Incubation of *Spirometra* eggs at laboratory conditions by Modified Harada-Mori method

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Abstract: *Incubation of Spirometra eggs was conducted in the helminthology laboratory Faculty of Veterinary Medicine, Sokoine University of Agriculture during the period July to September, 2012. Spirometra eggs from faeces of naturally infected lions (Panthera leo) from Tarangire National Park, Tanzania were cultured at laboratory condition using modified Harada-Mori method. The objective of the study was to hatch coracidia and use in a life-cycle experiment. The culture consisted of a thin film of washed eggs on a strip of filter paper inserted in an upright falcon tubes containing sterile sand and stones to the bottom up to 1 cm level and aquarium water up to just below the egg smear. Eggs were cultured at temperature 26-29ºC and light. On day 6 the eggs started hatching coracidia. Under the circumstance it was fruitful to culture Spirometra eggs using modified Harada-Mori method. The modified Harada-Mori culture technology showed a high rate of hatching of Spirometra eggs. We recommend using this modified Harada-Mori method for culture of Spirometra eggs being able to hatch many eggs, inexpensive and less time consuming.*

Key words: Harada-Mori, culture, eggs, *Spirometra*.

INTRODUCTION

Spirometra is a pseudophyllidean tapeworm of dogs, cats and other mammals (Mueller, 1974) with a worldwide distribution. Its plerocercoid larvae or spargana can infect humans causing sparganosis (Iwata, 1972). Although rare, sparganosis is endemic in many countries with the majority of cases were reported from Southeast Asia and Eastern Africa (Schmid, 1972; Cho, 1975; Sparks, 1976; Nobrega-Lee, 2006).

In Tanzania, the parasite has been reported by Schmid, in 1972. Its life-cycle requires two different intermediate hosts, the fresh water *Cyclops* as the first intermediate host and amphibians, reptiles, birds and mammals as the second intermediate hosts (Iwata, 1972; Mueller, 1974; Lee, 1990). Humans act only as the accidental host in this life-cycle. There are three ways by which humans can be infected with the parasite. Firstly, by ingestion of infected raw snakes, frogs and other animals that harbour spargana.

Secondly, by ingestion of infected *Cyclops* from contaminated drinking water and thirdly, by application of the flesh of infected frog (or poultices) to a wound or eye sores which may cause the spargana be transferred and then migrate to various visceral organs (Tanaka. et al., 1997) including subcutaneous tissues causing

swellings (Garin et al., 1997). 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, 2007).

A test-tube filter-paper method was introduced by Harada and Mori (1951) to culture hookworm (*Ancylostoma duodenale*). However, since its introduction there have been a number of modifications of the method. The modified Harada-Mori filter paper strip culture technique has been used for the diagnosis of other parasites *Strongyloides stercoralis* and hookworm infections (Sirima, 2008; Malinee, 2000). Also has been used in screening *Strongyloides stercoralis* during preparation of patients for transplantation (Nolan, 1996). Beaver (1964) used a simple culture method for *Ancylostoma duodenale* and to culture *Spirometra* eggs. The aim of this paper is to describe the modified Harada-Mori test-tube filter paper method used to culture *Spirometra* eggs to obtain coracidia for use in the life-cycle studies.

Materials and method

The experiment consisted of a thin film of washed eggs on a strip of filter paper in an upright 15 ml falcon tubes (Figure1) containing aquarium water prepared in the laboratory, stones and sand prepared by sterilizing in an oven at 180ºC for 1 hour. Eggs of *Spirometra* (Figure 2) were collected from faeces of infected lions by sedimentation method as described by Soulsby (1982).

The eggs were washed thoroughly 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 the other falcon tubes were control. Filter papers were inserted in falcon tubes, aquarium water added up to the level just below the egg smear. The falcon tubes including control were placed on a rack placed in the laboratory at temperature 26-29ºC and natural light.

Caps of falcon tubes were placed unscrewed at the top of each tube. 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 to observe hatched coracidia. The number of hatched coracidia was recorded daily. Pictures of egg and coracidia were taken by using a compound microscope Olympus BHT 210314.

RESULTS

A total of 6 falcon tubes were used to culture *Spirometra* eggs. The hatching of eggs is as shown in (Table 1). On day 6 eggs started hatching and the last day of hatching was (day 13). The hatched coracidia (Figure 4) measurements were in the range of 20-30 ε m in length and 20-30 ε m in width. The coracidia had cilia on the body surface which were used to swim. The hatched egg shells were seen with the operculum open (Figure 3).

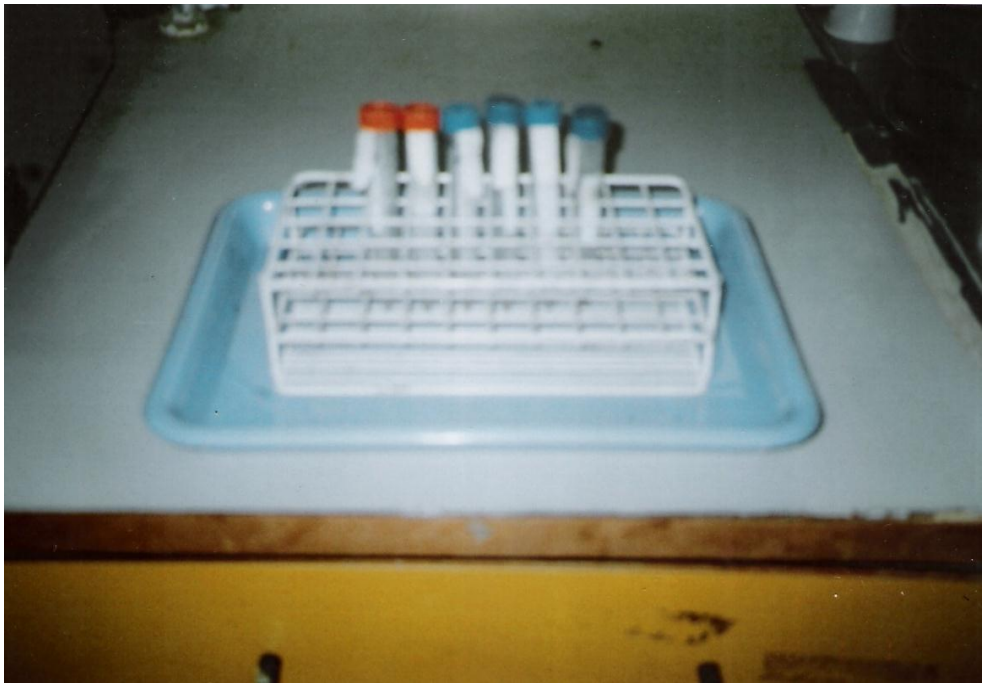

Figure 1: Incubation of *Spirometra* eggs by modified Harada-Mori method

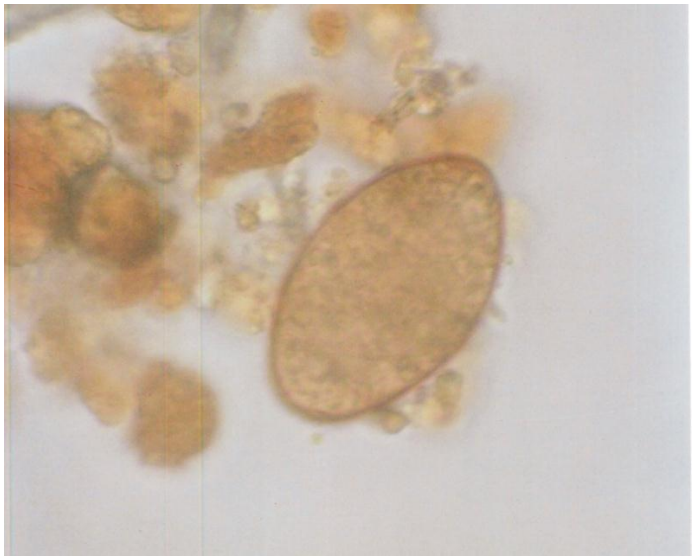

Figure 2. Tanzanian *Spirometra* egg from naturally infected lion, appearance under light microscope

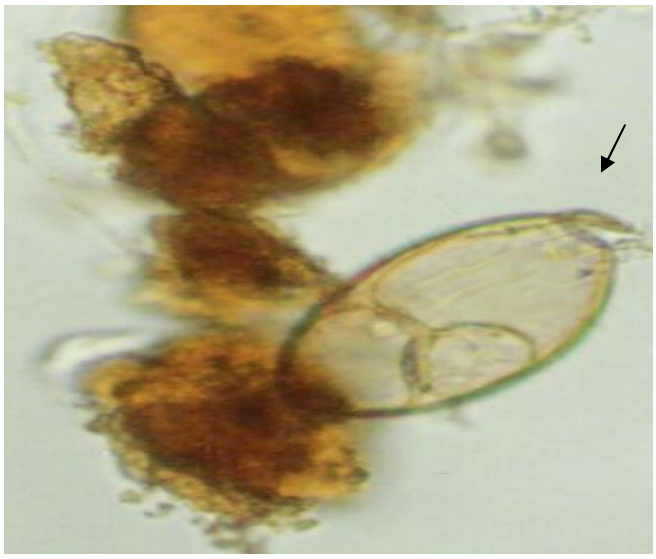

Figure 3. The egg which already hatched out operculum open (Arrow)

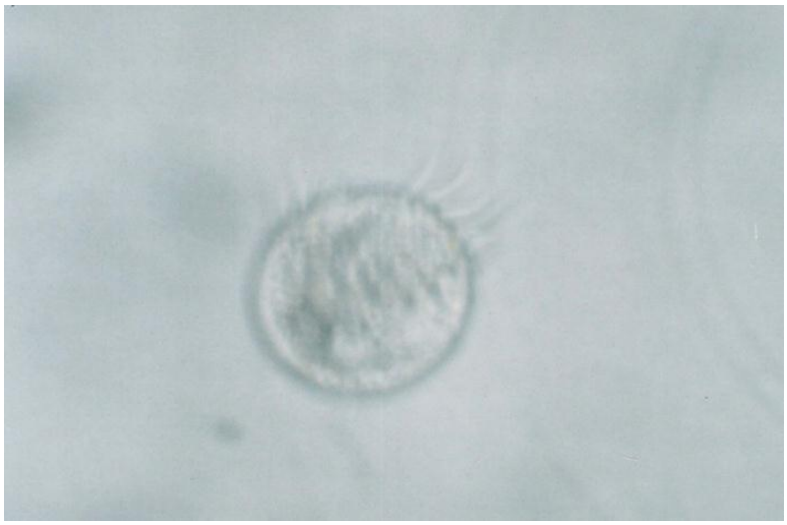

Figure 4. Hatched coracidia with cilia on the body surface

Table 1 Experimental observation on the hatching of *Spirometra* eggs using modified Harada-Mori method

S/N Of falcon tubes	Days of incubation at temperature 26-29ºC with light																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1.	0	0	0	0	0	12	9	1	0	1	0	1	0	0	0	0	0	0
2.	0	0	0	0	0	11	7	0	1	0	0	2	0	0	0	0	0	0
3.	0	0	0	0	0	8	12	0	0	1	0	1	0	0	0	0	0	0
4.	0	0	0	0	0	10	3	3	1	0	2	0	0	0	0	0	0	0
5.	0	0	0	0	0	7	5	1	1	1	3	0	1	0	0	0	0	0
6.	0	0	0	0	0	13	7	1	1	2	0	0	3	0	0	0	0	0
Time range 6-13 Days																		

DISCUSSION

The results in this study showed that there was hatching of *Spirometra* eggs. The eggs started hatching from day 6 to day 13 at temperature 26-29ºC. The result in the present study agrees with previous workers (Martin, 1999; Daniel, 2007). Martin (1999) reported to have obtained *Strongyloides stercoralis* larvae on day 10 after incubating faeces by using modified Harada-Mori filter paper strip culture technique at temperature 30ºC.

Daniel (2007) reported to have cultured hookworm *Ancylostoma ceylanicum* by using Agar Plate method which on day 7 the larvae were collected. In the present study, it was observed that hatching of eggs was highest on day 6, the following days the number of hatched eggs gradually decreased. Previous studies have reported that the larvae of *Ancylostoma ceylanicum* gravitate into the water from the faecal culture by Baermann method (Foreyt, 1989). In the present study coracidia were observed to be swimming in water in the falcon tubes. The result agrees with the previous worker that the hatched larvae migrate out from the faecal culture to water.

The present study demonstrated modification of the test tube filter-paper culture method for *Spirometra* eggs in which cleaned eggs and aquarium were used and gave similar environmental conditions to those found in the field.

CONCLUSION

In the present study cleaned *Spirometra* eggs and aquarium were used to modify Harada-Mori test tube filter-paper culture method. An interesting observation was the finding of massive hatching of *Spirometra* eggs. This method is simple, efficient, convenient and very easy to perform. It is suggested that the method

should be used in the laboratory for culturing of *Spirometra* eggs to obtain coracidia for other experimental studies.

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