The Pharma Innovation Journal 2018; 7(4): 657-660

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(4): 657-660

www.thepharmajournal.com
Received: 08-02-2018
Accepted: 10-03-2018

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A simple method of harvesting microfilaria of *Setaria digitata*

Lingala Raju Kumar and Manchukonda Udaya Kumar

Abstract
Adult female *Setaria digitata* worm incubated *In vitro* in PBS 7.2 has released eggs which are interconnected. Later embryonic mass in each egg developed into larvae arranged in coiled position inside the egg capsule. Coiled larvae inside the egg extended with round anterior and pointed posterior ends where as the egg shell remained on the larvae forming a sheath around it. Adult worms were found actively motile up to one day

Keywords: *Setaria digitata*, *In-vitro* incubation, microfilariae.

1. Introduction
Filarioid nematodes are the parasites of the tissues and tissue spaces of all vertebrates except fishes (Anderson and bain 1976) [1]. Among these filarial nematodes, genus *Setaria* are heteroxenic parasites dwells in peritoneal cavities of bovines which serves as definitive hosts and invertebrate mosquitoes are intermediate hosts. *Setaria* worms are unisexual in which males are small and slender where as females are long and stouter than males. Fertilized female worm releases sheathed microfilariae in to the blood as they are viviparous. These microfilariae in turn taken up by mosquitoes spp viz., *Aedes*, *Anopheles*, *Culex*, *Armigeres* during blood feeding (Tung *et al*., 2004), grows to infectious stages. Varma *et al*. (1971) [16], studied mf growth in mosquitoes and observed exsheathement in the stomach of the mosquitoes then, enters the haemocoel and migrated to the thoracic muscles in 4–5 h. Infective larvae, 1.95–2.52 mm in length, appeared in the mouthparts of the mosquitoes 11–13 days after the mosquitoes feed on infected rabbits. This infective stage (L3) transmitted to definitive host through mosquito bite, within 8–10 months these larvae reach sexual maturity, completing the life cycle. Adult female worm release microfilaria into circulation produces microfilariosis in natural bovine hosts. Accidental transmission of infective L3 stage to unnatural hosts viz., caprines and horses cause serious and often fatal cerebrospinal nematodiosis. *Setaria* worms and their microfilariae are extensively in used in diagnostic assays and drug studies of human filariois due to their morphological, histological and antigenic similarities (Muruganathan *et al.*, 2010)**.** Present study aimed to study the embryogenesis in *Setaria* worms and *In vitro* harvesting of host cell free microfilariae from adult worm for molecular studies.

2. Materials and methods
Setaria Worms of both sexes were collected from the peritoneal cavity of bovines slaughtered at Chengicherla, Hyderabad and Alana slaughter houses, Zaheerabad, A.P., in PBS pH 7.2 and transported to the laboratory. Worms were cleaned for several times in PBS pH 7.2 to remove host material and blood contamination (Dhas *et al* 1993) [3], etc,. Motile female worms were used for the study within 1 hr of collection. The worms were incubated in pbs7.2 in a clean sterile peridishes at room temperature (22 ^{0}C) for 18 hrs. In the early stages of incubation, incubating fluid transferred in to a small petridish to observe process of the formation of sheathed microfilariae.

3. Results
Harvesting of host cell free microfilariae in the present study revealed the development of microfilaria in a sequential manner starting from the stage of egg to fully developed microfilaria (L1). Initially, the fertilized female worm layed embryonated eggs in to the incubating medium. Eggs were seen as bunch of grapes (Fig 1).

The eggs were ovorectangular in shape containing embryonic mass inside it (Fig 2). The embryonic mass slowly developed in to coiled larval form (Fig.3). Larva inside the egg extended (Fig. 4&5) with round anterior and pointed posterior ends where as the egg shell remained on the larvae forming a sheath around it (Fig.6). Once the microfilariae exhibiting motility (Fig. 7) were seen in the incubating medium, the fluid was centrifuged to remove debris and pellet of microfilaria was washed thrice in PBS. (pH.7.2). The adult worms were found actively motile up to one day.

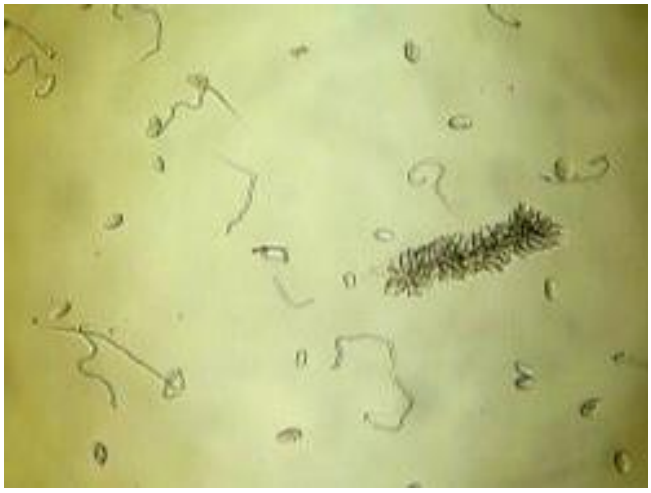

Fig 1: The eggs arranged as bunch of grapes (100X)

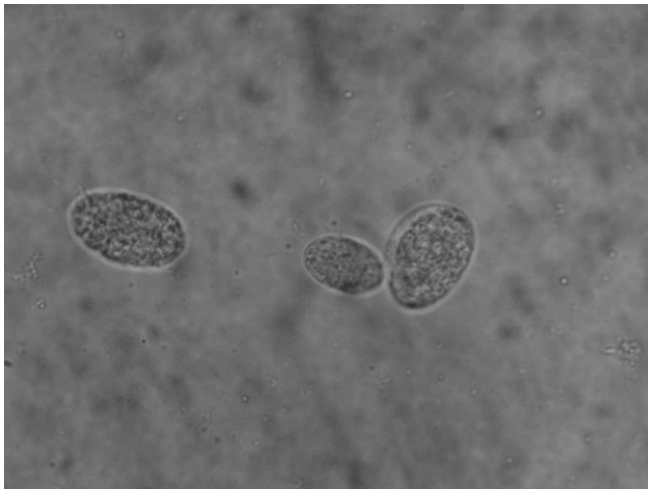

Fig 2: Embryonated egg of *S. digitata* (400X).

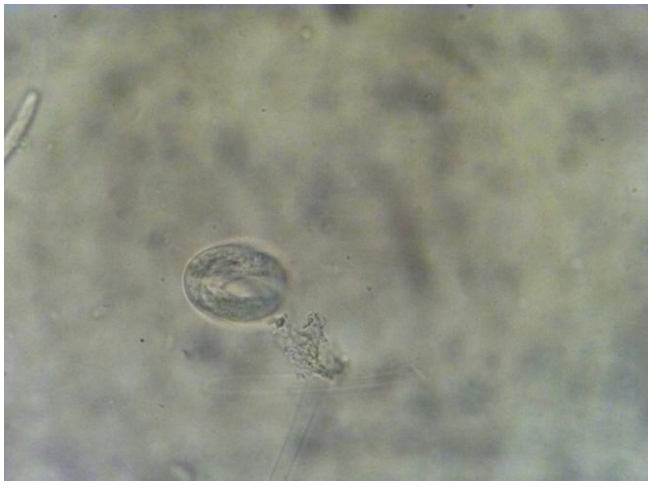

Fig 3: Egg with hatched larvae.

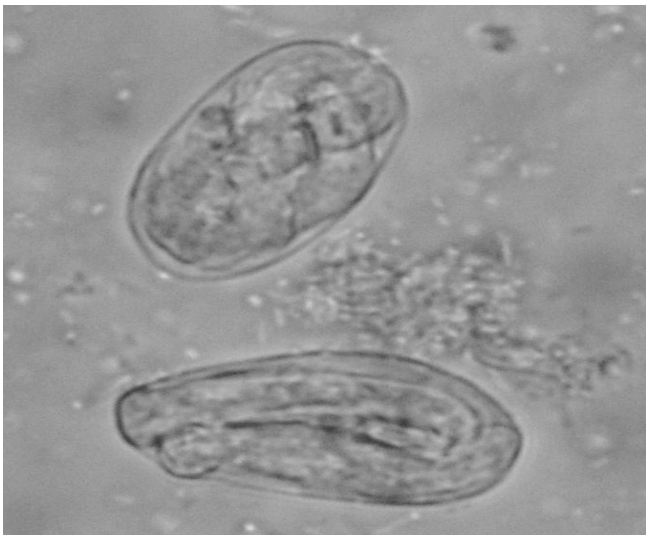

Fig 4: Egg with developing larvae in coiled position (400X).

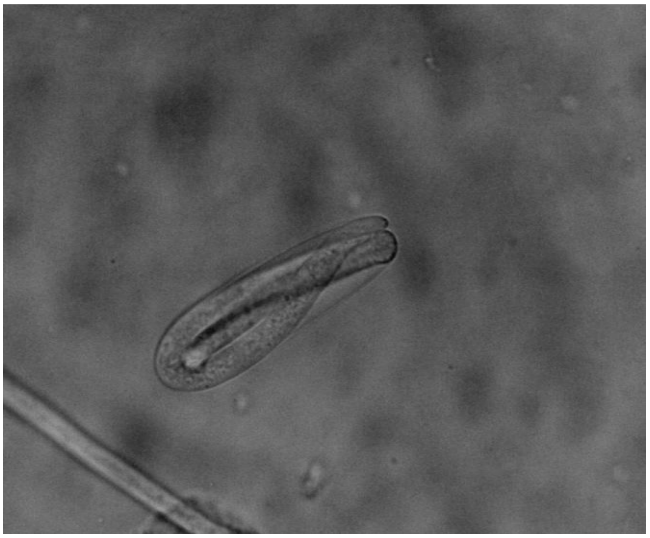

Fig 5: Egg developing larvae extending inside the egg (400X).

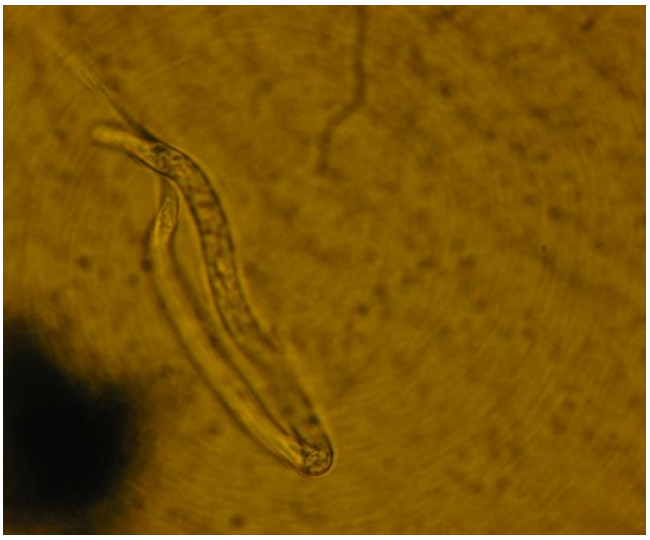

Fig 6:.Hatched, partially extended, Sheathed microfilariae (L 1) of *S. digitata* (400X).

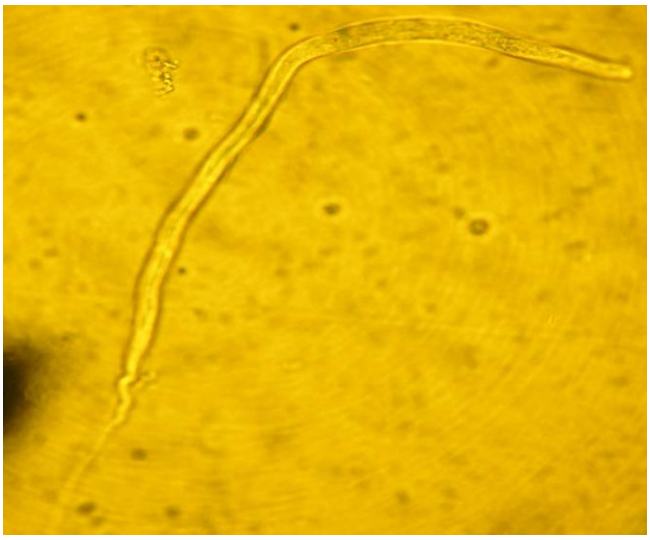

Fig 7: Sheathed, motile microfilariae (L1) of *S.digitata* (400X).

4. Discussion

In the present study the host cell free microfilaria were obtained from female *Setaria digitata* worms incubated individually in separate Petridishes containing PBS (pH 7.2) at room temperature (22 ^{0}C) for 18hr. A series of developmental stages starting from egg to first stage larva were recorded while harvesting microfilaria from adult worm. Initially, the ovo rectangular, embryonated eggs were released in to the incubating medium. Eggs were seen as bunch of grapes. This might be due to fact that, Decruse and raj. 1990 [11], while studying the histology of female *S.digitata* reported that the process of egg hatching to microfilariae was seen in the narrower region of uterus. Early embryos were seen in the posterior part of the uterus, fully embryonated eggs at the middle part of the uterus and microfilaria at anterior part of uterus and vagina. These developmental stages are interconnected and connected to the uterine wall.

In due course, the embryonic mass slowly developed in to first stage larva which was seen in coiled position at the beginning and later extended in to straight position with round anterior and pointed posterior ends. Finally, the larva was freed in to the medium following the formation of sheath by the egg shell. In sheathed microfilariae egg shell stretches to form sheath on the larva whereas in unsheathed species the mature microfilariae forces its way through the eggshells as opposed to molding it into a sheath (Wu *et al.,* 2008) [20], The present observations on the development of egg stage to L1 stage of *S. digitata* were in agreement with reports of Decruse and raj. 1990 but Dhas *et al.* (1993) [3], reported developmental stages like small morulae, big morulae, tadpole stage, coiled microfilaria and hatched microfilaria in the microdissected uterus of female *S. digitata*. Almost similar observations of different developmental stages in the dissected uterus of *S. digitata* made under light microscopy were reported by Kim *et al.* (2010) [5]. However, the tadepole stages recorded by Dhas *et al.* (1993) [3] in the dissected uterus of *S. digitata* were not observed in our study as we have directly collected the eggs released by live worm in to the incubating medium

Setaria adult worms are non-pathogenic, clinical manifestations are mainly associated with presence of microfilariae in blood. Direct relation between adult female worm load and number of microfilaria (mf) to circulating ES antigens was reported in filarial parasites (Reddy *et al* 1984 [13]; Weil 1987 [17]; Weil and Liftis 1987 [18]; Wenger *et al* 1988) [19]. These ES antigens are responsible for pathogenicity of microfilariosis. ES antigens were released during hatching of embryos however in the present study details regarding antigenic characterization was not studied as we were interested in isolating host cell free microfilariae for molecular studies. Sugunana and kaleysa raj 1986, demonstrated fluorescence with antisera against ES antigens was seen with entire uterine tissue, space between embryos and amniotic fluid. Fluorescence was not seen at excretory cells, excretory pore and esophagus of adult worm conforming antigenic source is female reproductive system. Dhas *et al* 1993 [3] studied fluorescent antibody binding with the antisera against ES antigens compared with different embryogenic stages. Fluorescence was almost absent at small morulae stage and increasing in intensity in the successive developmental stages with maximum at coiled microfilaria stage during. Hatched microfilaria did not show immunofluorescence. In *Setaria digitata*, ES antigens released *in vitro* have been shown to be directly-proportional to the number of mf released (Sugunan and Raj 1986) and originating from the female reproductive system (Decruse and Raj 1988) [10]. Hence, pathogenicity is mainly associated with release of ES materials during embryogenesis.

In India 3 distinct Setaria spp have been reported i.e., *Setaria digitata, Setaria labiatopapillosa* and *s.cervi* causing bovine microfilariosis in bovines (Mohan.1975 [6], Chauhan and Pande. 1980 [2], Patnaik. 1989 [7], Siddiqui *et al*., 1996 [8], Sunder and Ravindran., 2009) [9]. These species may occur individually or coexist in the peritoneal cavities. (Jayasinghe and Wijesundera., 2003) [4]. Further studies may be needed to study species specific variations during embryogenesis and antigenic characterization of different *Setaria* spp involving in bovine microfilariosis.

5. Acknowledgements

First author is thankful to Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India for the financial support in the form of P.G. stipend and for providing facilities to carry out the research work.

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