



Understanding the Morphology and Lifecycle of a Newly Reported Microscopic *Thysanosoma*/*Thysaneizia* Species from a Cattle Calf in Kashmir Valley (A Discovery)

Altaf Ahmad Reshi^{1*}, Hidayatullah Tak¹, KH Bulbul² and Zahoor Ahmad Wani²

¹Department of Zoology, University of Kashmir, Hazartbal, Srinagar, Jammu and Kashmir, India

²Division of Veterinary Parasitology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Shuhama, Ganderbal, India

*Corresponding Author: Altaf Ahmad Reshi, Department of Zoology, University of Kashmir, Hazartbal, Srinagar, Jammu and Kashmir, India

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Abstract

The aim of the present study was actually to examine the cattle faecal samples for *eimerian* oocysts to record their epidemiology. During the study one particular faecal sample from a calf in Ganderbal district of Kashmir valley showed some unique structures and changes in *eimerian* oocysts. The sample was then repeatedly collected on different dates from July 2021 to September 2021 to observe the changes and to note the unique structures. The particular sample was prepared by modified magnetic stirring technique in addition to normal salt floatation technique and revealed some amazing giant structures, which were photographed and their features recorded. The new technique had profound effect in revealing the features of the observed structures. On microscopic examination, enlarged *moneizia* type eggs, Trematode like larval stages, cestode like proglottids, fringed borders at certain stage and Taenia like adult with knob, rostellum but without hooks, neck and highly asymmetric adult body were observed which were bigger to their usual size using the new magnetic stirring technique. After thorough and repeated examination of different samples, it was observed that the organism is a new microscopic cestode species commonly known as fringe worms belonging to the order *Anoplocephalidae*, family *Thysanosomidae*, Genus *Thysanosoma* or *Thysaneizia* but seems to be the intermediate between Trematoda and Eucestoda. The new species as per its shape, morphology and understanding of the features has been named as *Thysanosoma/Thysaneizia camellia* (*T. camellia*). However in the present study it was not clear whether the worm should be categorized in the genus *Thysaneizia* or *Thysanosoma* though showing features more of the *Thysanosoma*. No sexual dimorphism could be reported in the present study. The worm reproduces by parthenogenesis and like other trematodes polyembryony is present in various larval stages.

Keywords: Life Cycle; Morphology; *Thysanosoma*; New Species; Cattle; Kashmir Valley

Introduction

The order *Anoplocephalidae* belongs to the class *Eucestoda*, phylum *Platyhelminthes* [1,2] and have neither rostellum nor hooks and the proglottids are usually wider than long [2]. In the family *Thysanosomidae* containing the main genera as *Thysanosoma* and *Thysaneizia*, the gravid uterus disappears and is replaced by par-uterine organs or capsules. The genus *Thysanosoma* contains fringed tape worms and so far only one species (*T. actinoides*) has been reported [2] and in genus *Thysaneizia*, a number of species have been reported from domestic and wild ruminants from different parts of the world but only few have been validated so

far [3-5]. *T. giarda* now *T. ovilla* [2,6] and *T. connochaeti* [7,8] are the main two reported species of the genus worldwide. They are also called as fringed tape worms because of their structure. *Thysanosoma actinoides* worms have so far been mainly reported in western parts of USA [9] and also in South America [10-12] and not much is known about their life cycle as their life has not yet been completely elucidated [2,6]. *Thysaneizia* worms are cosmopolitan in distribution [2] and adult tape worms inhabit the small intestine of ruminants mainly cattle and sheep [13-15]. *Thysaneizia ovilla* (*giarda*) has been reported in the small intestine of domestic ruminants in Europe, former USSR, Asia, Africa and America [6]. The

interest in the *Thysanosoma* species appears to be its controversial, complicated and misunderstandable life cycle. There is a general accepted paradigm that oribatid mites (*Acarina*) act as an intermediate host of the anaplocephalid cestodes, however with regards to genera *Avitellina*, *Thysanosoma* and *Thysaneizia*, it is still unclear whether oribatid mites are really the intermediate hosts as larval forms (cysticercoids) have also been found in collembolans and psocids [6]. Allen [6,9] experimentally demonstrated that larval stages of this cestode could develop in insects of the order Psocoptera. However, he was unable to infect sheep fed cysticercoids from infected psocids and postulated that two intermediate hosts were required, psocids being only the first one. Yannarella erroneously reported the larval stage of *Thysaneizia giarda* (*Helictometra giardi*) as *T. actinioides* from the oribatid mite *Zygori batulalata*. Later, Yannarella, et al. 1975 [16,17] corrected this misidentification.

Material and Methods

Faecal samples were brought from a particular calf of 7 months age in polythene bags from a particular locality in district Ganderbal on different dates which was found positive for *Eimeria*. The samples were brought to the laboratory on the same day and stored in the refrigerator at 4°C till further use. The sample was then subjected to magnetic stirring in clear water for varying times (5-45 minutes) in order to yield better results in oocyst pooling for DNA extraction over the traditional methods of sedimentation or floatation technique. In addition to the above mentioned method the normal procedure of salt floatation technique with centrifugation [18] and without centrifugation was also applied to the same sample and other samples suspected to show the same evidences at different occasions.

Results and Observations

On microscopic examination of the sample, following structures were observed.

Egg: An elongated hard shelled egg with beads all along the inner surface wall with a railway track like structure inside running diagonally from one corner to other was seen. The egg resembles a *Moniezia* egg in shape. The dimensions of the structure under 400x microscope using standard procedure of micrometry were 101.5 × 77µm. The structure was found both within and outside the reported worm. However, the outside structure was found to be larger in size. On subsequent studies for some days and with changed methodologies eggs of smaller size were also reported with shapes more or less resembling *Moniezia* eggs.

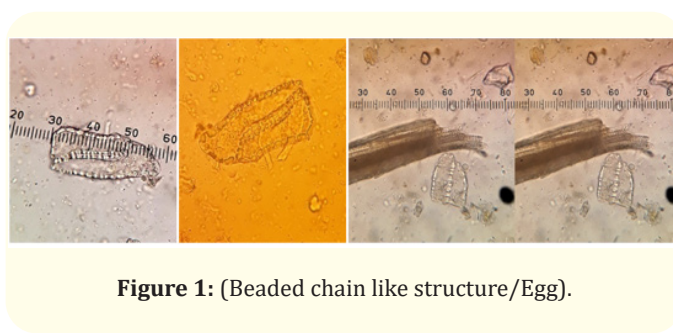


Figure 1: (Beaded chain like structure/Egg).

Miracidium: A motile canine tooth like structure or resembling early man’s stone tool or a cobra hood like structure but without cilia was observed with dimensions as 217 × 108.5µm. This was the only motile structure observed in the study which remained alive for some time. Inside the miracidium were also reported some stacked banana like structures and a hydra like structure.

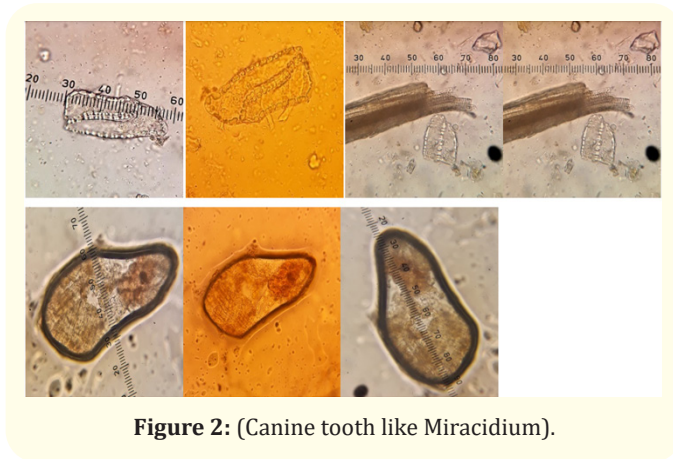


Figure 2: (Canine tooth like Miracidium).

Sporocyst: An individual spherical ball like structure was also reported the internal details of which could not be reported.

Redia: The redia stage was reported which looked like a hydra with two lappets and collars. The redia was also reported in a star fish like shape with six openings.

Cestode proglottids: A pack of 8 or more stacked bananas were reported both outside and inside the worm and also were seen in the miracidium larva. The bananas were stacked over each other and were attached to two parallel high way or stream like structures. The dimensions of the bananas were 140 by 73.5µm (Figure 5a,5b). A similar pack of bananas but smaller in size with fringes on its surfaces (Figure 5c) were also reported in the same sample with

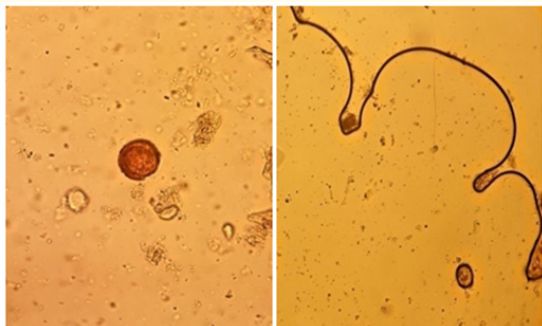


Figure 3: (Sporocyst).

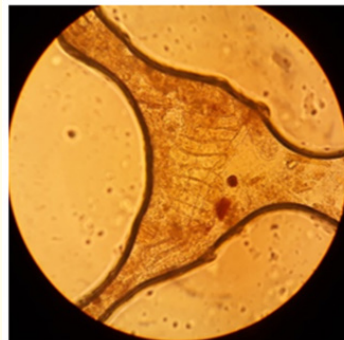


Figure 5b: (Proglottids inside the worm in camel hump).

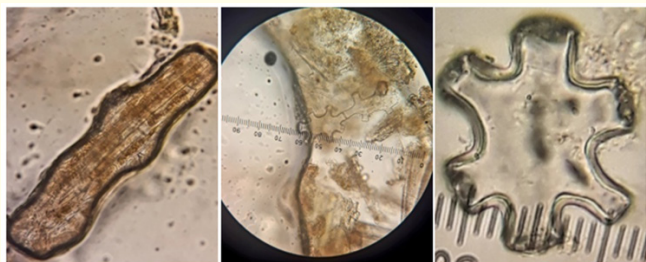


Figure 4: (Hydra or Star fish like Redia).

simple salt floatation technique with centrifugation and further another similar pack of bananas quite smaller in size was reported in another sample suspected for same infection in simple salt floatation technique without centrifugation (Figure 5d).

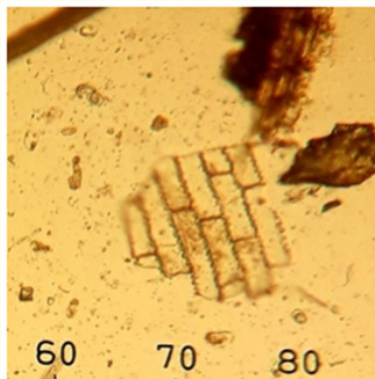


Figure 5c: (Fringe like borders of proglottids).

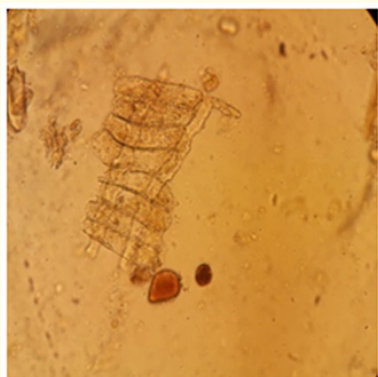


Figure 5: (Stacked banana like proglottids outside the worm).



Figure 5d: (proglottids reported in salt solution).

Sword: A beautiful sword like structure both at 100x and 400x with a hand fast and an outer covering like structure was also observed.

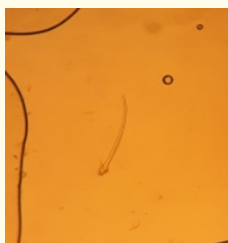


Figure 6a: (sword at 100x).

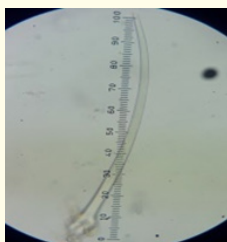


Figure 6b: (sword at 400x).

Larval stages: The magnetic stirred faecal solution was kept overnight at room temperature and on the second day, some larval stages quite resembling in shape to the *Fasciola* life stages were observed as shown in plate 1 figure 7.

Individual knobs: certain separated knobs cut at neck region were also observed.

Massive Sponge (Porifera) Skelton: A massive multi-spiculed sponge Skelton like structure was found containing both beaded chains and stacked bananas inside. The dimensions of structure could not be measured under 400 xs owing to its large size. The 100x vision revealed a long ribbon like structure with horns or spicules. A knob and mouth like structure with a dot in knob was observed at anterior end. The beaded chain like structure was observed inside it towards anterior side and a banana stack behind it. Individual bananas were observed towards posterior end. The dimensions of beaded leaf were 35 by 14µm. There was a mouth at one end facing bottom. Later on further analysis revealed that it was a broken worm.

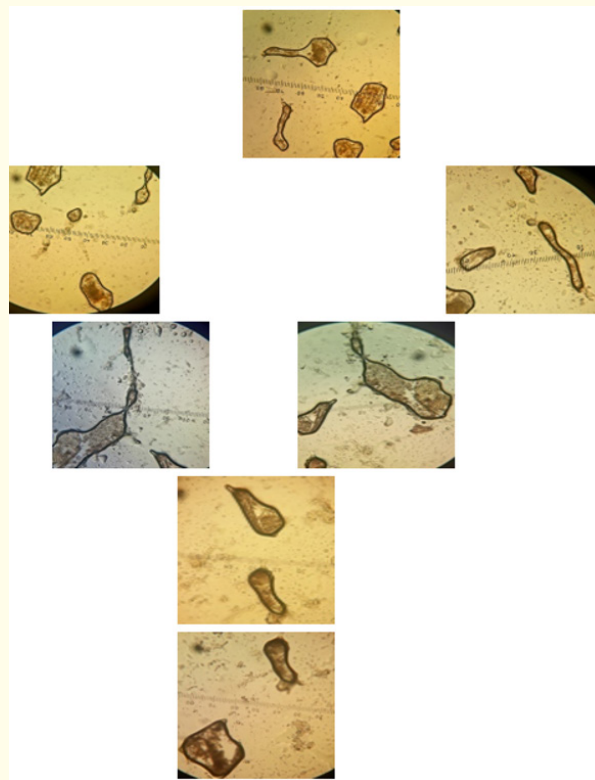


Figure 7: (Different larval stages).

Adult worm

While observing under microscope at 100x and 400x an intact worm was reported with a scolex, rostellum, neck and camel hump like structures. Camel humps were counted which were 4-5 in number alternating with each other to respective sides. The presence of humps makes this worm highly irregular and asymmetric and gives it an appearance of camel like with small narrow mouth at anterior side with rostellum and multiple humps. Some eggs were seen coming out of the worm through a pore located near neck region while some eggs seemed to get attached to highly delicate wall of the worm which actually forms the humps. The worm has two walls which are very delicate and highly fragile. The worm disappeared after 5-6 hours. A trail marked like structure was reported on the next day which seems to be the remnant of the outer wall. The worm was also observed under 400 x in parts and the parts were later joined to reveal the structure of intact worm

(Figure 10). In an another study carried out with same faecal sample (that was kept preserved at 4c in refrigerator) about one month later, the sample was processed in clear water without adding any salt and potassium dichromate and kept in incubator for about two days to notice the sporulation of oocysts if any. An asymmetric intact worm of similar morphology was observed that was photographed at 100x and size by micrometry also measured (Figure 9).



Figure 8: Adult worm with scolex and camel humps 100x (Magnetic stirred solution).

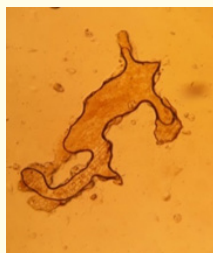


Figure 9: Adult worm in plain water without centrifugation or magnetic stirring.

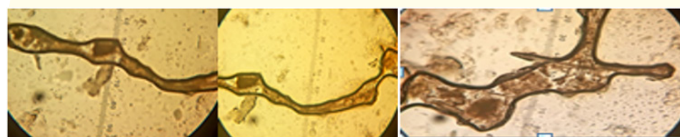


Figure 10: Adult worm at 400x (Magnetic stirred solution, parts Assembled together).

Discussion

In the present study all the structures and the life stages of the worm were reported under 100 x and 400 x microscope and majority of them in their giant size not reported earlier by any author. The giant size of the structures is attributed to the use of new magnetic stirring technique which enlarges, demorphs or even breaks certain structures both tissues and eggs as has been reported in other studies as well by the author.

The life cycle of *Thysanosoma* worms is quite complex and has not been completely understood yet. Various attempts have been made to interpret the misunderstood lifecycle of *Thysanosoma* worms but the puzzle has remained unsolved so far [6,9]. The life cycle of the worm is quite short spanning around 5-7 hours. The shape of egg is quadrangular with an internal bead like structure as if tiny rain drops are attached to side walls which seem to be developing miracidia at different stages. Miracidium was the only living structure reported in the present study but without cilia. Reporting of various trematode larva stages support the evidence of occurring these larva stages in the life cycle of newly reported worm. However besides trematode larva stages a chain of stacked bananas resembling *Taenia* proglottids were also reported. Fringe like outer borders were also reported in the larval stage (banana stage) but not in adult. The adult worm in its morphology and structure was also a typical cestode like with a scolex, knob, neck and asymmetrical unsegmented body. It is therefore preliminary interpreted that the reported worm is an intermediate between trematoda and cestoda with majority of larval stages resembling trematoda and the adult worm to cestoda. The worm was reported with 4-5 camel humps. Camel humps seem to be the sites of egg and larval development. The presence of humps makes this worm highly irregular and gives it an appearance of camel like with small narrow mouth and multiple humps. While some eggs get attached to highly delicate wall of the worm, some eggs also move outside through a pore located in the neck region and complete their life cycle outside. The worm has two walls which are very delicate and highly fragile. It seems that because of highly fragile walls, the life cycle of the worm is very short lived. A trail marked liked structure was reported which seems to be the remnant of the outer wall. No rostellum or rostellor hooks were observed in the adult. The adult worm also lacks true proglottids but same are present in the larval stage as

banana stacks which are wider than long and fringe on its borders. No sexual dimorphism could be reported in the present study and it is presumed that the worm reproduces by parthenogenesis. Also, larval reproduction was reported suggesting polyembryony in the worm. The classification of the worm as per its reported features and authors understanding is therefore given as:

- Phylum: *Platyhelminthes*
- Class: *Eucestoda* (South well 1930, Soulsby 1982)
- Order: *Anoplocephalidae* (Wardle 1974, soulsby)
- Family: *Thyosanomidae* (Fuhrman 1907)
- Genus: *Thyosanoma/Thysaneizia* (Skrjabin 1926)
- Species: *camellia* (Altaf., et al. 2021)

The probable cause of not reporting of this species is its microscopic nature and very short life cycle spanning only for few hours. The authors are of the opinion that the adult worm does not survive in the host or just dies at cysticercus/Cysticercoid stage while it fully completes its life cycle outside the host which is very short lived, 5-6 hours.

Conclusion

The worm is a microscopic cestode worm belonging to family *Thysanomidae* whose larval stages resemble both *Trematodes* and *Cestodes* and adult worm is a typical cestode like. Fringe like borders have been reported in larval (banana) stage but same are absent in adult. The adult worm is without proglottids but same have been reported in chain like fashion as stacked bananas at banana stage. This is probably the first comprehensive study to report the possible complex life cycle of *Thyosanoma* or *Thysaneizia* worms with more concrete findings and evidences. The worm seems to be the intermediate structure between *trematoda* and *cestode* with larval structures more resembling to *trematoda* and adult resembles a typical *Eucestoda* (*Thyosanoma* or *Thysaneizia* worm). The worm has a strong correlation with the *eimeria* oocysts as the evidences have shown.

Declaration of Competing Interest

The authors have no conflict of interest.

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