyara and Arusha Regions, Tanzania respectively. The main focus aimed towards understanding the knowledge, attitudes, practices and risk factors with respect to sparganosis.

3.28.2 Selection of study participants

Sampling method used was stratified random sampling. The wards were randomly selected each ward was served by a health facility. Participants were randomly selected from each ward to participate in the study. The participants were selected from outpatient and were grouped according to the wards they come from. Each participant selected to participate in the study was taken blood and answered questions from the questionnaire. However, some participants refused to be taken blood but agreed to answer questions from the questionnaire.

3.28.3 Sample Size Calculation

The study was carried out in two districts bordering with Tarangire National Park. The districts are Babati, Manyara Region and Monduli, Arusha Region. The blood sample for spargana antibodies was collected from patients attending Babati and Monduli districts health facilities. The sample size is calculated based on the prevalence of sparganosis as the main outcome measure. In this study, the prevalence of sparganosis in the study areas is not known.

The sample size was calculated using the formula:

$$n = \frac{z^2 pq}{d^2}$$

Level of confidence **z** = 1.96; Population variation **s** = 0.0475; Accepted error (precision) **d** = 0.03; Where s =SD of the mean p = 0.05; Sample size **n** =398; q = 0.95; pq = 0.0475

Therefore, the number of subjects in one district is 199 but there are two districts.

The sample size (**n**) would be 398**.**

Selection of study participants:

Sampling method used was stratified random sampling.

The wards were randomly selected each being served with a health facility. Three wards from each district were involved in the study. From each ward 66 participants were randomly selected to participate in the study. The participants were randomly selected from outpatient and were grouped according to the wards they come from.

3.28.4 Data collection

Questionnaires used for interviews had been translated into Swahili and translated back to English to confirm their meaning. The questionnaires were pre-tested before using. Each individual who came into the room was explained the purpose of the interview, a consent form was given to the participant to sign that has agreed to participate in the study (Appendix 1). Participant answered the questions asked by the interviewer from the questionnaire. Data collected by using questionnaires covered demographics, knowledge, attitude, and practices concerning sparganosis modes of transmission (Appendix 2). Some hospital staff were involved in this exercise and were first trained how to fill the questionnaires (Appendix 14). It was necessary and functional to collaborate with the hospital staff who gave support in allocating space to use and organized the patients to participate in the research.

3.28.5 Socio-economic and demographic characteristics

The study investigated in detail the socio-economic indices and population characteristics of inhabitants of Babati and Monduli Districts of which both are situated in northern Tanzania and surrounding Tarangire National Park. The most important variables were age, gender, marital status and Level of Education.

3.29 Statistics

Data generated in this part of study were analysed by using Epi Info Version 3 2003. Data analysis of relationships between knowledge, attitude, practices, demographics and risk factors was performed with the software program Epi Info version 3. Statistical significance and correlation were calculated using Chi-square, t-test and ANOVA. Significance was determined as $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Culture of *Paramecium* and *Cyclops*

Paramecium was cultured successfully in the laboratory by using pre-boiled wheat grains as a nutrient source (Fig.10). To produce sequential generations of *Cyclops*, the study started with a single female egg sack bearing *Cyclops* isolated from water collected from ponds in Tarangire National Park. The female egg sacked *Cyclops* hatched to nauplii at laboratory temperature 26-29ºC and grew to adult (Fig. 11a, b, c).

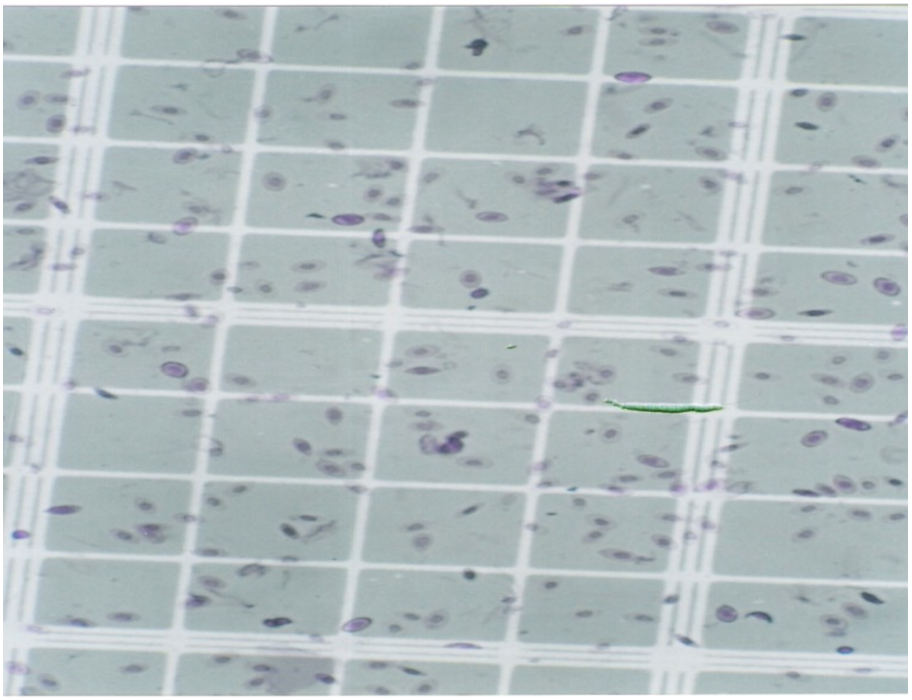


Figure 10: Laboratory cultured *Paramecium* that was used as food of *Cyclops* in this study.

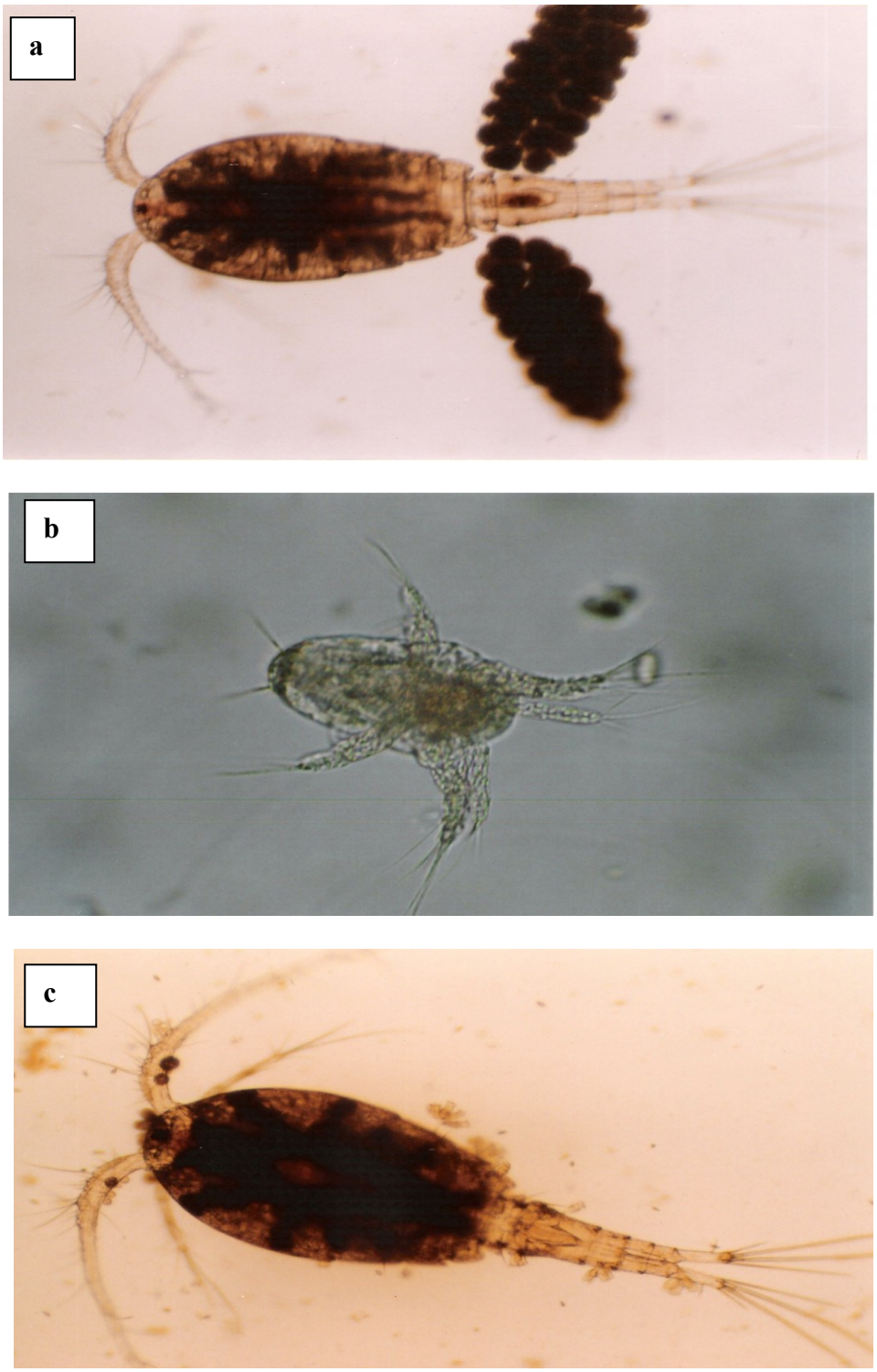


Figure 11: Culture of *Cyclops*

a = Egg sacked *Cyclops* species used in the study.
b = Naupliar hatched from egg sacked *Cyclops*.
c = Adult *Cyclops* developed from a naupliar.

4.2 A survey of *Spirometra* eggs from Faeces of Naturally Infected Lions

Lions are final hosts of *Spirometra* species. Faeces has been used to study the intestinal parasites harbored by the wild lions. In the present study, faecal examination of 7 wild lions (*Panthera leo*) from 3 prides in Tarangire National Park, Tanzania, revealed parasitic eggs, larvae and cysts after sedimentation and floatation methods. A total of 5 parasite taxa were detected in faeces by floatation method, 2 in saturated sugar solution and 3 in saturated salt. In saturated sugar solution, *Spirometra* eggs were detected in 5 (71.4%) individual lion faeces and *Strongyloides* larva in one individual lion. In saturated salt solution, 0 (0%) *Spirometra* eggs were detected but Taeniid eggs were detected in four individual lions and *Strongyloides* in five individual lions. *Toxocara* eggs in five individual lions and coccidium oocysts in one lion. By sedimentation method *Spirometra* eggs were detected in all 7 (100%) lion faeces as shown (Table 4).

Table 4: Comparison of efficiency of Floatation and Sedimentation methods to recover *Spirometra* eggs

Lion and hyena faeces	**Floatation method**		**Sedimentation method**
	Saturated salt solution	**Saturated Sugar solution**	
Lion 1	*Toxocara* eggs	*Spirometra* eggs	*Spirometra* eggs
Lion 2	*Toxocara* eggs	Taeniid eggs	*Spirometra* eggs
Lion 3	*Toxocara* eggs	*Spirometra* eggs	*Spirometra* eggs
Lion 4	*Toxocara* eggs	*Spirometra* eggs Taeniid eggs	*Spirometra* eggs
Lion 5	*Toxocara* eggs	Taeniid eggs	*Spirometra* eggs
Lion 6	*Toxocara* eggs	*Spirometra* eggs	*Spirometra* eggs
Lion 7	None	*Spirometra* eggs	*Spirometra* eggs
Hyena	Taeniid eggs		No parasite seen

4.3 A Survey of *Spirometra* Eggs from Faeces of Naturally Infected Domestic Animals (Dogs and Cats)

A total of 13 households were involved in this study, faeces of 59 dogs and 5 cats were collected. Out of these 25 (42.37%) dogs were positive and all cats were negative. Eggs and larvae of other intestinal parasites detected in dog faeces were *Ancylostoma* eggs, *Trichuris* eggs and *Strongyloides* larvae while in faeces of cats, eggs of *Ancylostoma* and *Trichuris* were detected as shown (Table.5).

Table 5: Faecal sample of dogs and cats examined for natural infection of *Spirometra*

Animal species	No. examined	Positive *Spirometra* eggs	Other intestinal parasites
Dog	59	25	*Ancylostoma*, *Trichuris*, *Strongyloides* larvae
Cat	5	0	*Ancylostoma*, *Trichuris*

4.4 Studies of the Morphology of *Spirometra* Egg

The morphology of the egg of *Spirometra* species recovered from faeces of lion after sedimentation and examination under microscope (Fig. 12a). The egg is ovoid, tapered at both ends, the shell is smooth, dark brown in color and the embryo is visible within the egg shell. Measurements of the 100 eggs were in the range of length 52-75 µm and the width between 30-45 µm. The mean was length 67.8 ± 3.71 and width 32 2± 3.65 µm.

4.5 Studies of Embryonic Development of *Spirometra* Eggs

Development of the embryo was observed daily under compound microscope and pictures were taken for every stage of development. On day 1 there was no much change on the central part of the egg (Fig. 12b). On day 3 of incubation the central part of the egg became more transparent and more oval (Fig.12c). On day 5 the central part of the egg

became continuously large and more formed (Fig.12d). On day 8 the coracidia was fully formed (Fig.12e). On day 12 the oncosphere formed with the appearance of embryonic hooks near its posterior end which are halberd-like shape (Fig.12f). The hatched egg shell was seen with the operculum open (Fig.12g).

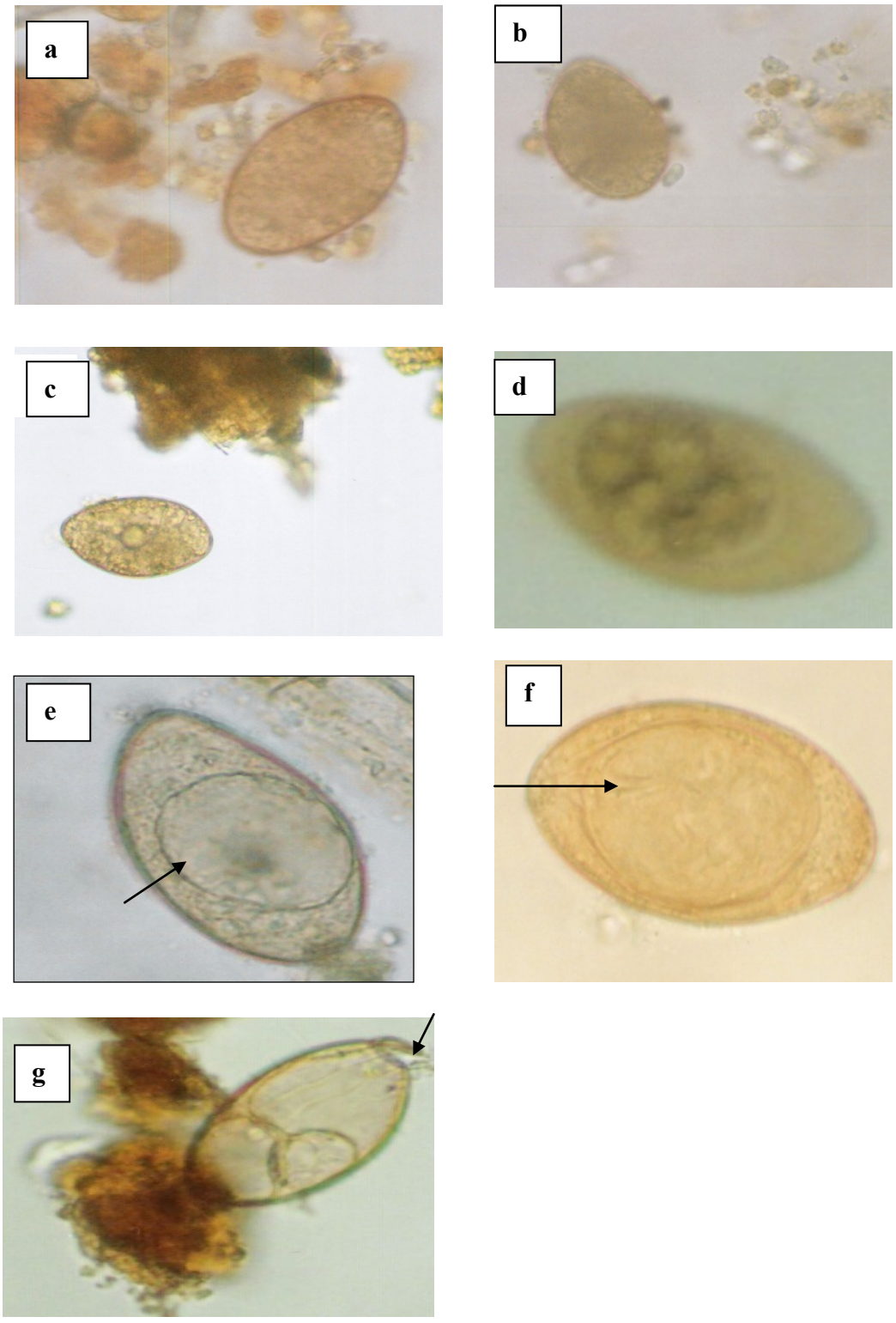


Figure 12: Developmental stages of *Spirometra* egg on incubation.

a = Tanzanian *Spirometra* egg from naturally infected lion, appearance under light microscope.
b = The egg after 1 day of incubation.
c = The egg after 3 days of incubation.
d = The egg after 5 days of incubation.
e = The egg after 8 days of incubation coracidia fully developed. Arrow showing fully developed coracidia.
f= The egg after 12 days of incubation coracidia with hooks. Arrow showing hooks which are halberd-like shape.
g =The egg which already hatched with the operculum open. Arrow showing open operculum.

4.6 The Importance of Optimum Temperature, Light and Duration of Storage of *Spirometra* Eggs in Relation to Hatching Time in Days

Eggs collected from lion faeces stored in the refrigerator at 4ºC for 1½ years were used in this study. The temperature range in the experiments was 26-30ºC, the experiments were carried out in two different conditions of light and darkness. The eggs exposed to light started to hatch from 6-13 days and in total darkness started to hatch from 7-16 days. Hatching was highest on day 7 in presence of light (Table 6) and on day 9 in darkness (Table 7).

Table 6: Observation on the effect of optimum temperature and light on the hatching of *Spirometra* eggs from lion faeces

Type Of eggs	No.of Obser-vation	No.of eggs	Days of incubation at temperature 26-29ºC with light												
			6	7	8	9	10	11	12	13	14	15	16	17	18
	1	30	12	9	1	0	1	0	1	0	0	0	0	0	0
1½	2	30	11	7	0	1	0	0	2	0	0	0	0	0	0
years	3	30	3	12	0	0	1	0	1	0	0	0	0	0	0
	4	30	10	3	3	1	0	2	0	0	0	0	0	0	0
	5	30	0	5	1	1	1	3	0	1	0	0	0	0	0
	6	30	5	7	1	1	2	0	0	3	0	0	0	0	0
Time range		6-13 days													

Table 7: Observation on the effect of optimum temperature and darkness on the hatching of *Spirometra* eggs from lion faeces

Type Of eggs	No.of Obser-vation	No.of eggs	Days of incubation at temperature 27-30ºC in darkness											
			7	8	9	10	11	12	13	14	15	16	17	18
	1	30	0	0	5	0	2	1	0	0	1	0	0	0
1½	2	30	0	4	2	1	1	2	1	0	0	0	0	0
years	3	30	0	0	6	0	0	2	0	1	2	0	0	0
	4	30	1	3	3	1	1	1	0	0	0	1	0	0
	5	30	0	2	4	2	2	1	1	0	0	0	0	0
	6	30	0	1	7	3	2	0	0	1	1	0	0	0
Time range		7-16 days												

4.7 Studies of Viability of *Spirometra* Eggs Hatched After Incubation at Temperature of 26-29ºC with Light to Assess the Hatching Percentage (%) Eggs Hatched

An experiment was carried out to assess the hatching rate of *Spirometra* eggs under room temperature with light. A total of 300 eggs of 1½ years old were incubated at laboratory temperature (26-29ºC) with light, it started hatching on day 6 of incubation. They were all hatched by day 13 and number of emerging coracidia was recorded. A total of 271 coracidia were recorded which amounted to 90.3% hatching percentage (%) eggs hatched (Table 8).

Table 8: Studies on the Viability of 1½ Years Old Eggs at Incubation of Laboratory Temperature (26-29ºC) With Light

Cavity block	Number of eggs	Hatched eggs	Percentage
1	50	48	96
2	50	47	94
3	50	42	84
4	50	46	92
5	50	45	90
6	50	43	86
Total	**300 (100%)**	**271**	**90.3**

4.8 Studies of *Spirometra* Eggs from Dog Faeces

In the life cycle of *Spirometra* it has been reported that dogs play part as a final host where an adult worm matures and produce eggs which are discharged out with faeces. A total of 22 dog's faecal samples were collected, processed by sedimentation method and examined under light microscope for the presence of *Spirometra* eggs. Of the dogs examined 3 (13.6%) were found to be positive of *Spirometra* egg (Fig.13a). The eggs were ovoid, dark brown in color and smooth surface. Two eggs were observed joined end

to end by apical filament (Fig.13b). Measurements of 100 eggs were taken and the length was in the range of 50-65 µm and width between 35-45 µm.

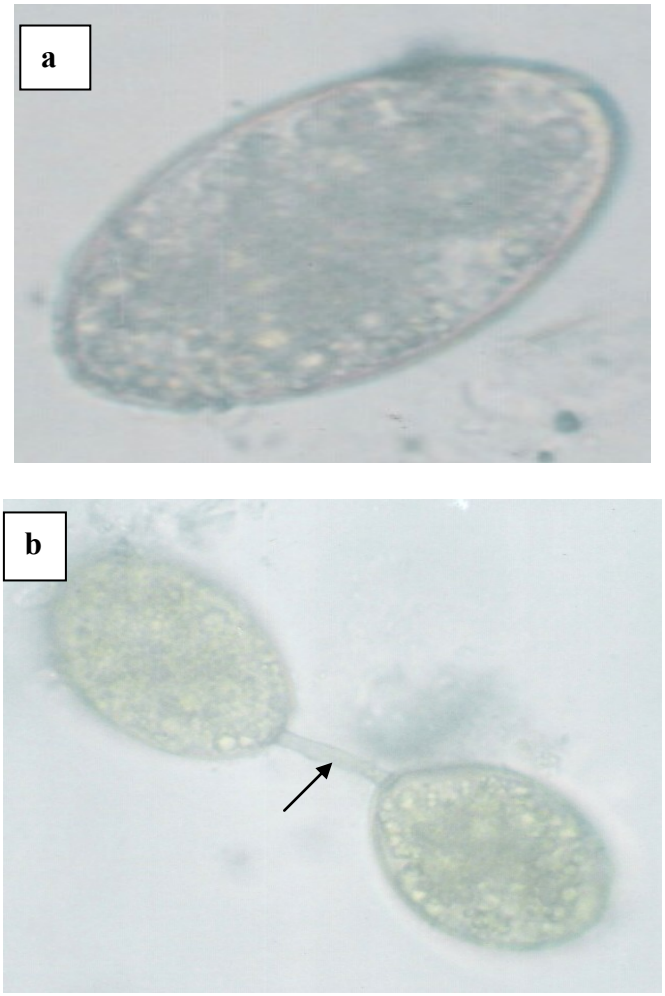


Figure 13: Normal and joined eggs of *Spirometra*

a = Normal *Spirometra* egg from dog faeces.
b = *Spirometra* eggs from dog feces joined with apical filament
Arrow shows joining apical filament.

4.9 Studies of Survival of Coracidia Observed Under Laboratory Condition (Temp. 26-29ºC)

The results of survival of the coracidia recorded at 6, 24, 30, 48 and 54 hours were: (1) At 6 hours all coracidia were alive and active. (2) At 24 hours all coracidia were alive. (3) At 30 hours 14% (7) had died. (4) At 48 hours 84% (42) had died and (5) At 54 hours 100% (50) had died (Table 9).

Table 9: Survival of coracidia observed under laboratory conditions (temperature 26-29ºC)

No cavity blocks	No .coracidia	6 hours Alive	6 hours Dead	24 hours Alive	24 hours Dead	30 hours Alive	30 hours Dead	48 hours Alive	48 hours Dead	54 hours Alive	54 hours Dead
1	10	10	0	10	0	8	2	2	8	0	10
2	10	10	0	10	0	9	1	1	9	0	10
3	10	10	0	10	0	7	3	3	7	0	10
4	10	10	0	10	0	9	1	1	9	0	10
5	10	10	0	10	0	10	0	1	9	0	10
Total	50	50 (100%)	0 (0%)	10 (100%)	0 (0%)	43 (86%)	7 (14%)	8 (16%)	42 (84%)	0 (0%)	50 (100%)

4.10 Studies on the Development and Infectivity of Coracidia to *Cyclops*

The coracidium is hatched from the egg in water, starts swimming by using cilia around its body. It can survive in water for about 48 hours if is not eaten by the first intermediate host (Arme, 1983). When eaten by the *Cyclops* (first intermediate host) it develops to procercoid in the abdominal cavity. In order to investigate the infectivity of coracidia to *Cyclops*, infectivity experiments were conducted in which *Cyclops* were mixed in small beakers with hatched coracidia. The *Cyclops* were observed under light microscope for the development of procercoids, and number of procercoids.

The experimentally hatched coracidia were observed under the microscope, swam actively by using their cilia (Fig.14a). The coracidia developed to procercoid in the body cavity of *Cyclops*. On day one post infection cilia on the body surface were shed out (Fig 14b). The morphological changes of coracidia occurred in the body cavity of *Cyclops*. They became elongate in appearance at day 4 post infection (Fig.14c). On day 9 there was indentation demarcation on apical disc (Fig.14d).

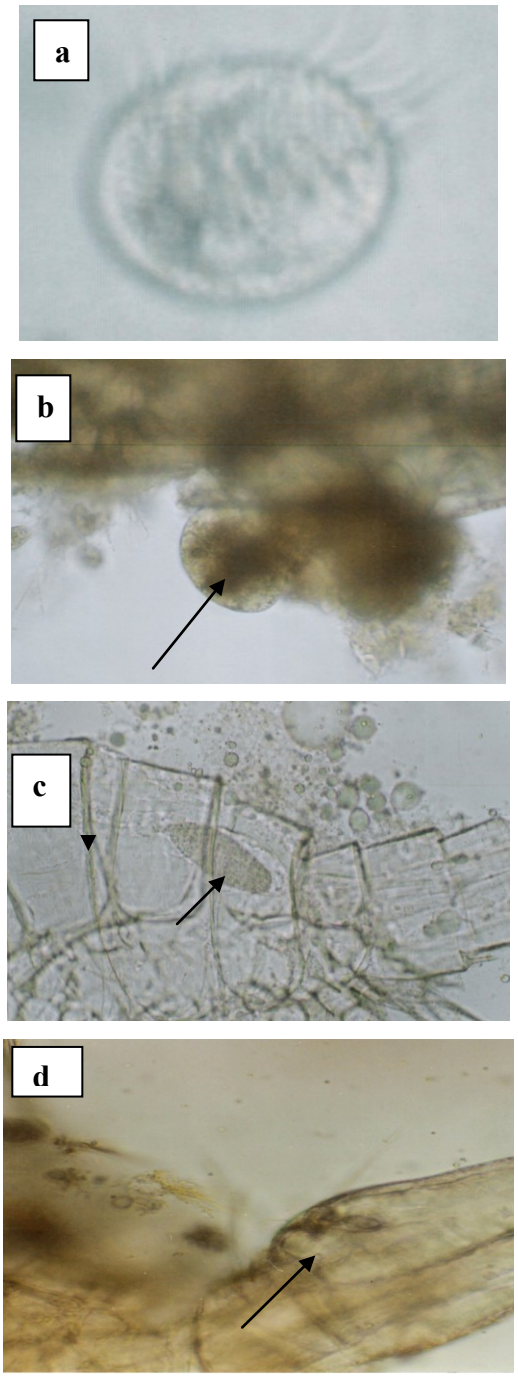


Figure 14: Hatched coracidia and developmental stages in the *Cyclops*.

a = Hatched coracidium with cilia on the body surface.
b = One day old procercoid extruded from the body cavity of *Cyclops*. Arrow showing all cilia on body surface has been shade out.
c = Arrow showing a four days old procercoid, elongated in the body cavity of *Cyclops*.
d = Arrow showing indented anterior end of procercoid 9 days old in the body cavity of *Cyclops.*

4.11 Measurements of Coracidia

A total of 20 coracidia from eggs collected from faeces of lion were taken measurements of length and width. The length was 30µm and width 20µm respectively.

4.12 Prevalence of Procercoids in Naturally Infected *Cyclops*

Examination of *Cyclops* for natural infection with procercoids was done in the laboratory. *Cyclops* collected from 8 water sources in Tarangire National Park were examined. A total of 84 *Cyclops* were examined, out of these 6 (7.14%) were infected with procercoids (Fig.15) and 78 (92.86%) were not infected with procercoids as shown in (Table 10).

Figure 15: *Cyclops* naturally infected with procercoid. Arrow showing procercoid with calcareous corpuscle

Table10: Natural infection of *Cyclops* with procercoids from Tarangire National Park

Site collected copepods	No.copepods examined	Positive	Negative
Sirale	16	0	16
Kuro	16	0	16
Boundary	14	0	14
River Tarangire	3	0	3
Public Area	12	4	8
Matete	7	0	7
Matete Bridge	6	0	6
Near camp Site	10	2	8
Total	**84 (100%)**	**6 (7.14%)**	**78 (92.86%)**

4.13 Establishment of Infection Rate of Procercoids in Experimentally Infected *Cyclops*

Cyclops were experimentally infected with hatched coracidia, they were examined under microscope for susceptibility. A total of 106 *Cyclops* were examined. There were 11 *Cyclops* infected, 8 *Cyclops* each harboured 1 procercoid and 3 each harboured 2 procercoids. The infection rate of coracidia in *Cyclops* was 10.4%.

4.14 Experimental Infection of Laboratory and Domestic Animals with Procercoids

A total of 122 animals (laboratory and domestic) animals were infected with procercoids. The animals infected orally were 97 and intraperitoneally 25. All 122 (100%) animals no single procercoid developed to plerocercoid (Table 11).

Table 9: Experimental infection of laboratory and domestic animals with procercoids

Animals	Number infected	Route of infection		Result
		Oral	Intraperitoneum	
Mice	49	38	11	All negative
Rats	46	35	11	All negative
Guinea pigs	22	20	2	All negative
N. Z. Rabbit	3	2	1	All negative
Pig	1	1	-	Negative
Goat	1	1	-	Negative
Total	**122**	**97**	**25**	**All negative**

4.15 A Survey of Birds for Natural Infection with Spargana

A total of 6 birds (duck 1, black snapper 1, helmented guinea fowl 4) from around Tarangire National Park were examined for natural infection with spargana. All birds examined were free from spargana infection.

4.16 A Survey of Rats for Natural Infection with Spargana

A total of 50 rats were caught at Minjingu village near Tarangire National Park and dissected to examine for natural infection of spargana. Three species of rats were identified: Octomis 1 (2%), Mastomis 17 (34%) and Africansis 32 (64%). All 50 rats examined were free from spargana (Table 12).

Table 10: Rats examined for natural infection with spargana from Minjingu village near Tarangire National Park

Rats	Number of rats	Pecentage	Infected rats
Octomis	1	2	0
Mastomis	17	34	0
Africansis	32	64	0
Total	**50**	**100**	**0 (0%)**

4.17 A Survey of Spargana in Naturally Infectected Wild Animals from Around Tarangire National Park

A total of 8 wild animals (wildebeests 5, warthog 1, tomi 2) around Tarangire National Park were killed and examined for natural infection of spagana. All animals were free from spargana.

4.18 Recovery of Adult Worm from Naturally Infected Dog

One adult worm of *Spirometra* was recovered from the small intestine of one naturally infected dog (Fig.16a, b, c). The adult worm recovered was without neck and scolex measured 89.5 cm long and 1.18 cm wide (Fig.16d). Mature proglottids were wider than long measured 1.02 cm (10.2 mm) wide and 0.17 cm (1.7 mm) in length. Stained uterus is conical in shape, with 6 coils (Fig.17a). The subterminal coil is the broadest while the terminal one is less broader. The male genital pore is on the upper ventral side (Fig.17b).

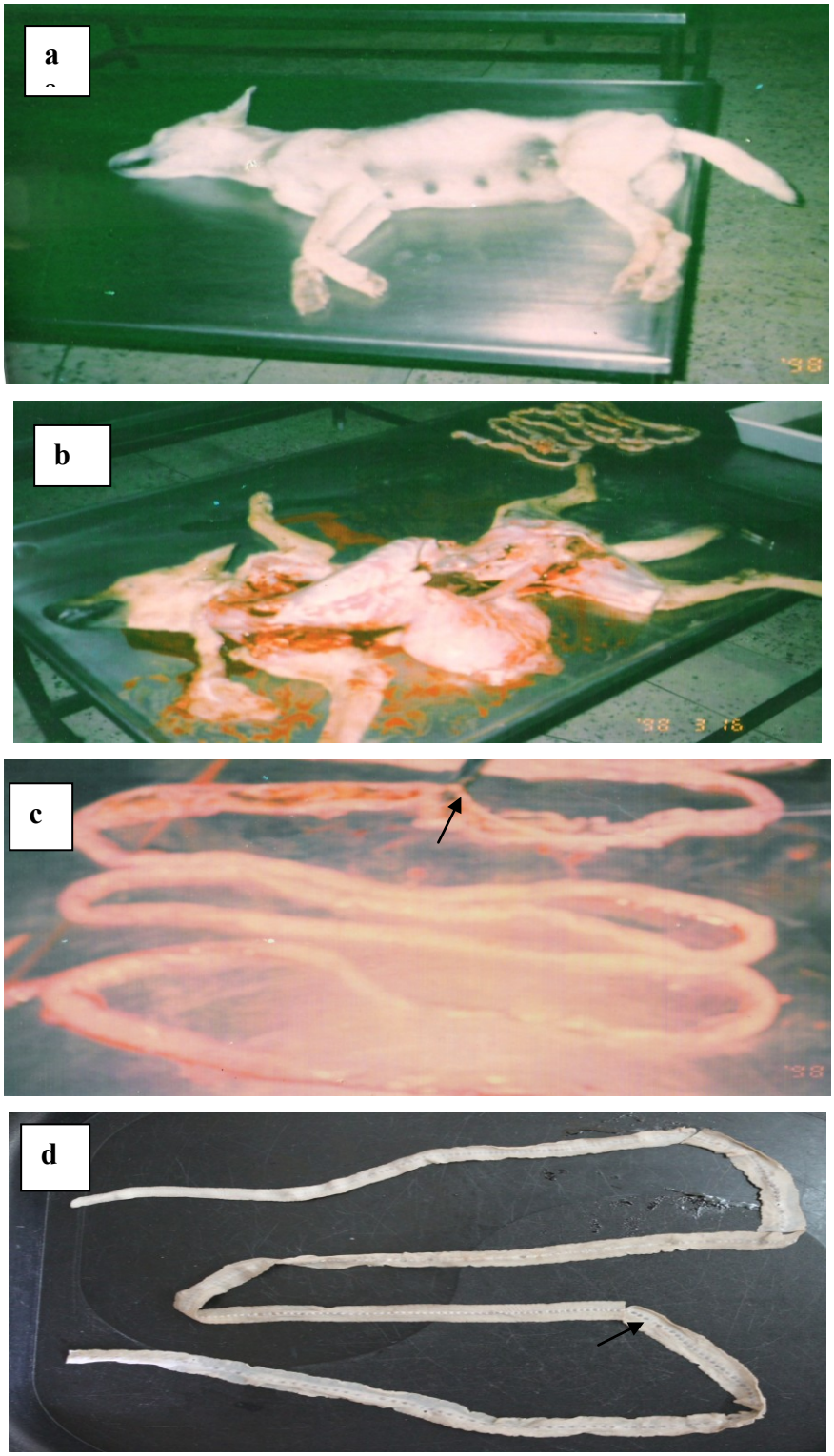


Figure 16: Recovery of an adult *Spirometra* from an infected dog

a = Dog infected with adult *Spirometra* authenized in post moterm room.
b =Carcas of a dog with infection of adult worm of *Spirometra*.
c = Adult *Spirometra* recovered from small intestine of a dog. Arrow shows lifted adult *Spirometra* with a forceps
d = Part of *Spirometra* strobila showing a chain of uterus centrally located proglottids. Arrow showing chain of uterus.

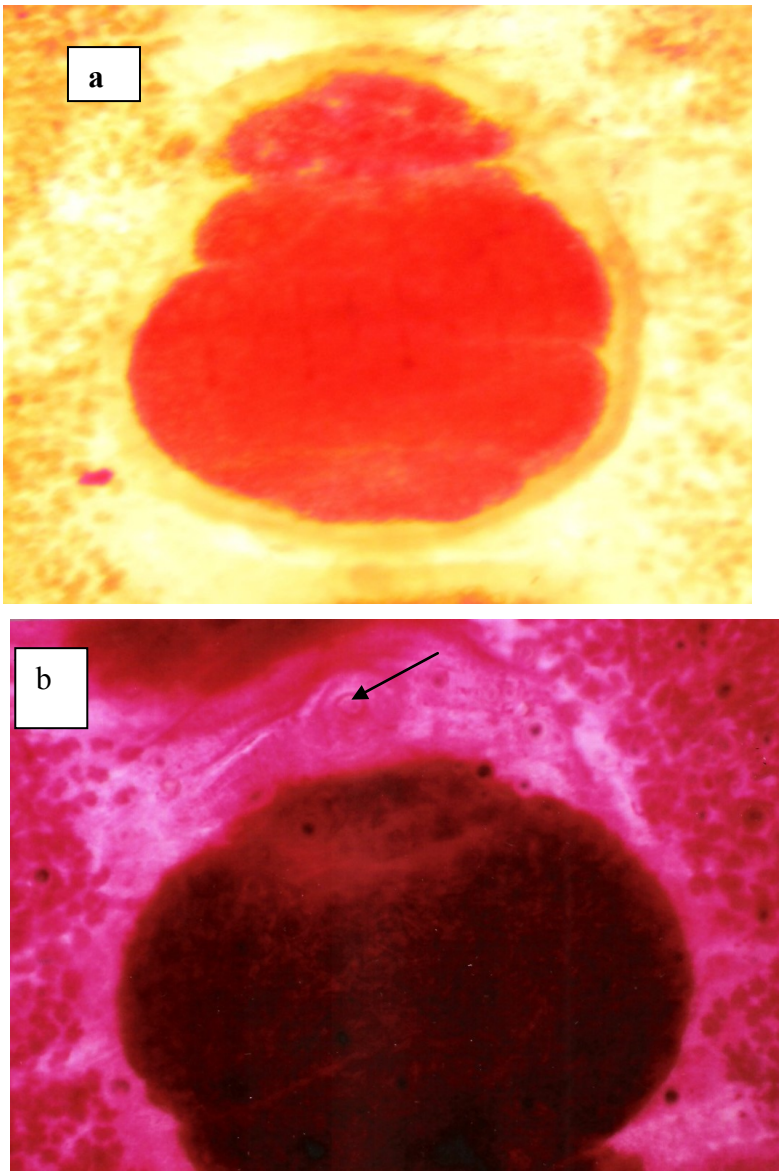


Figure 17: Appearance of *Spirometra* uterus after staining with carmine

a =Uterus of Tanzania *Spirometra*, conical in shape.
b = Uterus of Tanzanian *Spirometra*. Arrow showing male genital opening.

4.19 Identification of Tanzanian *Spirometra* spp. Using Morphological Characteristics

Morphological characteristics of different developmental stages of Tanzanian *Spirometra* spp. were compared to those of *S. erinacei* and *S. mansonoides* to identify the parasite (Table 13).

Table 11: Morphological characteristics of the different developmental stages of Tanzanian *Spirometra* spp., S. *erinacei* and *S. mansonoides.*

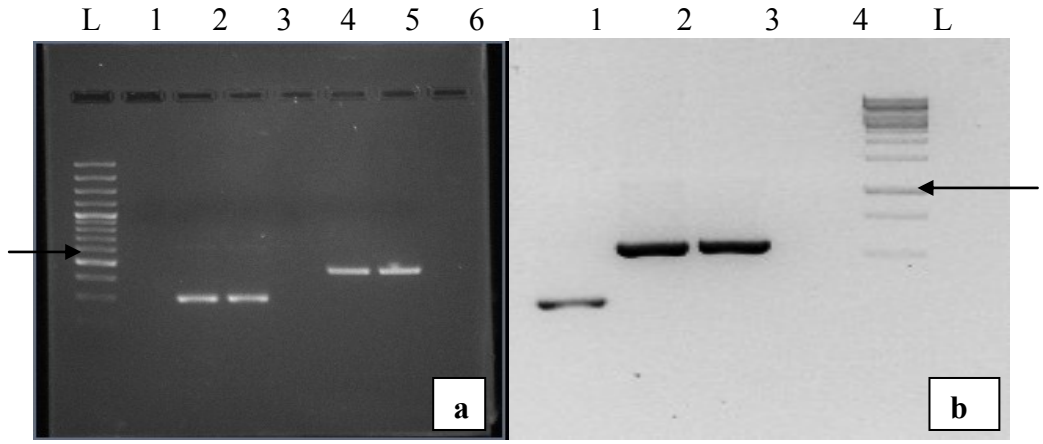
Stage and characteristic	Tanzanian *Spirometra* species (present study)	*S.erinacei* (Lee, 1990)	*S. mansonoides* (Muller, 1938)
Egg			
Shape	Ovoid	Ovoid	Ovoid
Operculum	Operculum (present)	Operculum (present)	Operculum (present)
Operculum suture	Operculum suture (present)	Operculum suture (present)	Operculum suture (present)
Length (µm)	50-65	55-76	48-83
Width (µm)	35-45	30-43	28-43
Hatching (days)	6-13	8-14	9
Pre-patent period (days)	Not observed	15-18	10-25
Coracidia			
Shape	Ovoid	Ovoid	Ovoid
Size (µm)	25.25±13.18 x 27.4±3.56	43.8	45
Surface	Cilia on the surface	Cilia on the surface	Cilia on the surface
Hooks	Hooks present	3 pairs of hooks	3 pairs of hooks
Shape of hooks	Halberd-like hooks	Halberd-like hooks	Halberd-like hooks
Procercoid			
Morphological changes (in days)			
1	Cilia on body surface lost	Cilia on body surface lost	Cilia on body surface lost
4	Procercoid elongated	Procercoid elongated	Procercoid elongated
9	Indentation anterior end	Indentation anterior end	Indentation anterior end
	Develop in cyclop	Develop in cyclop	Develop in cyclop
Plerocercoid			
	Not obtained	Club shaped anterior end (scolex) with tail	Club shaped anterior end (scolex) with tail
Adult			
Body length (cm)	89.5	30-95	25-100
External morphology:	Strobila	Scolex, neck, strobila	Scolex, neck, strobila
Scolex	Not obtained	Spatulate shaped with two lateral bothria.	Spatulate shaped with two lateral bothria.
Neck	Not obtained	Thin and long	Thin and long
Genital pores	Male genital pore present lies on upper ventral side.	Cirrus pore is present and located on the upper ventral side of the proglottid. Vaginal pore present. Broad opening a short distance infront of the terminal uterine coil. Uterine pore present lies on the terminal coil, some distance further from the cirrus pore.	Cirrus pore is present and located on the upper ventral side of the proglottid. Vaginal pore present. Broad opening a short distance infront of the terminal uterine coil. Uterine pore present lies on the terminal coil, some distance further from the cirrus pore.
Internal morphology:			
Uterus	Uterus has 4-6 coils, centrally located in the proglottid.	Uterus has 5-7 coils, centrally located in the proglottid.	Uterus coiled with **C**-shaped outer loop of the uterus, and its anterior limb constricted in midline forming a lateral expulsion chamber.
Testes	Testes not united with vitellaria anteriorly in the midline.	Testes not united with vitellaria anteriorly in the midline.	Testes not united with vitellaria anteriorly in the midline.
Vitellaria	Vitellaria not united with testes anteriorly in the midline.	Vitellaria not united with testes anteriorly in the midline.	Vitellaria not united with testes anteriorly in the midline.
Intermediate hosts:			
First intermediate host	*Cyclops*	*Cyclops* (*C.leuckarti* and *C.serrulatus*).	*Cyclops* (*C.leuckarti, C.viridans, C.biscupidus*).
Second intermediate host	Not determined	Amphibians, birds , reptiles, mammals	Amphibians, birds, reptiles, mammals
Definitive host	Canidae and Felidae	Canidae and Felidae, man is accidental	Canidae and Felidae, man is accidental
References	Present study (2013)	Lee (1990)	Mueller (1938).

4.20 Species Determination of *Spirometra*

A total of 7 samples were analysed by PCR reactions which composed of three types of primers JB3/4.5, JB10/9 and M13. After electrophoresis, drying the gel and autoradiography of the PCR products, 4 sample bands were positive on the gel (Fig.18 a). The sizes of the differential bands were ~300 bp to ~ 400 bp. In (Fig.18 b) revealed that clones harbored inserts, there were 3 colony bands on the gel. The sizes of the bands were ~250 bp to ~ 400 bp. Out of these one colony band was negative with ~250 bp and the other 2 were positive with colony bands ~ 400 bp. PCR screening and confirmatory results of *Spirometra* Tanzania isolate is shown (Table 14). In the present study there was no positive control used in the laboratory from any source more than the sample and colony. The phylogenetic trees were reconstructed using 11 representative nucleotide sequences as shown (Fig.19).

Table 12: PCR screening results and confirmatory sequencing results of *Spirometra erinaceieuropaei* isolates from Tanzania

Host	Origin	PCR	Result	Confirmatory sequencing	
		n	Species	n	species
Dog	Tanzania	7	*S.erinaceieuropaei*	3	*S.erinaceieuropaei*



L=DNA Ladder
1= Negative Control
2&3=JB3/4.5 primers (≈300bp)
N=Negative control
5&6=JB10/9 primers (≈400bp)
Arrow= 500bp

1=M13 primers
2&3=M13 primers (≈300bp)
4=Negative control
L=100bp DNA Ladder
Arrow=500bp

Figure 18: (a) Gel electrophoresis of PCR products for *Spirometra* spp. suspected samples from Tanzania and (b) Gel electrophoresis of colony PCR

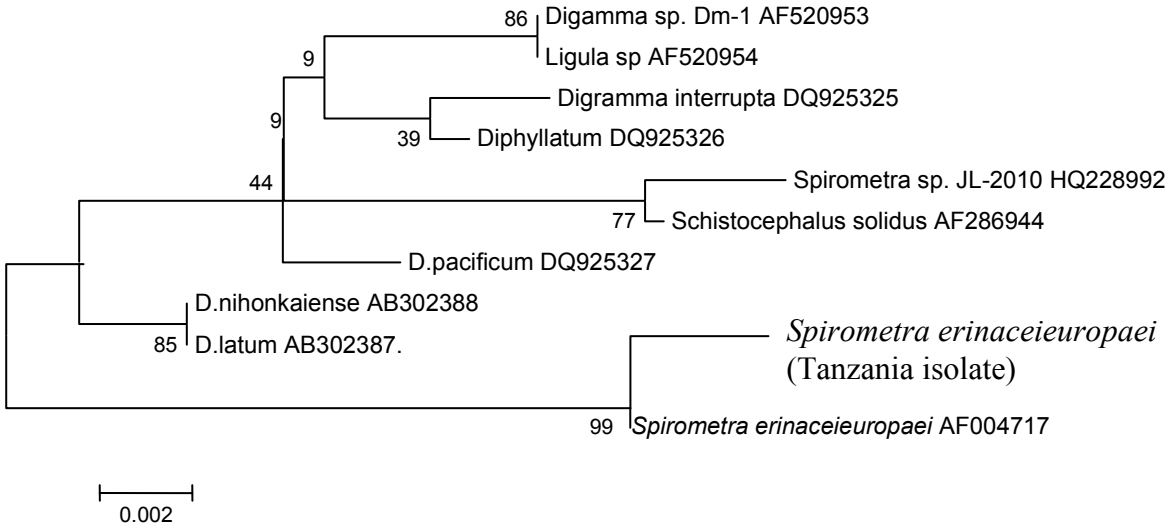


Figure 19: Phylogenetic tree of *Spirometra erinaceieuropaei* (Tanzania isolate) reconstructed using 11 representative nucleotide sequences by NJ method employing the Kimura – 2 – parameter option.

4.21 Seroprevalence Studies of Sparganosis

In this study, the antigenicity of crude extracts of adult *Spirometra erinaceieuropaei* Tanzania isolate was used for serodiagnosis of human sparganosis. A total of 216 sere were obtained for serologic tests from inhabitants of Babati and Monduli Districts. Serum levels of antibody specific to spargana were measured by ELISA. Antisparganum specific antibody (IgG) levels in sera were measured by using the crude extract of adult *S. erinaceieuropaei* as antigen. Cysticercosis serum was used as a positive control. Out of 216 sera samples of inhabitants tested, 135 (62.5%) sera showed positive reactions to the adult *S. erinaceieuropaei* antigen. There were 50 males, 139 females and 27 children (<18) years. In Monduli district females (18-80) years were 125 of these 80 were positive, males (18-81) years were 33 of these 17 were positive. Children (<18) years were 27 of these 19 were positive. Babati district females (18-80) years were 14 of these 7 were positive, males (18-95) years were 17 of these 12 were positive. The results are shown (Table 15).

Table 13: Summary of ELISA results showing positive and negative sera for sparganosis from inhabitants of Babati and Monduli Districts

	Total	Positive	(%)	Negative	(%)
Monduli district					
Male (18-81)	33	17	51.5	16	48.5
Female (18-80)	125	80	64.0	45	36.0
Children (<18)	27	19	70.4	8	29.6
Babati district					
Male (18-95)	17	12	70.5	5	29.5
Female (18-80)	14	7	50.0	7	50.0
Children (<18)	-	-		-	
	216 (100%)	135 (62.5%)		81 (37.5%)	

4.22 Knowledge, Atittude, Practices and Risk Factors Studies

A total of 345 participants from the two districts were included in the study. Of these, 46.4% were from Babati district and the rest were from Monduli district. Examination of socio-demographic characteristics did not disclose significant differences between the participants in the two districts. Sex of participants, 135(39.4%) were males and 209 (60.6%) were females. Age of participants, 15 (4%) were between 0-15 years, 128 (37%) were aged between 16-30 years, 99 (29%) were 31-45 years, 75 (22%) were 46-60 years old and 28 (8%) were older than 61 years. Marital status: married respondents were 234 (68.0%), unmarried 85 (24.7%), widow 13 (3.8%) and separated 12 (3.5%). Education of respondents, 36 (10.4%) Primary Education (I-IV), 171 (49.6%) Primary Education (V-VII), 31 (9.0%) Secondary Education (Form 1-4), 3 (0.9%) Secondary Education (Form 5-6), 8 (2.3%) Higher Education (University/College), 1 (0.3%) adult education and 95 (27.5%) had not attended school. The results are shown (Table 16).

Table 14: Socio-Economic and Demographic Characteristics of respondents

S/No	Study variable	No.of participants	Percentage
1	**Age**		
	0-15	15	4
	16-30	128	37
	31-45	99	29
	46-60	75	22
	61 and above	28	8
	Total	**345**	**100.0**
2	**Gender**		
	Female	209	60.6
	Male	136	39.4
	Total	**345**	**100.0**
3	**Marital status**		
	Married	234	68.0
	Unmarried	85	24.7
	Widow	13	3.8
	Separated	12	3.5
	Total	**345**	**100.0**
4	**Level of education**		
	Not attended	95	27.5
	Adult education	1	0.3
	Primary educ.(I-IV)	36	10.4
	Primary edu (V-VII)	171	49.6
	Sec. educ.(FI-IV)	31	9.0
	Sec.educ (FV-VI)	3	0.9
	Higher educ,(Un/College)	8	2.3
	Total	**345**	**100.0**

The disease sparganosis was described to the participants (Appendix 15) then were asked whether they ever have seen the disease condition, all 345 (100%) respondents denied to have seen sparganosis. Additionally, 345 (100%) participants reported that no relative had ever been sick or died of sparganosis.

The practice of the activities carried out to earn income (economic activities) by the participants was determined. The majority of the participants were involved in farm production followed by animal keeping. Few of them were involved in small business and a small number were employees (Table 17). Analysis of Variance (Two-Ways without replication) showed a significant difference ($p< 0.05$) in the practice of economic activities.

Table 15: Practices of participants on economic activities

Economic Activities	Most Participate (3 Scores)	Participate (2 Scores)	Less Participate (1 Score)
Farm Production	591	28	131
Animal keeping	117	94	259
Fishing	0	0	345
Employee	51	0	328
Business	96	66	280
Charcoal Burning	0	4	343
Others (Game Hunting)	147	6	293
Total (Scores)	**1002**	**198**	**1979**

Participants were asked if they keep dogs and cats, 157 (45.6%) participants responded to have been keeping dogs and cats; the other 188 (54.4%) responded not to be keeping dogs and cats. Participants were asked the aim of keeping the pets. The majority of the participants answered it was for protection (Table 18). The knowledge of how to keep dogs and cats was tested among the participants. Most of the participants responded that the pets were kept in a free-range system (Table 19).

Table 16: **Aim of participants on keeping dogs and cats**

Aim versus attitude in animal keeping	Like most (3 Scores)	Like (2 Scores)	Don't like (1 score)
Income generation	3	2	343
Protection	474	0	187
Hunting	6	8	339
Others	3	0	344
Total (Scores)	**486**	**10**	**1213**

Table 17: **Knowledge on how animals are kept**

Habits against attitude of Keeping dogs	Like most (3 scores)	Like (2 scores)	Dislike (1 score)
Not wondering	3	30	329
Limited free-range	198	14	272
Complete free-range	219	2	271
Total Scores	**420**	**46**	**872**

Sparganosis can be transmitted through drinking unsafe water. Participants were asked about boiling water for drinking. 71.3% of participants responded that they do not boil drinking water (Table 20). Participants were asked about the sources of water which they use. Most of the participants responded that they get water mainly from running springs followed by rivers (76.8%) and shallow local wells (43.8%) as in (Table 21) and (Appendix 16, 17).

Preparation of game meat, 39.4% of participants responded that they prepare game meat by boiling, 5.5% prepare by roasting and 0.3% consume raw meat (Table 21). In this study the life cycle, potential hosts in the environment and future investigations are represented in the flow chart (Fig. 6).

Table 18: Type of water which participants drink

Exposure versus risk factors	Boiling all the time	Boiling not always	Not Boiling	Total
Not attended	7(7.4)	12(12.6)	76(80.0)	95(100.0)
Adult Education	0(0.0)	1(100.0)	0(0.0)	1(100.0)
Primary Education (I-IV)	6(16.7)	5(13.9)	25(69.4)	36 (100.0)
Primary Education (V-VII)	30(17.5)	21(12.3)	120(70.2)	171(100.0)
Secondary Educ. (Form 1-4)	7(22.6)	5(16.1)	19(16.1)	31(100.0)
Secondary Educ. (Form 5-6)	2(66.7)	0(0.0)	1(33.3)	3 (100.0)
Higher Education (University, Colleges)	2(25.0)	1(12.5)	5(62.5)	8(100.0)
Total	**54(15.7)**	**45(13.0)**	**246(71.3)**	**345(100.0)**

Single Table Analysis $X^2 = 20.54$; df =12; P = 0.05

Table 19: **Types of Water supply sources**

Type of water sources versus uses	Most used	Used	Not used
Local Shallow wells	151	656	345
Deep Wells	15	0	340
River	265	2	259
Dam	21	4	336
Running springs	690	6	112
Others	0	0	345
Total	**1142**	**668**	**1737**

Table 20: Frequency distribution of respondents with different habits in preparing game meat before consumption

	Boiling	Roasting	Raw	Roasted bolus	Others	Total
Respondents	136	19	1	0	189	345
Percentage	39.4	5.5	0.3	0	54.8	100

CHAPTER FIVE

5.0 DISCUSSION

Spirometra is an intestinal cestode of cats and dogs which causes the disease in humans known as sparganosis. Different stages in the life cycle of the parasite is used for characterization. Serological tests are used to diagnose sparganosis infection in humans. The objective of these studies was to characterize the parasite by using morphological characteristics of different stages in the life cycle and molecular techniques for the adult worm. Serological tests were carried out to determine sparganosis infection in humans. Questionnaire survey was used to examine Knowledge, Attitudes, and Practices on sparganosis among inhabitants in two districts (Babati and Monduli) in Tanzania. Morphological characterization revealed the parasite to be *Spirometra erinaceieuropaei*. Molecular techniques used were Polymerase Chain Reaction (PCR), sequencing and Phylogenetic tree construction confirmed to be *S. erinaceieuropaei*. Enzyme linked immunosorbent assay (ELISA) showed positive human sera to spargana antibodies. Questionnaire survey revealed all participants had no knowledge, poor attitudes and practices on sparganosis.

5.1 Studies on Culture of *Paramecium* and *Cyclops*

Paramecium and *Cyclops* were cultured in the laboratory. *Paramecium* provided food source of *Cyclops* used for infection with coracidia which developed to procercoid in the body cavity. The food organism *Paramecium* proliferated in the *Cyclops* culture and supported a rapid growth and reproduction of the *Cyclops.* These findings are in agreement with those of Mueller (1935) who established a mass culture of *Cyclops* which was used to complete the life cycle of *Spirometra mansonoides* in the laboratory by feeding hay infusion. Other workers reported laboratory maintenance of *Cyclops* (Poulin

et al., 1992; Nie and Kennedy, 1993; Pasternak *et al.,* 1995; Oyamada *et al*., 1996; Okino, 1996) but little effort was made to determine food organisms. Riviere *et al*. (1987) successfully cultured *Mesocyclops aspericornis* by giving *Chlorella* to naupliar and early copepodite stages. Lewis *et al*. (1971) cultured *Cyclops abyssorum* by feeding *Euglena gracilis* plus *Artemia*.

5.2 Collection of *Spirometra* Eggs from Lion and Dog Faeces

Spirometra infection of lions and dogs was determined by screening the presence of *Spirometra* eggs in their faeces by using Sodium chloride (Specific Gravity 1.210) and sugar solutions (Specific gravity 1.459). In sugar solution *Spirometra* eggs were easily detected than in Sodium chloride solution (Specific Gravity 1.210). The presence of *Spirometra* eggs in lions and dogs faeces is similar to the observations of Round (1968); Rodgers (1974); Bwangamoi (1970); Müller-Graf *et al*. (1995) who reported *Spirometra* eggs to be common in wild lions and Lee *et al*. (1990), Little and Ambrose (2000); Bowman (2009); Zajac and Conboy (2012) reported *Spirometra* eggs in dogs. The present result shows that sugar solution is efficient for detection of *Spirometra* eggs in definitive hosts. This conforms to the results of Engh *et al*. (2003) who detected *Spirometra* eggs in a coprological survey of spotted hyenas using sugar solution. Sugar solution method is efficient in detection of *Spirometra* eggs in definitive hosts because it has higher Specific Gravity than Sodium chloride solution. Formal-ether method has been used by Cheesbrough (1987); Müller-Graf (1994) to detect *Spirometra* eggs in lion faeces from Serengeti National Parks and Ngorongoro Crater, Tanzania which showed similar results as Sugar solution in the present study. The method easily detected *Spirometra* eggs and other intestinal parasites in lion faeces.

During microscopic examination of faecal sample of dog an abnormal egg of Tanzanian *Spirometra* sp. was observed. The eggs were joined end to end with apical filament on the posterior ends. The finding agrees with that of Mueller (1959, 1961, 1966) who reported a double egg of *Spirometra mansonoides*. Two eggs were joined end to end. One end had operculum, the embryo had 12 hooks and two flame cells on one end.

Spirometra infection in lions of Tarangire National Parks was determined by screening their faeces for the presence of *Spirometra* eggs. All seven lions screened were found to be infected with *Spirometra*. The results agrees with Nelson *et al*. (1965); Dinnik and Sachs (1972); Müller-Graf (1995) who reported *Spirometra* infection in lions of Serengeti National Park and Ngorongoro Crater in Tanzania. This could be explained that, lions in the National Parks feed on wild animals infected with plerocercoid larvae (spargana), the plerocercoid develop to adult worm in the small intestine of the lion, produce eggs which are passed out in faeces.

Dogs in Minjingu ward which borders Tarangire National Park were found infected with adult worms of *Spirometra* spp. The two definitive hosts lion and dog species in the area sampled are in the same environment, they share water sources and game meat. Both animal species defecate on the ground, the faeces are carried by water to the water ponds where the eggs develop and hatch to coracidia which are eaten by the *Cyclops*. When other animals come to these water sources they drink water with infected *Cyclops* which results in infection with spargana. Lions feed on these animals and get infection. Dogs in this area go in the National Park once they sense a smell of carcases which have been killed by the lions and other predators they eat the same carcases. Sometimes they take home the remaining parts of the carcases where other dogs feed especially puppies which can also get the infection. The dogs in this area are taken together with cattle when cattle

are sent for grazing. Sometimes the dogs are used for hunting of small animals which might be infected with spargana. The results agree with Lee *et al*. (1990); Little and Ambrose (2000) who reported *Spirometra* infection in dogs.

5.3 Studies on the Morphology of *Spirometra* Egg

Spirometra develop to adult stage in the small intestine of the final host. They produce and liberate large numbers of eggs which are voided with the host´s faeces into the environment (Uchida *et al*. 1980). The eggs of *Spirometra* and other diphyllobothriids have a thick egg shell of tanned proteins (phenol) of which the main function is to protect the developing coracidium from adverse environmental conditions (Smyth and Clegg, 1969). Other cestodes such as cyclophyllidea have no thick egg shell. The egg shell is the only hardened structure in life-cycle of the *Spirometra.* It has often been used for taxonomic purposes. The characters which are commonly used as taxonomic criteria are the egg measurements, colour, pitted surface, operculum, operculum suture, pre-patent period and periodicity of egg output.

The eggs of *Spirometra* recovered from faecal samples of lions and dogs were ovoid, pointed at one end, egg shell smooth. The results agree with those of the previous workers (Berntzen and Mueller, 1964; Ash and Orihel, 1990; Ugarte *et al.,* 2005; Schar *et al.,* 2014) who reported *Spirometra* eggs to be ovoid, pointed at one end and egg shell smooth.

5.4 Measurements of *Spirometra* Eggs

Measurements of eggs of Tanzanian *Spirometra* spp. from lion faeces had the length 52 - 75 µm and width 30-45 µm. The mean length was 67.8 ± 3.71 and width was 32 2 ± 3.65 µm and eggs from dog faeces were 50 - 68 x 30 - 42 µm. The results of

Spirometra eggs measurements reported by previous workers. Okumura (1919): 57 x 37 µm; Li (1929): 54 to 66.4 x 33.8 to 37.1 µm; Odening and Bockhadt (1980): 56 to 65 x 33 to 35 µm; Lee *et al*. (1990): 66.8 x 36.1 µm; Opuni (1974): 67 ± 5.6 x 41 ± 2.5 µm; Miyazaki (1991): 55 to 76 x 30 to 43 µm; Müller-Graf (1999): length 54.7-76.0 µm; mean 64.9 µm± 1.0; width 30.4 - 44.4, mean 37.5 ± 0.6 µm. The results in the present study agree with the previous workers.

5.5 Incubation of *Spirometra* Eggs

The Harada-Mori method of incubation of eggs was redesigned by using aquarium this resulted to massive hatching of coracidia which was used for the study of coracidia and experiment of life cycle of *Spirometra* by infecting the first intermediate host (*Cyclops*). This method is simple and very effective in hatching eggs to obtain massive coracidia for use in the study of life cycle. The principle is the same as for the Harada-Mori except in this method you use aquarium (boiled water, clean eggs, sterile sand and stones) at the bottom of the 15 ml falcon tube. Sand creates a natural environment like that in water ponds and rivers. Cleaning the eggs by sedimentation reduces the amount of bacteria which can have effect on hatching of eggs (Mueller, 1966). In rivers and ponds there is natural cleaning of eggs reducing the amount of bacteria in faeces. This is the same principle in the method used in this study. In the present study two experiments were set separately, one in the presence of light and the other in darkness which the sample was placed in a box to ensure darkness. Hatching of eggs was not affected by darkness, the eggs hatched normally like in the presence of light. The results agree with those of Opuni (1974) who reported that there was no significant difference in the hatching ability of eggs exposed to light or darkness.

In the present study eggs were incubated several times by using methods of Stephanson (1985) and Beaver *et al*. (1964) but the experiments were not successful. Incubation of the eggs of *Spirometra* is difficult because the rate of development depends on the temperature and water (Mueller, 1959). Development of eggs occurs in the presence of water. Harada-Mori culture provided a simple and efficient method of culturing *Spirometra* eggs. In this method, there is capillary film of water through filter paper which maintains moist habitat for the eggs to develop. In the National Parks, lions defecate faeces containing *Spirometra* eggs which come in contact with water bodies and mud-ooze medium at the margins of streams providing a natural moist habitat for the eggs to develop to coracidia (Beaver *et al.,* 1964). The incubated eggs are gradually seeded into the water by rain and wave action (Beaver *et al.,* 1964). This maintains hatched coracidia in water, finally infection of the first intermediate host occurs.

5.6 Studies on Sedimentation of *Spirometra* Eggs

The process of sedimentation was carried out according to Soulsby (1982). *Spirometra* eggs were collected from the bottom of sedimentation flask. This indicates that *Spirometra* eggs settle down in water. It has been reported that in natural condition *Spirometra* eggs settle down in mud and sand in the ponds and rivers where they embryonate and hatch to coracidia which swim in water bodies using cilia (Beaver *et al.,*1964). The result in the present study agrees with the study of Beaver *et al*. (1964).

5.7 Studies on Effect of Temperature on Hatching of *Spirometra* Eggs

In this study, the optimum temperature ranging from 26 to 29ºC with light hatching of eggs occurred from 6 to 13 days post incubation. At temperature ranging from 27 to 30ºC in darkness hatching of eggs occurred from 7 to 16 days and there was no hatching of eggs when they were placed in a refrigerator at 4ºC. It has been reported that temperature

is an important factor for hatching of *Spirometra* eggs. Effect of temperature on hatching of *Spirometra* eggs has been studied by Li (1929) the eggs hatched from 6 to 9 days at 35ºC, Kobayashi (1931) the eggs hatched from 12 to 14 days at 28ºC and Lee *et al*. (1990) the eggs hatched from 8 to 14 days at 29ºC. Opuni (1974) recorded that hatching of eggs of *S .theileri* occurred on 21st days at temperature ranging from 22 to 25ºC in darkness. Therefore, the results of the present study and previous studies have shown that the optimum temperature for hatching of *Spirometra* eggs is between 22 and 35ºC and at low temperature of 4ºC no development of eggs and thus do not hatch.

5.8 Studies of the Viability of the Eggs

The viability of eggs collected at a point of time from naturally infected lions was established. Hatching ability of 1½ years old eggs observed under optimum temperature and light, revealed 90.3% of the eggs to be viable. The time of storage in the refrigerator at 4ºC did not reduce viability of eggs. The results in the present study agree with Stephanson (1985) who reported 98% of fresh *Spirometra* eggs hatched.

5.9 Studies of the Survival of Coracidia

The survival of the coracidia was observed under laboratory conditions of temperature and light. The coracidia continued to swim until their glycogen content was depleted ending in death. It was observed that coracidia survived for a maximum of 54 hours. Coracidia less than 24 hours old were more active and there was no death. Death of coarcidia was highest at 48 hours. The present study clearly shows the observations to be similar to the observations reported by Mueller (1974), Arme and Pappas (1983) that the maximum survival period of a free swimming coracidia is 48 hours irrespective of species.

5.10 Measurements of Coracidia

The measurements of coracidia hatched from *Spirometra* eggs collected from lion faeces were 30 µm and 20 µm respectively. The measurements of coracidia hatched from *Spirometra* eggs reported by Li (1919) was 54 µm and Lee *et al*. (1990) was 43.8 µm. The present study has smaller measurements than those obtained by the previous workers.

5.11 Studies on the Role of *Cyclops* as First Intermediate Host

The *Cyclops* are fresh water dwellers which are an important part of aquatic food chain, therefore, their role as vectors of disease cannot be overlooked. The faeces from infected animals contaminate water sources and the *Spirometra* eggs are released into the water system where they hatch into coracidia. Coracidia are free swimming, which in turn infect the free living *Cyclops*. Once the coracidium is ingested by *Cyclops*, it penetrates the intestinal wall into the abdominal cavity, where it develops into the procercoid. The number of procercoids in each *Cyclops* varies. In the present study it was observed 1-2 procercoids in a single *Cyclops*, the number had no impact on the mobility of the *Cyclops*. Lee *et al*. (1990) established the role of *Cyclops* as the first intermediate host for *Spirometra* infections. He also reported that *C. affinis, C. leuckarti, C.serrulatus* served as the first intermediate hosts of *Spirometra.* It was observed that not all *Cyclops* exposed to coracidia were infected, only 10.4% of the *Cyclops* carried procercoid. The number of procercoid in each *Cyclops* varied between one and two. In the present study, identification of the *Cyclops* was not done. *Spirometra* contrasts with *Diphyllobothrium latum* the plerocercoid which develops only in fish. The preferred copepod host of *Diphyllobothrium latum* is cold water *Diaptomus*, while that of *Spirometra* is the warm water *Cyclops* (Mueller, 1966). This suggests that *Cyclops* examined for natural infection had *Spirometra* procercoids and only 7.14% of the *Cyclops* carried procercoid.

5.12 Survey of Spargana in Natural Infection of Wild Animals, Rats and Birds As Second Intermediate Hosts

Wild animals and birds have been reported to be second intermediate hosts in the life cycle of *Spirometra* species. In the present study all animals, rats and birds caught and examined from around Tarangire National Park were free from spargana infection. Sachs and Sachs (1968) reported to have examined about 500 wild animals for parasitic infestations in the boundaries of the Serengeti National Park in Tanzania. Three forms of tapeworm larvae encountered in the wild herbivorous hosts examined are: cysticerci, *Echinococcus* cysts and spargana but no percentage of infection was given. Opuni (1974) reported to have recovered spargana from warthog from Serengeti National Park. Mastura *et al*. (1995) reported infection of spargana in the bird common myna (*Acridotheres tristis tristis*). The results of the present study can be explained that there is low infection rate in these animals also the number of animals and birds examined was not big enough due to restriction of the Tanzania National Parks (TANAPA). However, a survey of *Cyclops* in the same area was carried out to establish natural infection with procercoids. It was found 7.14% of *Cyclops* were infected with procercoids of S*pirometra*. This proves that the second intermediate host is present in this area because the *Cyclops* collected from water ponds in the National Park where the animals drink water. It is obvious that they drink water which is contaminated with infected *Cyclops*.

5.13 Studies of Dogs as Definitive Hosts

The adult worm of *Spirometra* is found inside the small intestine of dog, the micro-environment in the small intestine is important for survival of the adult worm where produce eggs which are discharged in faeces. In the present study, dogs at Minjingu village which borders with Tarangire National Park had their faeces screened for *Spirometra* eggs. Out of the number of dogs examined, 42.4% were infected with

Spirometra spp. One of the infected dogs was sacrificed, a single adult worm was recovered from the small intestine. The measurement of the adult worm without neck and scolex was 89.5 cm. The result in the present study agrees with the previous workers (Muller, 1938; Lee *et al*., 1990; Miyazaki, 1991) who reported that dogs are definitive hosts for *Spirometra* spp.

5.14 Studies of Adult Worm of *Spirometra*

Morphology of adult *Spirometra* recovered from an infected dog was studied. The identification was based on a single adult worm lacking neck and scolex. Uterus had 6 coils, centrally located in the proglottid. The proglottids were wider than long. Carmine was used to stain internal organs which showed clearly male genital pore, the other pores were not clearly seen. Testes were not united with vitellaria in the midline. The adult worm of *Spirometra* has been studied by previous workers Mueller (1938); Lee *et al*. (1990) and Miyazaki (1991) who reported that the measurements of adult *Spirometra* were length of 95 cm, proglottids with width of 1 cm and length of 9.5 mm(wider than long). Lee *et al*. (1990) and Miyazaki (1991) reported that the scolex of *Spirometra* had two bothria, neck was long, uterus coiled with 5 to 7 coils, and occupied a narrow field along the median line. The genital pore is located on the upper ventral side, the vaginal pore a short distance in front on the terminal uterine coil and uterine pore lies on the ventral side of the terminal coil some distance from male genital pore. Testes and vitellaria do not unite anteriorly in the midline. Mueller (1938) reported that the uterus of *S. mansonoides* differs from *S. erinacei* as it is coiled with **C**-shaped outer loop of the uterus and its anterior limb constricted in midline forming a lateral expulsion chamber. Stephanson (1985) reported that the two forms differed from each other for their final host range and morphology (measurements of the eggs, scolex, proglottids and overall size) but with the exception of the size of the scolex of *D. pretoriensis* all other

characteristic values fell within the range of that found for the Western Australian *Spirometra erinacei.* He concluded that both species are more similar to *S. erinacei* than they are to either of the type specimen of *D .pretoriensis* or *D. theileri*. Although the *Spirometra* spp.in Tanzania have been considered as belonging to the species "*theileri*", Dinnik (1969) suggested that other species of this genus might be involved. In the present study, the biological, morphological and molecular data of the species studied correspond with *Spirometra erinacei.*

5.15 Molecular Characterization of *Spirometra*

In the present study, PCR and DNA sequencing techniques were used to identify the species. Primers JB3/4.5, JB10/9 for sample and M13 primer for colony were used. Amplicons for both sample and colony provided similar results for amplification. To confirm the positive clones, PCR was conducted using M13 primer. This revealed clones harbored inserts of approximately 400bp similar to sample amplicons. Nucleotide sequencing was performed as a confirmatory method. Sequences from positive bands of sample and clones were identical. The sequences were compared with the sequences deposited in the GenBank using the BLAST programs and databases. Nucleotide sequence identity was 100% similarity to *S. erinaceieuropaei.* The sequence was grouped in the same cluster with AF004717 from Korea. *S. erinaceieuropaei* (Tanzania isolate) showed to be genetically related to *S. erinacei* (Korea isolate). Previous workers (Olson, *et al.,* 2001; Lockyer *et al.,* 2003) used PCR and DNA sequencing to identify parasites of similar morphological characteristics. The results of the present study agree with the previous workers.

In the present study, the methods used to construct phylogenetic tree were Maximum Parsimony (MP) and Neighbour-Joining (NJ). The methods have been used by Previous

workers (Olson *et al.,* 2001; Vilas *et al*., 2005; Guo-Hua *et al.,* 2012) used the same methods to construct phylogenetic tree. The results of the present study agree with the previous workers. In conclusion, the present study determined and characterized the complete mtDNA sequence of *S. erinaceieuropaei* from Tanzania. These mtDNA sequences should provide novel genetic data for addressing further questions in systematics and population genetics of this and other cestodes of soci-economic significance.

5.16 Determination of Seroprevalence of Sparganosis

Human sparganosis is a chronic infection of long duration (Cho and Seo, 1975). However, the exact period of infection can hardly be assessed in individual cases because of modes of daily life such as drinking unsafe water from streams and ponds give rise to the infection. Game meat eaters have histories of eating different animal meats, and it is difficult to determine which of the game meat has been the source of infection. In addition, most cases manifest no symptoms. Serological diagnosis of the infection can be done because the antigenic epitopes recognize the antibodies produced as early as two weeks post infection (Young *et al.,* 2000).

Diagnosis of human sparganosis largely depends on the identification of the worms recovered from excisional biopsy. Imaging diagnosis can be used when the larva invades the central nervous system (Chang *et al*., 1992; Moon *et al*., 1993), while ultrasound is used in the diagnosis of subcutaneous sparganosis (Chung *et al*. 1995). The antibody test is supplementary to confirm the diagnosis, also is a useful seroepidemiological survey tool (Kong *et al.,* 1994). Crude saline extract of plerocercoid (spargana) has been reported to be useful for serologic test of sparganosis (Kim *et al*., 1984).

In the present study, no spargana was recovered to be used in the serologic test for sparganosis also there was no serum of positive control group because no patient had been diagnosed before or during collection of sera in the study areas. Therefore, adult crude extract antigen was used in the present study for serological screening of individuals to sparganosis in two districts of Babati and Monduli in Northern Tanzania. Hyun *et al*. (1998) reported the use of crude extracts of adult *Spirometra erinacei* in serodiagnosis of sparganosis. The present study revealed 62.5% positive reaction to spargana antibodies in sera of inhabitants in the study area. In South Korea, serologic examination showed positive rates 1.6% to 3% (Kong *et al*., 1994; Park *et al*., 1994; Lee *et al.,* 2002; Lee *et al.,* 2003). The present study has shown that crude extract of *S. erinaceieuropaei* is suitable for use in the serodiagnosis of human sparganosis. This study confirm that sparganosis is highly prevalent in the two districts of Babati and Monduli.

The present study, shows that there is no specific age group for sparganosis infection. Previous study in Korea reported that there is no specific age group for infection of sparganosis (Lee *et al*., 2002). The results in the present study agree with previous study. This is the first time in Tanzania to report seroprevalence of human sparganosis.

This study was hospital based, samples were collected from participants who came for health services. Women are responsible for health care of the families by sending children to underfive clinics and treatment when they fall sick. The number of women with positive reaction to spargana antibodies was higher in Monduli District than women in Babati District. The data shows that more women participated in the study in Monduli district than in Babati District. Monduli district is inhabited with the Masai tribe, these

are pastoralists they share water sources with animals. If water sources contain infected *Cyclops* they easily get infected with procercoids.

In the present study, no physical examination for cutaneous mass or history taking for focal neurological manifestations and other symptoms associated with sparganum was carried out to the inhabitants involved in the study. It is not certain whether these positive cases had or may develop sparganosis symptoms in future. Therefore, it is recommended further studies to be carried out to invetstigate on the development of sparganosis among infected individuals.

5.17 Determination of Knowledge, Atittudes, Practices and Risk Factors of sparganosis

(a) Knowledge

The adult worm of *Spirometra* develops in the small intestines of dogs and cats and start discharging eggs in their faeces. The faeces can be washed with water and contaminate the source of water. Human is an accidental second intermediate host, who can get the infection through drinking unboiled or untreated water containing *Cyclops* infected with procercoids. In the present study, 345 (100%) of the respondents did not know the signs and symptoms, mode of transmission and prevention of sparganosis. Previous investigations have demonstrated that dogs and cats are definitive hosts in the life cycle of *Spirometra* spp. (Faust *et al.*, 1929; McIntosh, 1937; Mueller, 1938; Opuni, 1974; Lee *et al.,* 1990). The results of the present study, shows that there is lack of knowledge on sparganosis among the inhabitants of Babati and Monduli districts.

(b) Atittude

The attitude towards keeping dogs and cats was examined in this study. An ANOVA analysis (Two-ways without replication) indicate that there is a significant difference ($P<0.05$) among respondents on the knowledge of keeping dogs and cats. Dogs easily get infected with *Spirometra* because they are not confined in one place. They get prey from small animals and carcasses within the area which some of them may be infected with spargana. They contaminate the environment with eggs of *Spirometra* because they defecate in areas around household and on pastures as they are not regularly treated with antihelminthics. The best way of keeping pets is in a confined manner restricted in one place, fed well, dewormed regularly and vaccinated with antirabbies and against other diseases and proper disposal of their faeces. In the present study the respondents lacked appropriate awareness of the dangers associated with the dogs on transmission of sparganosis.

(c) Practices

Dogs are kept for guarding home and hunting, they need to be fed well and proper disposal of faeces. In the present study, the result shows that the practice of keeping dogs among the respondents is not in a confined manner, disposal of faeces is not proper and they are not dewormed with antihelminthics.

(d) Risk factors

The association of level of education and the risk factor of drinkig unsafe water was examined. Most of the respondents had attained education level of Standard VII. According to the policy of the country standard seven is universal education which every Tanzanian should reach so as to be able to read and write.

The water supply to the villages of the respondents is by pipes which are connected from the sources of water then distributed direct to the villages. There is no stage where water is treated. Villagers are aware of this, the water supplied to them is not treated at any stage but no emphasis has been put on improving access to clean water, adequate sanitation and changing hygiene behaviour, although these interventions are key factors for sustainable control. The water for drinking is not safe because is used without boiling or filtering with a clean cloth. The other water sources are not protected can easily be contaminated with faeces of wild animals which are definitive hosts of *Spirometra* and the *Cyclops* which are found in natural water bodies can be infected with coracidia. Therefore, users of this water can easily get infection through unsafe water. Jing *et al.* (2011) reported that 3.53% of *Cyclops* examined in Henan Province, China, were found infected with procercoids. The present study agree with previous worker that raw water containing *Cyclops* which has not been boiled or filtered poses a high risk for sparganosis. Participants in this study were from areas surrounding Tarangire National Park. It was revealed that illegal and legal hunters sell game meat, the meat sold is not inspected by any authorized meat inspector. Therefore, people in these areas are predisposed to high risk of diseases which can be transmitted through meat like Sparganosis, Brucellosis and Trichinellosis. In the present study it was found that the preferred methods of preparation of meat is boiling, followed by roasting and eating raw meat is the least preferred method. In China it has been reported many people acquire the infection mainly by eating raw or insufficiently cooked meat of frog and snake or by placing frog or snake flesh on open wounds for treatmrnt of skin ulcers or on eyes to treat (Qui and Qui, 2009; Li *et al.,* 2009). Jing *et al*. (2011) reported that 11.93% of tadpoles examined in Henan Province, China had infection with plerocercoids.

The result in the present study shows that people in this area of study are at risk of getting sparganosis infection from partially boiled or roasted meat infected with spargana. The present result agree with previous workers that sparganosis can be transmitted through eating partially cooked game meat or untreated water contaminated with infected *Cyclops*, eating partially cooked meat of frogs and snakes. Previous studies on parasitic worm infections support that both individual and community perceptions, attitudes, prevention and treatment are important factors (Uchoa *et al.,* 2000; Mwanga *et al.,* 2004). Therefore, health education and promotion campaigns are essential for any change in behaviour to be made.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Wild lions and domestic dogs are definitive hosts in the life cycle of Tanzanian *Spirometra* species. Eggs of *Spirometra* were recovered from faeces of naturally infected lions and dogs. Morphology and measurements of eggs were suggestive of *Spirometra* species.

The eggs developed after incubation at laboratory Temperature 26-29ºC in presence of light and 27-30ºC in darkness, hatched coracidia which were oval in shape, covered with cilia on the body surface which was used to swim and had hooks. Viability studies of the *Spirometra* eggs showed eggs to be viable. Survival studies of coracidia showed to be at most 54 hours of survival in water.

Cyclops collected from ponds in Tarangire National Park were examined for natural infection with procercoids. The *Cyclops* were found to be infected with procercoids. Experimental infection of *Cyclops* with coracidia was carried out in the laboratory. Coracidia developed to procercoid in the body cavity of *Cyclops*. This indicated that *Cyclops* in Tarangire National Park are used as first intermediate host of the *Spirometra* species in this study.

Determination of second intermediate host in which various animals mice, guinea pigs, rats, New Zealand rabbit, goat and pig were experimentally infected with *Cyclops* infected with procercoids. The plerocercoids were not recovered from these animals. But, this cannot ascertain whether plerocercoids developed or not.

Determination of plerocercoid from naturally infected wild animals as second intermediate host was carried out. A few animals, rats and birds from around Tarangire National Park were killed and searched for plerocercoids. All were free from plerocercoids.

Adult worm was recovered from small intestine of a naturally infected domestic dog from Minjingu village boardering Tarangire National Park. Mophological data and measurements of the whole worm were taken. To study the internal sexual organs some proglottids were stained with Carmine. The uterus showed to be centrally located, spiral with male genital opening. However, uterine and vaginal openings were not clearly seen. To determine the species of *Spirometra* which exist in Tanzania, molecular biology studies of the adult worm was carried out. PCR and sequencing revealed to be *Spirometra erinaceieuropaei*. The species of *Spirometra* in Tanzania is now characterized.

The reconstructed phylogenetic tree using 11 representative nucleotide sequences displayed close genetic relatedness to *Spirometra erinaceieuropaei*.

Seroprevalence for spargana antibodies among inhabitants in two districts of Babati and Monduli was determined. The results showed (62.5%) positive reactions with adult worm antigen. This shows that some inhabitants in the two districts of Babati and Monduli are infected with spargana.

Questionnaire survey was conducted among the inhabitants in two districts of Babati and Monduli. The aim was to determine the knowledge, attitudes, practices and risk factors related to sparganosis. The study showed that inhabitants in the two districts of Babati and Monduli have no knowledge on sparganosis, the mode of transmismission of the disease

is not known to them. They have no knowledge of risk of drinking unsafe water and eating of game meat which is not inspected by the authorized meat inspectors.

6.2 Recommendations

The health significance of *Spirometra* infection needs further investigation to establish relevance in health programmes in Tanzania. The Tanzanian rural life is very closely interwoven with agriculture. From this study, it was found that national parks are surrounded by humans who are farmers and pastoralists. There is a great interaction with animals from the parks as a result water sources are shared.

(i) There is a need for research to be carried out in order to establish the second intermediate host. Due to time and financial constraints this stage was abandoned after failing to establish the suitable second intermediate host in the field.

(ii) Further studies are needed to investigate the evolutionary characteristics of this worm.

REFERENCES

Adrian, A. R. and Frost, T. M. (1993). Omnivory in cyclopid copepods: comparisons of algae and invertebrates as food for three, differently sized species. *Journal Plankton Research* 15: 643 – 658.

Andrews, P., Thomas, H., Polke, R. and Seubert, J. (1983). Praziquantel. *Medicinal Research Review* 3(2): 147 – 200.

Ausayakhun, S., Siriprasert, V., Morakote, N. and Taweesap, K. (1993). A case report: Ocular sparganosis in Thailand. *Southeast Asian Journal of Tropical Medicine Public Health* 7(3): 683 – 697.

Arme, C. and Pappas, P. W. (1983). *Biology of the Eucestoda.* Academic Press, London. 628pp.

Ash, L. C. and Orihel, T. C. (1990). *Atlas of Human Parasitology*. ASCP Press, Chicago. 530pp.

Becker, B., Melhorn, H., Andrews, P. and Thomas, H. (1981). Ultrastructural investigation on the effect of praziquantel on the tegument of five species of cestodes. *Zeitschrift Fur Parasitenkunde* 64: 257 – 269.

Baer, J. G. (1925). Contributions to the helminth-fauna of South Africa. Mammalian Cestodes. Thesis for Award of PhD Degree at University of Pretoria, South Africa, 79pp.

Baer, J. G. and Fain, A. (1955). Exploration du Park National de I´Upemba. *Cestodes* 36: 1 – 38.

Beaver, P.C., Malek, E.A. and Little, M.D. (1964). Development of *Spirometra* and *Paragonimus* Eggs in Harada-Mori Cultures. *The Journal of Parasitology* 50(5): 664 – 666.

Berntzen, A. K. and Mueller, J. F. (1964). In vitro cultivation of *Spirometra mansonoides* (Cestoda) from the procercoid to early adult. *Journal of Parasitology* 50(6): 705 – 711.

Beveridge, I., Friend, S.C. and Jeganathan, N. (1998). *Proliferative sparganosis* in Australian dogs. *Australian Veterinary Journal* 76: 757 – 759.

Bowles, J. and McManus, D.P. (1994). Genetic characterization of the Asian Taenia, a newly described taeniid cestode of humans. *American Journal of Tropical Medicine and Hygiene* 50: 33 – 44.

Bowman, D. D. (1999). *Helminths, in Georgi´s Parasitology for Veterinarians*. (7th Ed.), WB Saunders Co., Philadelphia. 129pp.

Bowman, D. D. (2009). *Helminths, in Georgi's Parasitology for Veterinarians*. (9th Ed.), WB Saunders Co., Philadelphia. 137pp.

Bwangamoi, O. (1970). A checklist of helminnth parasites of animals in Tanzania. *Bulletin of Epizootic Diseases of Africa* 18: 229 – 242.

Buergelt, C. D., Greiner, E. C. and Senior, D. F. (1984). *Proliferative sparga*nosis in a cat. *Journal of Parasitology* 70: 121 – 125.

Burrows, R. B. and Lillis, W. G. (1966). Treatment of canine and feline tapeworm infections with Butamide Hydrochloride. *Annals Journal of Veterinary Research* 27: 1381 – 1384.

Campbell, E. and Beals, C. (1977). Striking eosinophilia in sparganosis. *Postgraduate Medicine* 62(6): 138 – 140.

Chai, D., Farah, I. and Muchmi, G. (1997). Sparganosis in non-human primates. *Onderstepoort Journal of Veterinary Research* 64: 243 – 244.

Chamadol, W., Tangdumrongkul, S., Thagphaisal, C., Sithithaworn, P. and Chamadol, N. (1992). Intracerebral haematoma caused by sparganum: *Journal of Medical Association Thai* 75(10): 602 – 605.

Chang, K. H., Cho, S.Y. and Chi, J. G. (1987). Cerebral sparganosis: CT characteristics. *Radiology* 165: 505 – 510.

Chang, K. H., Chi, J. G. and Cho, S. Y. (1992). Cerebral sparganosis: Analysis of 34 cases with emphasis on CT features. *Neuroradiology* 34: 1 – 8.

Cheesbrough, M. (1987). *Medical Laboratory Mannual for Tropical Countries*. English Language Books Society Co., Cambridge. 420pp.

Chen, C. W. and Chow, H. W. (1971). A case of sparganosis. *Transactions of Ophthalmology Society* 10: 123 – 127.

Cho, S. Y., Bae, J. H. and Seo, B. S. (1975). Some aspects of human sparganosis in Korea. *Korean Journal of Parasitology* 13: 60 – 77.

Cho, S. Y., Kim, S. I., Kang, S. Y., Choi, D. Y., Suk, J. S., Choi, K. S., Ha, Y. S. and Chung, C. S. (1986). Evaluation of enzyme-linked immunosorbent assay in the serologic diagnosis of human neurocysticercosis using paired samples of serum and cerebrospinal fluid. *Korean Journal of Parasitology* 24: 25 – 41.

Cho, K. J., Lee, H. S. and Chi, J. G. (1987). Intramural sparganosis manifested as intestinal obstruction: A case report. *Journal of Korean Medical Science* 2: 137 – 139.

Cho, Y. D., Huh, J. D., Hwang, Y. S. and Kim, H. K. (1992). Sparganosis in the spinal canal with partial block an uncommon infection. *Neuroradiology* 34: 241 – 244.

Choi, S. H., Kang, S. Y., Kong, Y. and Cho, S. Y. (1988). Antigenic protein fractions reacting with sera of sparganosis patients. *Korean Journal of Parasitology* 26(3): 163 – 167.

Chowdhury, A. G., Dasgupta, B. and Ray, H. N. (1955). Kernechrot or nuclear fast red in the histochemical detection of calacareous corpuscles in *Taenia saginata. Nature* 176: 701 – 702.

Chung, W. K., Wu, W. C., Kao, M. T., Chen, C. C. and Huang, Y. W. (1990). Sparganosis associated with chronic renal failure: *Journal of Nephrology* 4: 70 – 73.

Chung, K. H., Park, K. S., Lee, Y. and Park, C. K. (1995). Breast sparganosis: mammographic and ultrasound features. *Journal of Clinical Ultrasound* 23: 447 – 451.

Chung, Y. B., Kong, Y., Cho, S. Y. and Yang, H. T. (2003). Purification and localization of 10 kDa calacareous corpuscle binding protein of *Spirometra mansoni* plerocercoid. *Parasitology Research* 89: 235 – 237.

Corkum, K. C. (1966). Sparganosis in some vertebrates of Louisiana and observations on human infection. *Journal of Parasitology* 52: 444 – 448.

Dinnik, J. A. and Sachs, R. (1972). Taeniidae of lions in East Africa. *Zeitschrift für Tiermedizin und Parasitologic* 23: 197 – 210.

Engh, A. L., Nelson, K. G., Peebles, R., Hernandez, A. D., Hubbard, K. K. and Holekamp, K. E. (2003). Coprologic survey of parasites of spotted Hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve, Kenya. *Journal of Wildlife Diseases* 39(1): 224 – 227.

Eslami, A. and Bazargani, T. T. (1986). The efficacy of praziquantel against *Coenurus cerebralis* in naturally infected sheep. *Veterinary Medical Review* 21: 97 – 99.

Faust, E. C., Campbell, H. E. and Kellog, C. R. (1929). Morphological and biological studies on the species of *Diphyllobothrium* in China. *American Journal of Hygiene* 9: 560 – 583.

Fung, C. F., Ng, T. H. and Wong, W. T. (1989). Sparganosis of the spainal cord. *Journal of Parasitology* 78: 735 – 738.

Garcia, L. and Bruckner, D. A. (2007). *Diagnostic Medical Parasitology*. (Edited by Herndon, V. A.), ASM Press, London. 560pp.

Garin, Y. J., Frottier, J. and Lavergne-Slove, A. (1997). Cutaneous sparganosis in France: the second case described from Europe. *APMIS* 105(1): 14 – 16.

Georgi, J. R. (1987). Tapeworms. Veterinary Clinics of North America. *Small Animal Practice* 17: 1285 – 1305.

Gray, M. L., Rogers, F., Little, S., Puette, M., Ambrose, D. and Hoberg, E. P. (1999). Sparganosis in feral hogs (*Sus scrofa*) from Florida. *Journal of American Veterinary Medicine Association* 215: 204 – 208.

Grifin, M. P., Tompkins, K. J. and Ryan, M. T. (1996). Cutaneous sparganosis. *American Journal of Dermatopathology* 18(1): 70 – 72.

Groll, E. (1980). Praziquantel for cestode infections in man. *Acta* 37: 293 – 296.

Guo-Hua, L., Chun, L., Jia, Y. L., Dong, H. Z., Rong, C. X., Rui, Q. L., Feng, C. Z. and Xing, Q. Z. (2012). Characterization of the complete Mitochondrial Genome sequence of *Spirometra erinaceieuropaei* (Cestoda: Diphyllobothriidae) from China. *International Journal of Biological Science* 8: 640 – 649.

Ha, K. Y. and Oh, I. S. (2011). Case report: Lower extremity sparganosis in a bursar. *Clinical Orthopaedic Relat Research* 46(7): 2072 – 2074.

Hersberg, A. J., Boyd, P. R. and Gutierrez, Y. (1995). Sucutaneous dirofilariasis in Colliercounty, Florida, USA. *American Journal of Surgical Pathology* 19: 934 – 939.

Ho, Y. S., Tsai, M. D. and Wong, C. W. (1992). *Intraoperative Diagnosis of Cerebral Sparganosis: Challenge to Pathologist (Abstract).* Transactions of the Pathological Society, Taipei. 381pp.

Huang, C. T. and Kirk, R. (1962). Human sparganosis in Hong Kong. *Journal of Tropical Medicine and Hygiene* 65: 133 – 138.

Hughes, A. J. and Biggs, B. A. (2001). Parasitic worms of the central nervous system an Australian perspective. *Internal Medicine Journal* 32(11): 541 – 543.

Hyun, J. Y., Yoon, K., Soo, U. L. and Sun, H. (1998). Applicability of crude extracts of adult *Spirometra erinacei* for serodiagnosis of sparganosis. *Journal of Helminthology Society Washington* 65(1): 122 – 124.

Iwatani, K., Kubota, I., Hirotsu, Y., Wakimoto, J., Yoshioka, M. and Mori, T. (2006). Sparganum mansoni parasitic infection in the lung showing nodule. *Pathology International* 56: 674 – 677.

Jeong, S. C., Bae, J. C. and Hang, S. H. (1998). Cerebral sparganosis with intracerebral hemorrhage: A case report. *Neurology* 50: 503 – 506.

Jing, C., Xi, M. L., Hong, W. Z., Bian, L. X. and Zhong, Q. W. (2011). Sparganosis, Henan Province, Central China. *Emerging Infectious Disease* 17(1): 146 – 147.

Jirawattanasomkul, S. and Noppakun, N. (2000). Human sparganosis in Thailand: *Thai Journal of Dermatology* 16: 131 – 135.

John, D. T. and Petri, W. A. (2006). *Medical Parasitology*. (9th Edition), St. Louis, Saunders Elsevier, USA. 580pp.

Joyeux, C. and Houdemer, E. (1928). Recherches sur la fauna helminthologique de I´Indochine (Cestodes et Tre´matodes). *Annales de Parasitologie* 6: 27 – 58.

Kee, S. E., Seung, H. K. and Han, J. R. (1988). Efficacy of praziquantel (cesocide injection) in treatment of cestode infection in domestic and laboratory animals. *The Korean Journal of Parasitology* 26(2): 121 – 126.

Kim, H., Kim, S. I. and Cho, S. Y. (1984). Serological diagnosis of human sparganosis by means of micro ELISA. *Korean Journal of Parasitology* 22: 222 – 228.

Kim, C. H. and Yang, J. (1988). Immunological characterization of antigens from *cysticercus* and sparganum and their application to immunodiagnosisI. Immunocharacteristics of crude antigenic components from *Cysticercus cellulosae*. *Korean Journal of Parasitology* 26: 245 – 254.

Kim, C. Y., Cho, B. K. and Kim, I. O. (1997). Cerebral sparganosis in a child. *Paediatric Neurosurgery* 26: 103 – 106.

Kim, J. and Lee, J. (2001). A case of intramuscular sparganosis in the sartorius muscle. *Journal of Korean Medical Science* 16: 378 – 80.

Kim, S. H., Park, K. and Lee, E. S. (2001). Three cases of cutaneous sparganosis. *International Journal of Dermatology* 40: 656 – 658.

Kiremerwa, D.N., Byaruhanga, D.B. and Raper, A. B. (1956). Sparganosis with report of two cases. *East African Medical Journal* 33(20): 37 – 42.

Kirkpatrick, C. E. (1983). *Spirometra* sp in a domestic cat in Pennsylvania. *Journal American Veterinary Medicine Association* 183: 111 – 112.

Kittiponghansa, S., Tesana, S. and Ritch, R. (1988). Ocular sparganosis: A case of subconjuctiva tumor and deafness. *Tropical Medical Parasitology* 39: 247 – 248.

Kobayashi, E. (1931). Studies on the development of *Diphyllobothrium mansoni* Cobbold, 1882 (Joyeux, 1927) V. The first intermediate host. *Taiwan Igakkai Zasshi* 30: 286 – 311.

Kong, Y., Cho, S. Y. and Kang, W. S. (1994). Sparganam infection in normal adult population and epileptic patients in Korea: A seroepeidemiologic observation. *Korean Journal of Parasitology* 32: 85 – 92.

Kudesia, S., Indira, D., Sarala, D., Vani, S., Yasha, T., Jayakumar, P.and Shankar, S. (1988). Sparganosis of brain and spinal cord: unusual tapeworm infestation (report of two cases). *Clinical Neurology Neurosurgery* 100: 148 – 152.

Kuhlow, F. (1953). Über die Entwicklung und Anatomic von *Diphyllobothrium dendrticum* Nitzsch 1824. *Zeitschrift für Parasitenkunde* 16: 1 – 35.

Kuntz, R., Myers, B. and Katzberg, A. (1970). Sparganosis and ‘‘proliferative’’ spargana in vervets (*Cercopithecus aethiops*) and baboons (Papio sp.) from East Africa. *Journal of Parasitology* 56: 196 – 197.

Kwa, B. H. (1972a). Studies on the sparganum of *Spirometra erinacei*-I. The histology and histochemistry of the scolex. *International Journal of Parasitology* 2: 23 – 28.

Kwa, B. H. (1972b). Studies on the sparganum of Spirometra erinacei-II. Proteolytic enzymes in the scolex. *International Journal of Parasitology* 2: 29 – 33.

Lee, S. Y., We, J. S., Bae, J.and Seo, B. S. (1975). Some aspects of human sparganosis in Korea. *Korean Journal of Parasitology* 13: 60 – 77.

Lee, H. B., Lee, K. W. and Lee, S. B. (1987). Clinical observation on cerebral sparganosis. *Journal of Korean Neurology Association* 5: 64 – 69.

Lee, S. H., We, J. S. and Sohn, W. M. (1990). Experimental life history of *Sporometra erinacei. Korean Journal of Parasitology* 28: 161 – 173.

Lee, K. J. and Ahn, Y. K. (1996). The status of sparganosis in the western areas of Kangwon do, and experimental identification of the adult worm. *Journal Wonju College of Medicine* 9: 139 – 148.

Lee, S. U., Huh, S. and Phares, C. K. (1997). Genetic comparison between *Spirometra erinacei* and *S. mansonoides* using PCR-RFLP analysis. *Korean Journal of Parasitology* 35(4): 277 – 282.

Lee, K. J., Bae, Y. T., Kim, D. H., Deung, Y. K. and Ryang, Y. S. (2002). A seroepidemiologic survey of human sparganosis in Gangweon-do. *Korean Journal of Parasitol*ogy 40: 177 – 180.

Lee, S. H., Kim, M. N., Back, B. Y., Chai, J. Y, Kim, T. H. and Hwang, Y. S. (2003). Analysis of parasite-specific antibody positive patients for *Clonirchis sinensis*, *Paragonimus wetermani*, cysticercosis and sparganum using ELISA. *Korean Journal of Laboratory Medicine* 23: 126 – 131.

Lee, S. U., Chun, H. C. and Huh, S. (2007). Molecular phylogeny of parasitic Platyhelminthes based on sequences of partial 28S DNA DI and mitochondrial cytochrome C oxidase subunit 1. *Korean Journal of Parasitology* 45(3): 181 – 189.

Lewis, B. G., Luff, S. and Whitehouse, W. (1971). Laboratory culture of *Cyclops abyssorum* Sars, 1863 (Copepoda, Cyclopoida). *Crustaceana* 24: 176 – 182.

Li, C. H. (1929). The life history of *Diphyllobothrium decipiens* and *D.erinacei*. *American Journal of Hygiene* 10: 527 – 555.

Li, M. W., Lin, H. Y., Xie, W. T., Gao, M. J., Huang, Z. W. and Wu, J. P. (2009). Enzootic sparganosis in Guuangdong, People‘s Republic of China. *Emerging Infectious Diseases* 15: 1317 – 1318.

Li, M. W., Song, H. Q., Li, H. Y., Xie, W. T., Lin, R. Q. and Zhu, X. Q. (2011). Review sparganosis in mainland China. *International Journal of Infectious Disease* 15(3): 154 – 156.

Liao, S. W., Lee, T. S., Shih, T. P., Ho, W. L. and Chen, E. R. (1984). Proliferating sparganosis in lumbar spine- a case report. *Journal of the Formosan Medical Association* 83: 603 – 611.

Lin, T. P., Su, I. J., Lu, S. C.and Yang, S. P. (1978). Pulmonary proliferating sparganosis A case report. *Journal of the Formosan Medical Association* 77: 467 – 472.

Little, S. and Ambrose, D. (2000). *Spirometra* infections in cats and dogs. *Compendium on Continuing Education for the Practicing Veterinarian* 22: 299 – 305.

Liu, W., Liu, G. H., Li, F., He, D. S., Wang, T., Sheng, X. F., Zeng, D. L, Yang, F. F.and Liu, Y. (2011). Sequence variability in three mitochondrial DNA regions of *Spirometra erinaceieuropaei* spargana of human and animal health significance. *Journal of Helminthology* 20: 1 – 5.

Lo, Y. K., Chao, D. and Yan, S. H. (1987). Spinal cord proliferative sparganosis in Taiwan: A case report. *Neurosurgery* 21: 235 – 238.

Lockyer, A. E., Olson, P.D. and Littlewood, D. T. (2003). Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodamata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal Linn Society* 78: 155 – 171.

Macdonald, B. (1998). *Disposition of Game Porcine Carcases Infested With Spargana, In AQIS Notice Meat.* Working Paper No. 29. Australian Quarantine Inspection Service, Canberra, Australia. 210pp.

Manson, P. (1882). Case of lymph scrotum associated with filariae and other parasites. *Lancet* 1: 289 – 290.

Manson, P., Manson-Bahr, P. and Wilcocks, C. (1921). *Manson's Tropical Disease*. William Wood and Company, New York. 564pp.

Mariana, A., Ho, T. M. and Tan, S. (1998). Life-cycle, longevity and fecundity of *Suidasia pontifica* (Acari: Saproglyphidae) in a tropical laboratory. *International Medical Research Journal* 2(2): 75 – 80.

Mastura, A. B., Ambu, S., Chandra, S., Kiew, B. H. and Rosli, R. (1995). A preliminary survey of frogs for *Spirometra* sp. infection a food borne human parasite. *Tropical Biomedicine* 14: 81 – 84.

McIntosh, A. (1937). New host records for Diphyllobothrium mansonoides. *Journal Parasitology* 70: 121 – 125.

Min, D.Y. (1990). Cestode infections in Korea. *Korean Journal of Parasitology* 28: 123 - 144.

Mitchell, A., Scheithauer, B.W. and Kelly, P.J. (1990). Cerebral sparganosis a case report. *Journal of Neurosurgery* 73: 147 – 150.

Miyazaki, I. (1991). *Helminthic Zoonoses*. Working Paper No. 62. International Medical Foundation, Tokyo, Japan. 489pp.

Moon, W. K., Chang, K. H. and Cho, S. Y. (1993). Cerebral sparganosis: MR imaging versus CT features. *Radiology* 188: 751 – 757.

Moreira, M. A., de Freitas, J. R. and Gerais, B. B. (1997). Granulomatous mastaitis caused by sparganum. A case report. *Acta Cytology* 41: 859 – 862.

Morton, H. (1969). Sparganosis in African green monkeys (Cercopithecus aethiops). *Laboratory Animal Care* 19: 253 – 255.

Moulinier, R., Martinez, E. and Torres, J. (1982). Human proliferative sparganosis in Venezuela: Report of a case. *American Journal of Tropical Medicine and Hygiene* 31: 358 – 363.

Mueller, J. F. (1935). The laboratory propagation of *Spirometra mansonoides* as an experimental tool, II. Culture and infection of the copepod host, and harvesting the procercoid. *Transactions American Microscope Society* 78: 245 – 255.

Mueller, J. F. (1937). A repartition of the genus *Diphyllobothrium*. *Journal of Parasitology* 23: 308 – 310.

Mueller, J. F. (1937). The hosts of *Diphyllobothrium mansonoides* (Cestoda: Diphyllobothriidae) Washington. *Helminthology Society* 4: 68 – 69.

Mueller, J. F. (1938). The life history of Diphyllobothrium mansonoides Mueller, 1935, and some considerations with regards to sparganosis in the United States. *American Journal of Tropical Medicine* 18: 41 – 66.

Mueller, J. F. (1938). Studies on *Sparganum mansonoides* and *Sparganum proliferum*. *American Journal of Tropical Medicine* 18: 303 – 324.

Mueller, J. F. (1959). The laboratory propagation of *Spirometra mansonoides* as an experimental tool. I. Collecting, incubation and hatching of eggs. *Journal of Parasitology* 45(4): 353 – 360.

Mueller, J. F. (1960). *The Immunologic Basis of Host Specificity in The Sparganum Larva of Spirometra Mansonoides.* In Libro Homenaje al Doctor Eduardo Caballero, Caballero, Mexico. 142pp.

Mueller, J. F. (1961). The laboratory propagation of *Spirometra mansonoides* as an experimental tool, IV. Experimental inversion of the primary axis in the developing egg. *Experimental Parasitology* 11: 311 – 318.

Mueller, J. F. (1974). The biology of *Spirometra. Journal of Parasitology* 60(1): 3 – 14.

Müller-Graf, C. D. M. (1994). Ecological parasitism of baboons and lions. Thesis for Award of PhD Degree at University of Oxford, London, UK, 180pp.

Müller-Graf, C. D. M. (1995). A coprological survey of intestinal parasites of wild lions (*Panthera leo*) in the Serengeti and Ngorongoro Crater, Tanzania, East Africa. *Journal of Parasitology* 81: 812 – 814.

Müller-Graf, C. D. M., Woolhouse, M. E. J. and Parker, C. (1999). Epidemiology of an intestinal parasite (*Spirometra* spp.) in two populations of African lions (*Panthera leo*). *Parasitology* 118: 407 – 415.

Mwanga, J. Q., Magnussen, P., Mugashe, C. L, Gabone, R. M. and Aagaard-Hansen, J. (2004). Schistosomiasis related perceptions, attitudes and treatment seeking practices in Magu District, Tanzania: Public Health implications. *Journal of Biosocial Science* 36: 63 – 81.

Myers, B. J. and Kuntz, R. E. (1967). Parasites of baboons taken by the Cambridge Mwanza expedition (Tanzania 1965). *East African Medical Journal* 44: 322 – 324.

Nakamura, T., Hara, M. and Matsuoka, M. (1990). Human proliferative sparganosis: A new Japanese case. *American Journal of Clinical Pathology* 94: 224 – 228.

Neiland, K. A. (1952). A new species of Proteocephalus eiland, 1858, (Cestoda), with notes on its life history. *Journal of Parasitology* 38: 540 – 542.

Nelson, G. S., Pester, F. R. N. and Rickman, R. (1965). The significance of wild animals in the transmission of cestodes of medical importance in Kenya. *Transactions of Royal Society of Tropical Medicine and Hygiene* 59: 507 – 524.

Nie, P. and Kennedy, C. R. (1993). Infection dynamics of larval *Bothriocephalus claviceps* in *Cyclops vicinus*. *Parasitology* 106: 503 – 509.

Nobrega-Lee, M., Hubbard, G., LoVerde, P., Carvalho-Queiroz, C., Conn, D. B., Rohde, K., Dick, Jr, E.J., Nathanielsz, P., Martin, D., Siler-Khodr, T. and Schlabritz-Loutsevitch, N. (2006). Sparganosis in wild-caught baboons (*Papio cynocephalus anubis*). *Journal of Medical Primatology* 36: 47 – 54.

Noya, O., Alarcon, De Noya, B., Arrechedera, H., Torres, J. and Arguello, C. (1992). *Spargana proliferum*: an overview of its structure and ultrastructure. *International Journal of Parasitology* 22: 631 – 640.

Odening, K. and Bockhadt, I. (1980). Discovery of the tapeworm *Spirometra mansonoides* in Europe. *Proceedings of the 3rd European Multicolloquium of Parasitology*, Cambridge, UK. pp. 7 – 13.

Oh, S. I., Chi, J. G. and Lee, S. E. (1993). Eosinophilic cystitis caused by vesical sparganosis: A case report. *Journal of Urology* 149: 583 – 583.

Odening, K. and Bockhardt, I. (1980). Discovery of the tapeworm *Spirometra mansonoides* in Europe. *Proceedings of the 3rd European Multicolloquium of Parasitology*, Cambridge UK. 7 - 13 September 1980. 123pp.

Okumura, T. (1919). An experimental study of the life-history of sparganum mansoni Cobbold. *Kitasato Experimental Medicine* 191 – 197.

Okino, T. (1996). Surface ultrastructure in developmental stages of Spirometra erinaceieuropaei (Rudolphi, 1819) Mueller, 1937 (Cestoda, Pseudophyllidea). *Japanese Journal of Parasitology* 45: 112 – 122.

Olson, P. D., Littlewood, D. T., Bay, R. A. and Mariaux, J. (2001). Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). *Molecular Phylogenetic Evolution* 19: 443 – 467.

Oyamada, T., Ohta, Y., Noguti, S., Kudo, N. and Yoshikawa, T. (1996). Assessment of three species copepods as the first intermediate host to *Gnathostoma nipponicum,* in Aomori Prefecture, Japan. *Japanese Journal of Parasitology* 45: 234 – 237.

Opuni, E.K. (1973).Laboratory studies on *Spirometra theileri* (Baer, 1925) n.comb. (Cestoda, Pseudophyllidea) from East Africa. PhD Thesis, London School of Hygiene and Tropical Medicine, UK, 187pp.

Opuni, E. K. (1974). Studies on *Spirometra theileri* (Baer, 1925) n.comb. I. Identification and biology in the laboratory. *Journal of Helminthology* 48: 15 – 23.

Pampiglione, S., Fioravanti, M. and Rivasi, F. (2003). Human sparganosis in Italy. A case report and review of the European cases. *APMIS* 111(2): 349 – 354.

Park, K. S., Lee, Y. and Chung, S. Y. (1993). Soft tissue sparganosis. *Journal of Korea Radiology Society* 29: 1288 – 1294.

Park, H. Y., Lee, S. U., Kim, S. H., Lee, P. C., Huh, S., Yang, S. Y. and Kong, Y. (1994). Epidemiological significance of sero-positive inhabitants against sparganum in Kangwon do, Korea. *Yonsei Medical Journal* 42: 371 – 374.

Pasternak, A. F., Huntingford, F. A. and Crompton, D. W. T. (1995). Changes in metabolism and behaviour of the fresfwater copepod *Cyclops strenuous* abyssorum infected with *Diphyllobothrium* spp. *Parasitology* 110: 395 – 399.

Phunmanee, A., Boonsawat, W., Indharapoka, B., Tuntisirin, C. and Kularbkeaw, A. (2001). Pulmonary sparganosis: A case report with five years follow-up. *Journal of Medical Association Thai* 84: 130 – 135.

Poole, J. B., Dooley, K. L. and Rollins, L. D. (1971). Efficacy of niclosamide for the removal of tapeworms (*Dipylidium caninum* and *Taenia pisiformis*) from dogs. *Journal American Veterinary Association* 159: 78 – 80.

Poulin, R., Curtis, M. A. and Rauk, M. E. (1992). Effects of *Eubothrium salvelini (Cestoda)* on the behaviour of *Cyclops vernalis* (Copepoda) and its susceptibility to fish predators. *Parasitology* 105: 265 – 271.

Qiu, M. H. and Qiu, M. D. (2009). Human plerocercoidosis and sparganosis. A historical review on pathology, clinics, epidemiology and control in Chinese. *Chinese Journal of Pathology and parasitic Diseases* 27: 251 – 260.

Rees, G. (1941). The musculature and nervous system of the plerocercoid larva Dibothriorhynchus grossum. *Parasitology* 33: 373 – 389.

Rim, H. J., Lyu, K. S., Lee, J. S. and Joo, K. H. (1981). Clinical evaluation of the efficacy of praziquantel (Embay 8440) against *Clonorchis sinensis* infection in man. *Annal Tropical Medical Parasitology* 75(1): 27 – 33.

Riviere, F., Kay, B. H., Klein, J. M. and Secham, Y. (1987). *Mesocyclops aspericomis* (Copepoda) and *Bacillus thuringiensis var Israelensis* for the biological control of *Aedes* and *Culex* vectors (Diptera: Culicidae) breeding in crab holes, tree holes and artificial containers. *Journal of Medical entomology* 24: 425 – 430.

Rodgers, W. A. (1974). Weights, measurements and parasitic infestation of six lions from Southern Tanzania. *East African Wildlife Journal* 12: 157 – 158.

Round, M. (1968*). Checklist of helminth parasites of African mammals of the orders Carnivora, Tubulidentata, Proboscides, Hyracoidea, Artiodactyla and Perssodactyla.* Commonwealth Agricultural Bureau International, Wallingford, London. 38pp.

Rudolphi, C. A. (1819). *Entozoorum Synopsis Cui Accidunt Mantissa Duplex Et Indeces Locupletissimi*. Berolini. 811pp.

Sachs, R. and Sachs, C. (1968). A survey of parasitic infestation of wild herbivores in the Serengeti region in northern Tanzania and the Lake Rukwa region in Southern Tanzania. *Bulletin of Epizootic Diseases of Africa* 16: 455 – 472.

Sakamoto, T. (1977). The antihelmintic effect of Droncit on adult tapeworms of *Hydatgena taeniaeformis*, *Mesocestoides corti*, *Echinococcus multilocularis*, *Diphyllobothrium erinacei* and *D. latum*. *Veterinary Medical Review* 1: 64 – 74.

Sakamoto, T., Gutierrez, C., Rodriguez, A. and Sauto, S. (2003). Testicular sparganosis in a child from Uruguay. *Acta Tropical* 88: 83 – 86.

Schar, F., Inpankaev, T., Traub, R. J., Khieu, V., Dalsgaard, A., Chimnoi, W., Chhoun, C., Sok, D., Marti, H., Muth, S. and Odermatt, P. (2014). The prevalence and Diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. *Parasitology International* 63(4): 597 – 603.

Schmid, H. and Watchinger, H. (1972). Sparganosis in the Masailand. *Acta Tropica* 29: 218 – 230.

Schmidt, G. D. (1974). The taxonomic status of *Spirometra* Faust, Campbell et Kellog, 1929 (Cestoideaiidae) (1974). *Journal of Helminthology* 48: 175 – 177.

Sen, D. K., Muller, R. and Gupta, V. P. (1989). Cestode larva (spargana) in the anterior chamber of the eye. *Tropical Medicine* 41: 270 – 273.

Sharp, G. J. E., Secombes, C. J. and Pike, A. W. (1990). The laboratory maintenance of *Diphyllobothrium dendriticum*. *Parasitology* 101: 153 – 161.

Shmidl, J. A., Cox, D. D. and McCurdy, H. D. (1981). Summary of safety evaluations for praziquantel in dogs. *Veterinary Medicine Small Animal Clinic* 76: 692 – 697.

Shmidl, J. A., McCurdy, H. D. and Mozier, J. O. (1982). Summary of safety evaluations for praziquantel in dogs. *Veterinary Medicine Small Animal Clinic* 76: 692 – 697.

Sim, S., You, J., Lee I., Im, K. and Yong, T. (2002). A case of breast sparganosis. *Korean Journal Parasitology* 40: 187 – 189.

Smyth, J. D. (1957). Studies on tapeworm physiology IX. A histochemical study of egg shell formation in *Schistocephalus solidus* (Pseudophyllidea). *Experimental Parasitology* 5: 519 – 540.

Smyth, J. D. and Clegg, J. A. (1959). Egg-shell formation in trematodes and Cestodes. *Experimental Parasitology* 8: 286 – 323.

Smyth, J. D. (1959). Maturation of larval pseudophyllidean cestodes and strigeid trematodes under axenic conditions; the significance of nutritional levels in platyhelminth development. *Annal New York Academy of Science* 77: 102 – 125.

Smyth, J. D. (1962). *Introduction to Animal Parasitology*. The English University Press, London. 470pp.

Smyth, J. D. and McManus, D. P. (1989). *The Physiology and Biochemistry of Cestodes.* Cambridge University Press, Cambridge. 470pp.

Sohn, W. M., Hong, S. T. and Chai, J. Y. (1993). Infectivity of the sparganum treated by praziquantel, gamma-irradiation and mechanical cutting. *Korean Journal of Parasitology* 31: 135 – 139.

Song, T., Wang, W. S. and Mai, W. W. (2007). CT and MRI characteristics of Cerebral sparganosis. *American Journal of Neuro Radiology* 12: 1700 – 1705.

Soulsby, E.J.L. (1982). *Helminths, Arthropodes and Protozoa of Domestic Animals*. 7th Edn. The English Language Book Society and Bailliere Tindall, London. 420pp.

Sparks, A. K., Neafie, R. C. and Connor, D. H. (1976). Pathology of tropical and extraordinary diseases. *Sparganosis* 2: 534 – 538.

Stephanson, J. M. (1985). Biology and immunology of *Spirometra* in Western Australia. Thesis for Award of PhD Degree at Murdoch University, Australia, 215pp.

Stunkard, H. W. (1965). Variation and criteria for generic and specific determination of diphyllobothriid cestodes. *Journal of Helminthology* 39(3): 281 – 296.

Sun, T. (1999). *Parasitic Disorders: pathology, Diagnosis, and Management.* (2nd Ed.), Williams and Wilkin, Pennysylvania. 398pp.

Sundaram, C., Prasad, V. S. and Reddy, J. J. (2003). Cerebral sparganosis. *Journal Association of Physicians India* 51: 1107 – 1109.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology Evolution* 28: 2731 – 2739.

Tanaka, S., Maruyama, H., Ishiwata, K. and Nawa, Y. (1997). A case report of pleural sparganosis. *Parasitology International* 46: 73 – 75.

Tansurat, P. (1966). Human sparganosis in Thailand. *Journal of Medical Association Thai* 49: 391 – 395.

Tashiro, K. (1924). Clinical, pathologic-anatomical and experimental studies on *Plerocercoid proliferum* Ijima (1905) Sparganum proliferum Stiles (1906) Kyushu University. *Faculty of Medicine* 9: 1 – 42.

Tesjaroen, S. (1991). Sparganosis in Thais. *Siriraj General Hospital* 43(10): 743 – 749.

Torres, J. R., Noya, O. O. and Noya, B. A. (1981). Treatment of Proliferative sparganosis with mebendazole and praziquantel. *Transactions Royal Society of Tropical Medicine and Hygiene* 75: 846 – 847.

Threadgold, L.T. (1984). Parasitic platyhelminths. In: *Biology of the Integument.* (Editors by Bereiter-Hahn, J. A., Maltoltsy, G. and Richards, K. S.), Springer-Verlag, Berlin. pp. 132 – 191.

Threadgold, L. T. and Robinson, A. (1984). Amplification of the cestode surface: A sterological analysis. *Parasitology* 89: 523 – 535.

Tsai, M. D., Chang, C. N. and Ho, S. (1993). Cerebral sparganosis diagnosed and treated with sterotactic techniques: Report of two cases. *Journal of Neurosurgery* 78: 129 – 132.

Tsou, M. H. and Huang, T. W. (1993). Pathology of subcutaneous sparganosis: report of two cases. *Journal of the Formosan Medical Association* 92: 649 – 653.

Uchida, A., Fukase, T. and Itagaki, H.(1980). Biology of pseudophyllidean tapeworm *Diphyllobothrium erinacei* (I) oviposition in a cat infected with a single tapeworm. *Japanese Journal of Parasitology* 29: 108 – 102.

Uchoa, E., Barreto, S. M., Firmo, J. O., Guerre, H. L. and Pimenta, F. G. (2000). The control of Schistosomiasis in Brazil an ethnoepidemiological study of the effectiveness of a community mobilization program for health education. *Social Science Medicine* 51: 1529 – 1541.

Ugarte, C. E., Thomas, D. G., Gasser, R. B., Hu, M. and Scott, I. (2005). *Spirometra erinacei*/*S.erinaceieuropaei* in feral cat in Manawatu with chronic intermittent diarrhoea, *New Zealand Veterinary Journal* 53(5): 347 – 351.

Vilas, R., Criscione, C. D. and Blouin, M. S. (2005). A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Pathology* 131: 839 – 846.

Von Brand, T., Meracado, T. I., Nylen, M. U. and Scott, D. B. (1960). Observation on function, composition and structure of cestode calcareous corpuscles. *Experimental Parasitological* 9: 205 – 214.

Walker, M. D. and Zunt, Z.R. (2005). Neuroparasitic Infections: Cestodes, Trematodes and Protozoans. *Seminars in Neurology* 25(3): 262 – 277.

Wardle, R. A. and McLeod, J. A. (1952). *The Zoology of Tapeworms*. University of Minneapolis Press, Minneapolis. 780pp.

Wiwanitkit, V. (2005). A review of human sparganosis in Thailand. *International Journal of Infectious Diseases* 9(6): 312 – 316.

Yamaguti, S. (1959). *Systema Helmintum*. The Cestodes of Vertebrates. Interscience, New York. 860pp.

Yamashita, K., Akimura, T. and Kawano, K. (1990). Cerebral sparganosis mansoni: report of two cases. *Journal of Surgical Neurology* 33: 28 – 34.

Yang, H. J. (2000). Separation of calcareous corpuscles from plerocercoids of *Spirometra mansoni* and their binding proteins. *Parasitology Research* 86: 781 – 782.

Yang, J. W., Lee, J. H. and Kang, M. S. (2007). A case of Ocular Sparganosis. *Korean Journal of Ophthalmology* 21(1): 48 – 50.

Yokogawa, M., Yoshimura, H., Okura, T. and Sato, M. (1962). Effect of bithionol on a *Taenia saginata,* especially with reference to the detection of eggs with faecal examination and the perianal cellophane tape method. *Journal Chiba Medical Society* 37: 451 – 455.

Yong, T. S., Seo, J. H. and Yeo, I. S. (1993). Serodiagnosis of human paragonimiasis by ELISA-inhibition test using monoclonal antibodies. *Korean Journal of Parasitology* 3: 141 – 147.

Yoon, K., Seo, M., Park, S. and Park, Y. (2004). Eyelid sparganosis. *American Journal of Ophthalmology* 138: 873 – 875.

Young, E. (1975). Some important parasitic and other diseases of lion, *Panthera leo*, in the Kruger National Park. *Journal of the South African Veterinary Association* 46: 181 – 183.

Young, B. C., Yoon, K., Hyun, J. Y. and Seung, Y. C. (2000). IgG antibody responses in early experimental sparganosis and IgG subclass responses in human sparganosis. *Korean Journal of Parasitology* 38(3): 145 – 150.

Zajac, A. M. and Conboy, G. A. (2012). *Veterinary Clinical Parasitology*. (8th Ed.), Wiley Blackwell, Ames, Iowa. 368pp.

Zhong, H. L., Shao, L. and Lian, D. R. (1983). Ocular sparganosis caused blindness. *Chinese Medical Journal* 96: 73 – 75.

Zhou, P., Chen, N., Zhang, R. L., Lin, R. Q. and Zhu, X. Q. (2008). Foodborne parasitic zoonoses in China: perspective for control. *Trends Parasitology* 24: 190 – 196.

APPENDICES

Appendix 1: Informed Consent Form

Principal investigator: Dr. Nicholas J. Kavana

Name of the organization: Sokoine University of Agriculture, Morogoro, Tanzania

Research Title: Experimental life cycle and molecular studies of *Spirometra* species in Tanzania.

Purpose of the study

Spirometra species are parasites of the canidae and felidae. Its larvae, the plerocercoid (sparganum) causes sparganosis in human. The parasite is worldwide distributed.

In Tanzania no work has attempted to establish seroprevalence of sparganosis in human. The present study is therefore designed to develop a tool for serological diagnosis of human sparganosis.

Procedures (Methodology)

This study will be conducted in Tarangire, Manyara region. Permission to do the research on human blood will be requested from Regional and District Administrative Officers. A preliminary survey of the study has been conducted on lion (final host) faeces of Tarangire National Park, Manyara to isolate *Spirometra* eggs. Information on seroprevalence of sparganosis on human will be recorded during the study.

Benefits from the study

This study on its completion will provide useful data including the extent of the problem of sparganosis on human and a diagnostic tool will be developed.

Confidentiality

The present study will be conducted with high degree of confidentiality. Participant´s information including names and test results will be kept with secrecy.

Incentives to research participants

Free of charge consultation will be provided to the study participants at all levels of the study.

Participation rights

Participants will be involved in the study following their own will to participate. There will be no barriers for the participants to withdraw from the research in case they want to do so.

Contact person

Principal investigator: Dr. Nicholas J. Kavana

Mobile phone number: 0786139078

E-mail address: nkavana@yahoo.com

Certificate of consent

I have read the above information/the above information has been read to me and I have understood it. I have had the opportunity to ask question/questions about the research. The question/questions has/have been answered to my satisfaction. I consent voluntarily that I will participate in this study.

Name_____

Date and signature _____

_____

If illiterate

Name of independent literate witness: _____ Date and signature of witness

_____

Name of researcher _____

Dr.Nicholas J. Kavana

Appendix 2: Questionnaire Survey

A seroprevalence survey for human sparganosis in villages surrounding Tarangire National Park, Tanzania

Questionnaire for cross-sectional survey: Patients attending health facilities

A General information

i.District ——————— Ward ——————— Village ———————

ii.Date of interview ——————— Name of enumerator ———————

B Participant characteristic

i .Name of participant ——————————Gender 1=Male, 2=Female ———

ii. Age of participant ————(yrs) Tribe ——————————————

iii. Marital status: 1=Married, 2=Single, 3=Widowed, 4=Divorced.

iv. Education level of the participant.

1=No formal education, 2=Adult education, 3=Primary: standard 1-4,

4=Primary:standard 5-7, 5= Secondary: O-level, 6=Seconadry, A-level,

7=College/University, 8=Others (specify) ——————————

C General information regarding economical activities

i. What is your main economical activities

S/N	**Type of economical activity**	**Indicate (tick)**	**Order of importance (rank)**
1	Crop farming		
2	Livestock keeping		
3	Fishing		
4	Salary employment		
5	Business		
6	Charcoal making		
7	Others (specify)		

ii. What are the main types of crops do you grow?.

Type of crop →

Order of importance

(rank)

D General information regarding pets keeping

i. Do you keep pet animals (dog/cat)?. 1=Yes, 2=No (If yes go to question ii).

ii. When did you start keeping pets? (year) ————————

iii. What is your purpose of keeping pets?

Purpose	Income generation	Protection	Hunting	Others (specify)
Indicate (tick)				
Order of importance				

iv. What is your current pet size (number) 1= Dog ————Cat ————

v. How do you keep your pets?

Pet management	Tick	Rank
1.Total confinement		
2.Semi confinement		
3.Free range		

vi. What are the main food resources for your pets? ————————————————

E Awareness and knowledge of sparganosis

i. What are the major health problems you normally experience in your area?

Type of health problem experienced	Order of importance (rank)
1.	
2.	

3.

---

ii. Have you ever heard or experienced about sparganosis? 1=Yes, 2=No

iv. If yes, when did you get aware of the disease for the first time? (year)

iv. Briefly explain your understandingof the disease.

_____

_____

v. What is the local name of the disease. _____

Vi. Do you know how people get infected with spargana?.1=Yes, 2=No

Vii. If yes, please indicate the causes of the infection.

1. _____
2. _____

Viii. If yes, where did you get the information on the disease.

1=from my relative, 2=health workers, 3=from researchers, 4 = others (specify)

_____

ix. Can spargana cause any problem to human being? 1=Yes, 2=No

X. If yes, briefly explain how.

_____

Xi. How serious is sparganosis in your area.

1=no-existence, 2=it is present but not serious, 3=moderate serious

5=it is serious problem, 5=I am not aware.

Xii. Have you ever encountered cases of sparganosis in your household?

1.=Yes, 2.=No, 3.=not sure.

Xiii. If yes, which action do you take take to the person with sparganosis

1._____

2._____

Xiv. Have you experienced any deaths from sparganosis? 1=Yes, 2=No

Xv. If yes, how many members of the household have died from sparganosis.

_____

xvi. If yes, mention which years they died: _____

xvii. Do you know how to prevent human from getting infected with sparganosis?

1=Yes, 2.=No.

Xiii. If yes, what are the methods used in prevention.

1._____

2._____

F Drinking water and eating behaviour

i. Does your members of the household boil drinking water?

1.=always, 2.=sometimes,3.=never

ii. If no, what other method do you use to treat your drinking water

_____

iii. What sources of water do you use in your area?

Source of water	Order of importance (rank)
Local wells	
Deep bore hole	
River	
Ponds	
Springs	
Others (specify)	

iv. Location of water source.

1=within the household, 2=within the village, 3=outside the village.

v. If outside the household, what is the distance to the mostused water source for your household: _____ (km).

vi. Do you and members of your household eat game meat? 1=Yes, 2=No.

vii. If no, what reasons made you not to eat:

_____

viii. If yes, which places do you get game meat for home consumption

1. _____

2. _____

ix. If "ever"how did you know wheather or not it was fit for human consumption.

1.= by using our traditional inspection methods

2.= by using official meat inspector

3.= no any consideration made

4.= Others (specify) _____

x. Within your household, which is the game meat preparation method mostly preferred

1.= boiling, 2.= frying, 3.= raw, 4.= barbecure, 5.= Others (specify)

_____

THIS IS THE END OF THE INTERVIEW

THANK YOU VERY MUCH FOR YOUR PARTICIPATION

Appendix 3: Ethical Clearance

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
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Tel: 255 22 2121400/390
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26th January 2012

Dr Nicholas J Kavana
Sokoine University of Agriculture
Faculty of Veterinary Medicine
Dept. Veterinary Microbiology and Parasitology
P O Box 3019, MOROGORO

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Experimental Life Cycle and Molecular Studies of *Spirometra* Species in Monduli in Arusha Region, Babati and Simanjiro in Manyara Region, Tanzania (Kavana N J *et al*), has been granted ethics clearance to be conducted in Tanzania.
The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Approval is for one year: 26th January 2012 to 25th January 2013.

Name: Dr Mwelecele N Malecela

Signature

CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Deo M Mtasiwa

Signature

CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL WELFARE

CC: RMO
DMO

Appendix 4: A pride of lion resting in Tarangire National Park.

Appendix 5: Sampling of lion faeces deposited on the ground

Appendix 6: Preparation for faecal sampling from the rectum of immobilized lion

Appendix 7: Sampling of lion faeces from the rectum after immobilization.

Appendix 8: Sampling of faeces from the rectum of a carcase of spotted hyena (*Cocruta cocruta*) at Makuyuni.

Appendix 9: Collection of *Spirometra* eggs from faeces of lion by Sedimentation method in the laboratory

Appendix 10: Collection of dog faeces per rectum.

Appendix 11: Dissecting water duck to search for natural infection of spargana.

Appendix 12: Carcase of warthog to be dissected to search for spargana.

Appendix 13: Carcase of wildebeest dissected to search spargana

Appendix 14: A researcher teaching a Clinical Officer how to fill questionnaires at Mto wa Mbu Health Centre, Monduli District

Appendix 15 : Researcher describing the disease sparganosis to villagers at Mamire Ward, Babati District

Appendix 16: Water pond near houses bordering with Tarangire National Park.

Appendix 17: Masai woman carrying a bucket of water from a pond bordering Tarangire National Park