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(RESEARCH ARTICLE)

A survey of lions (*Pantheraleo*) as definitive host of *Spirometra* species

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Abstract

The aim of the study was to carry out a complete life-cycle of the tapeworm *Spirometra* (Cestoda: Pseudophyllidea) under experimental conditions in the laboratory. A total of 7 lions from Tarangire National Park were examined their feces for presence of *Spirometra* eggs. Two methods were used to collect feces of lions: collection of feces from the ground where has been deposited and manual removal of feces from the rectum of the lion after immobilization. Eggs collected from the feces were found to be ovoid, tapered at both ends, the shell is smooth, dark brown in color and the embryo is visible within the egg shell. The measurements were in the range of length 52-75 µm and the width between 30-45 µm. The means were length 67.8 ± 3.71 and width 32 2± 3.65 µm. The eggs collected conforms with the eggs of *Spirometra* species.

Keywords: Lion; S*pirometra*; Egg; Tarangire

1. Introduction

Spirometra is a pseudodiphyllidean cestode of dogs and cats [1]. Lions are final hosts of *Spirometra* species also they harbor other intestinal parasites. Feces has been used to study the intestinal parasites harbored by the wild lions. The lions act as definitive hosts of *Spiromtra* species. The life cycle of *Spirometra* 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 [2, 3]. The first stage in the life cycle of *Spirometra* is the egg which hatches to coracidia, the egg is not infective to man but the coracidia is infective to *Cyclops.* The other two larval stages procercoid and plerocercoid (sparganum) are infective stages. Human can be infected with sparganum by three possible routes: First route is ingestion of raw snakes, frogs or other animals that harbour the spargana [4]. Second route is ingestion of infected cyclops in drinking water [4]. Third route is the application of the flesh of infected frogs to a wound or eye sores [5]. The spargana develops to adult worm in the small intestine of dog, cat and lion (definitive host). Lions can be infected from eating mammals which are infected with spargana. 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 Kenya [6], Tanzania [7] and Uganda [8]. In Tanzania, various researchers reported sparganosis from the Maasai of Serengeti [7], sparganosis in baboons [9] and infection of *Spirometra* in two populations of lions in the Serengeti National Park and Ngorongoro Crater [10]. The aim of the present study was to survey *Spirometra* eggs in lions (*Pantheraleo*) of Tarangire National Park in Tanzania.

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2. Material and methods

2.1. Study Area

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 2850 km^2, making the 5th largest park in Tanzania. It lies about 120 km southwest of Arusha.

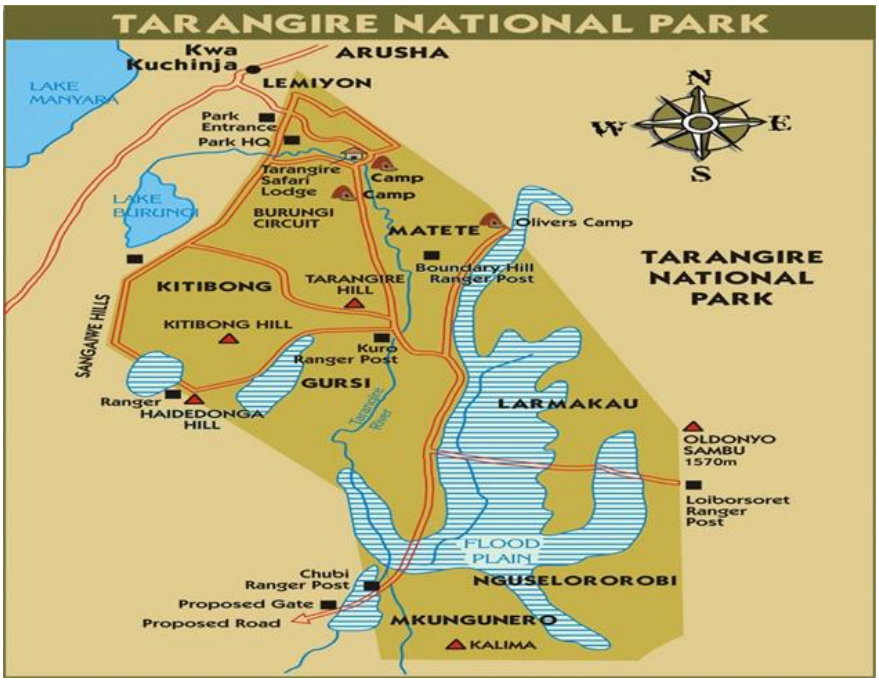

Figure 1 Tarangire National Park (Source: Tanzania National Parks)

2.2. Faecal sampling from lions

Figure 2 A pride of lion resting in Tarangire National Park

Sampling of faeces from lions (*Pantheraleo*) of Tarangire National Park {Fig.1) was carried out with the help of staff from Tanzania Wildlife Research Institute, Arusha and Tarangire Lion Research Project, Tarangire. Lions of Tarangire National Park are in prides (Fig 2) which are well known to Tarangire Lion Research Project. In each pride one lion has a collar which makes easy to trace by frequencies from the tracking box and antenna used in the research car. Three prides (Tarangire Hill, Altipiano and Wazi) were identified. Places where lions were resting and the left over carcase were thoroughly searched for the feces deposited (Fig 3). Two methods were used to collect feces (i) Collection of feces

from the ground where the lion was seen depositing (Fig 4). (ii) Manual removal of feces from the rectum of the lion after immobilization (darting) (Fig 5, 6). A total of 7 fecal samples were collected. Feces of one lion from Tarangire Hill pride was collected from the rectum after immobilization by darting and the other 6 fecal samples were collected from the ground using gloved hands. Ketamin for animals was used (Dose 3 ml, route Intramuscular, Manufacturer-Kyron Laboratories (Pty) Ltd) (Table1 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.

Figure 3 Carcase of Zebra killed and partially eaten by the lions in Tarangire National Park

Figure 4 Sampling of lion feces deposited on the ground

Figure 5 Lion has been immobilized using animal Ketamin

Figure 6 Sampling of lion feces from the rectum after immobilization

Table 1 Sampling of lion (*Pantheraleo*) faeces at Tarangire National Park, Manyara, Tanzania

Pride	Lion S/No	Sample	Site collected	Method of collection
Tarangire Hill	1	Faeces	From rectum	Immobilization by darting
	2	Faeces	On the ground	Collected on the ground
Altipiano	3	Faeces	On the ground	Collected on the ground
	4	Faeces	On the ground	Collected on the ground
	5	Faeces	On the ground	Collected on the ground
	6	Faeces	On the ground	Collected on the ground
Wazi	7	Faeces	On the ground	Collected on the ground

2.3. Screening for the presence of *Spirometra* eggs by Floatation Method

Small amount about 5g of lion feces was dissolved separately in saturated salt, sugar and Zinc sulfate solutions in small beakers (preparation of saturated solution: 400g of salt sugar and Zinc sulfate in water up to 1lt level). The mixture stirred well to break the lumps, filtered through tea strainer. Solution poured in centrifuge tubes which was placed on a rack then more saturated salt, sugar and zinc solution was added up to the brim, cover slide applied on top for 3 minutes. Cover slide was removed and placed on the slide without changing the side which was in contact with the solution. The slide was examined under compound microscope.

2.4. Collection of *Spirometra* eggs by Sedimentation Method

Small amount about 5g of lion feces was taken suspended in tap water in a beaker, filtered through tea strainer, and sediments collected in a sedimentation flask. Tap water was added in the flask up to the level of 1000 ml [Fig.7]. It was left to stand for 30 minutes then supernatant poured off, water was added and left to stand for another 30 minutes. The process was repeated until the supernatant was clean. When poured the last supernatant of the sediment was poured into a Petri dish, small amount of water was added to dilute it. The specimen screened on a dissecting microscope. Identification and confirmation was done by examining the sample under compound microscope (x10). Small amount of sediment from Petri dish was taken 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 morphological study and taking measurements.

Figure 7 Collection of *Spirometra*eggs from lion feces by Sedimentation method in the Laboratory at Sokoine University of Agriculture

2.5. Sedimentation rate of *Spirometra* eggs

The aim of this experiment was to determine if eggs settle to the bottom of the sedimentation flask.

Eggs, Sedimentation flasks, timing watch, tap water, Petri dishes and Pasteur pipette were used. Four sedimentation flasks marked 1000 ml, 800 ml, 600 ml, 400 ml and 200 ml were used in this experiment. Washed eggs were mixed in 1000 ml of tap water, shaken well and poured in sedimentation flasks. After every 5 minutes about 5 ml of water was drawn from each level of marking, placed in Petri dish examined under dissecting microscope. First study water was drawn from the bottom level of 200 ml then gradually upwards to 1000 ml. Second study water was drawn vice vesa.

3. Results

In the present study fecal examination of 7 wild lions (*Pantheraleo*) from Tarangire National Park, Tanzania, revealed parasitic eggs, after sedimentation, and floatation methods (Table 2).

In saturated sugar solution,*Spirometra* eggs were detected in 5 (71.4%) individual lion faeces, In saturated salt solution, no (0%) *Spirometra* eggs were detected. By Sedimentation method *Spirometra* eggs were detected in all 7 (100%) lion feces.

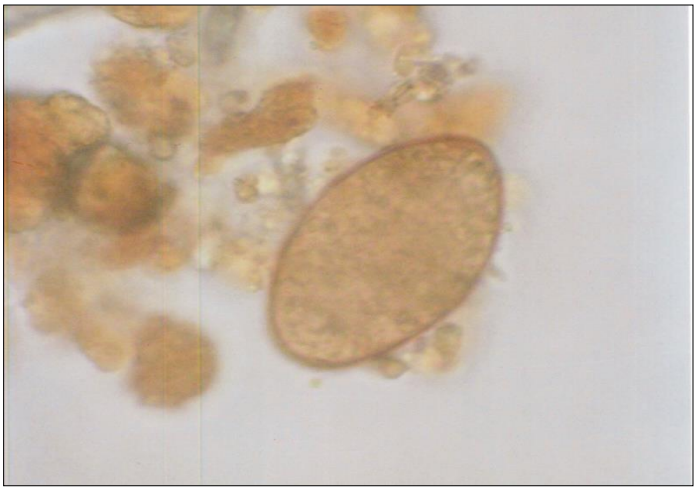

Figure 8 Egg of *Spirometra* species from faeces of lions

Spirometra develop to adult stage in the small intestine of the final host. They produce and liberate large number of eggs which are voided with the host´s faeces into the environment. 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. 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 morphology of the egg of *Spirometra* species recovered from lion faeces after sedimentation and examination under microscope is shown (Fig 8) and the relationship between length and width (Fig 9). 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 means were length 67.8 ± 3.71 and width 32 2± 3.65 µm.

Table 2 Measurements of 100 *Spirometra*Egg (in µm) from 7 different lions (in 3 prides)

S/N	Mean1	Mean2	SD1	SD2		
					µ±SD1	µ±SD2
1	37.00	62.85	2.801	5.475	37.00±2.8^{b}	62.85±5.5^{b}
2	33.57	62.00	4.089	6.421	33.57±4.1^{b}	62.00±6.4^{b}
3	38.21	65.64	2.293	5.852	38.21±2.3^{b}	65.64±5.8^{b}
4	38.78	65.00	1.476	4.607	38.78±1.5^{b}	65.00±4.6^{b}
5	39.14	66.14	2.213	5.815	39.14±2.2^{b}	66.14±5.8^{b}
6	39.92	62.92	8.561	7.931	39.92±8.6^{b}	62.92±7.9^{b}
7	35.50	60.81	3.464	4.636	35.5±4.6^{b}	60.81±3.5^{b}

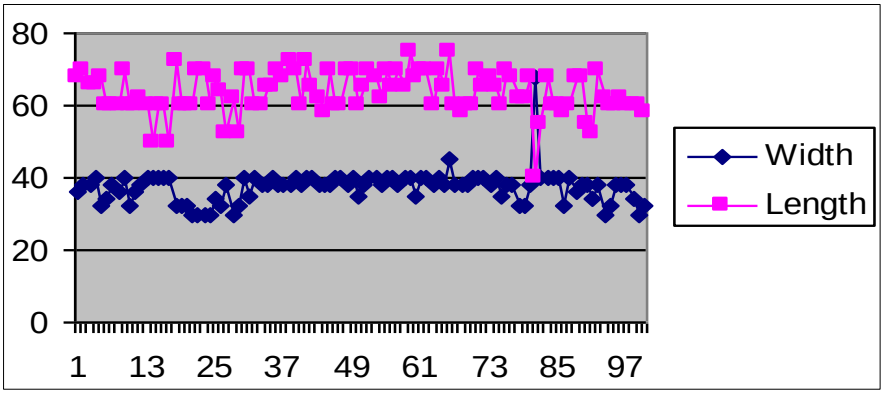

Figure 9 The relationship between length and width measurements of *Spirometra* eggs from lion feces

4. Discussion

Spirometra has been reported to be the common parasite in wild lions [11, 12, 13, 10]. In the present study eggs of *Spirometra* were observed in the feces of lions of Tarangire National Park, Tanzania. The morphology was oval and measurements were were in the range of length 52-75 µm and the width between 30-45 µm. The means were length 67.8 ± 3.71 and width 32 2± 3.65 µm.This corresponds with the eggs of parasites reported by Müller-Graf [10] in lions of the Serengeti and Ngorongoro Crater National Parks, Tanzania.

Screeningof parasite eggs in fecal samples of lions have been done by using Formol-ether method [14,10]. The methods easily detected *Spirometra* eggs in lion feces. Engh[15] used sugar solution for screening parasite eggs of spotted hyenas. In the present study saturated salt, sugar and Zinc sulfate solutions were used for screening of lion feces for *Spirometra* infection. In saturated sugar solution *Spirometra* eggs were easily detected. While in saturated salt solution *Spirometra* eggs were not easily detected. In Zinc sulfate solution the *Spirometra* eggs were found to be deformed. The present result shows that sugar solution is the best for detection of *Spirometra* eggs because it has higher Specific Gravity than

salt solution. This conforms with the results of Engh[15]. who used sugar solution in coprologic survey of parasites of spotted hyenas and easily detected *Spirometra* eggs.

Spirometra infection in lions of Serengeti National Parkand Ngorongoro crater has been reported by Müller-Graf [10]. In the present study fecal sample from lions of Tarangire National Park revealed infection of *Spirometra* sp. The infection rate is very high all lions sampled were found to be infected with *Spirometra* (definitive host). This could be explained that, lions in the National Parks feed on wild animals only which are second intermediate hosts of *Spirometra* [16,17]. In the present study the eggs of *Spirometra* were found to settle down in the sedimentation flask. This indicates that in natural condition when the lion defecates the egg settle in the mud where hatches to coracidia. Then coracidia is eaten by *Cyclops* where develops to procercoid. Then animals get infected by ingesting water contaminated with infected *Cyclops*. These animals which are the prey of lions if happens they are eaten by the lion, the lions easily get infection.

5. Conclusion

Wild lions are the definitive host in the life-cycle of Tanzanian *Spirometra* species. Eggs of *Spirometra* were recovered from feces of naturally infected lions. Morphology and measurements of eggs were suggestive of *Spirometra* eggs.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

In this study there is no conflict of interest.

Statement of ethical approval

This study involved the use of animals. The animals used were not killed, only fecal sample was collected. Permission was granted by Tarangire National Park Authority to enter in the National Park and collect feces from lions.

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