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Autogenicity and Precocious Development of *Sturdynema multiembryonata* (Upadhyay, 2017) in *Xenentodon cancila* (Osteichthyes: Belonidae) from the Gangetic Ecosystem

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Abstract:
The five years (2007–2012) consecutive parasitological investigations on freshwater crowfish, *Xenentodon cancila* conducted to learn the events in autogenic life cycle of *Sturdynema multiembryonata*. The *S. multiembryonata* is a roundworm with sturdy, spiny body in fresh water garfish peculiarly found taxonomically closer to gnathostomatoid roundworms. The developmental patterns and migratory behavior within the host was worked out critically. The findings were experimentally validated by oral feeding of eggs and developmental stages in dehelminthized experimental Swiss albino mice. The findings reflected exceptionally large body of advanced 3rd stage larva ($AdvL_3$) displayed strong possibility of precocious development, which was at least four times in size than any of the gnathostomatoid worms reported so far. The presence of caudal papillae resembling roundish bosses described in *Gnathostoma lamothei* and *G. miyazakii* with peculiar single-spined ornamentation all over the body. The study concludes that the larvae of *S. multiembryonata*, reported here, may not enter into 4th stage of development, and advanced 3rd stage larvae ($AdvL_3$) might transform into mature forms in a newer habitat, *i.e.* liver, within the body of newly reported definitive hosts, *X. cancila*. The establishment of larvae, without moulting, beyond the 3rd stage to attain maturity in the liver of experimental rodent hosts, corroborated the findings in natural fish definitive hosts. In the current investigation author wish to explore the precocious and autogenic development of *S. multiembryonata*, a roundworm with zoonotic potential which may provide a new dimension in the maintenance of healthy and disease free society based upon aquaculture.

Keywords: *Sturdynema multiembryonata*, Autogenic life cycle, Precocious development, Zoonoses, *Xenentodon cancila*

INTRODUCTION

The strategy to transfer of larvae from one paratenic host to another has invariably been adopted by parasitic helminthes of superfamily Gnathostomatoidea to gain acceleration in production of gametes and embryos in the final host (Amleyda-Artigas et al., 2010; Bertoni-Ruiz et al., 2011). The excessive growth and development in *Gnathostoma* larvae to represent precocity has been documented in several earlier studies (Ash, 1962). The co-infection of 2nd stage (L_2), advance 3rd stage larvae ($AdvL_3$) and mature worms in hepatic cavity formed on the surface of liver of fish, under natural conditions, has been unique. The completion of life cycle in experimental mice and role of fish as intermediate and natural definitive hosts of gnathostomatoid worms (Anderson, 1988, 2000; Anderson and Bartlett, 1993) under experimentation is reported in this investigation. The occurrence of multiple binary fission stage in muscles of natural intermediate fish host and experimental mice, have been brought on record. The autogenic life cycle and precocious development of the target specimen were peculiarly found closer to member of Gnathostomatoidea corroborated experimentally and documented as first record in Indian fresh water crowfish of the Gangetic ecosystem so far. The exceptionally short and single host specific life cycle of worms provides an outstanding way in zoonotic potential of this round worm. The nemic worms of the current investigation did not enter into 4th stage (L_4) of development. The advanced 3rd stage might transform into mature stage in liver, within body of the definitive hosts, *X. cancila*. Since a larger human population in north India is dependent on fish as staple food. Therefore, utmost care should be taken to educate people regarding ill effects of such parasitic infections. Because these worms have zoonotic potential to cause harms to human beings. This study of currently investigated gnathostomatoids is peculiarly significant because of association to homeotherms. It may also provide a new mile stone in the maintenance of healthy and disease free society based upon aquaculture.

MATERIALS AND METHODS

The findings were based upon 5 years consecutive investigation (2007–2012) in river Ganges and Yamuna at Allahabad (now known as Prayagraj), Uttar Pradesh, India (81°49′06.28″E (Lon), 25°24′53.24″N (Lat), 74m (Alt)). The *in vivo* and *in vitro* experiments were conducted in Parasitology Laboraotry, University of Allahabad (earlier known as Oxford of East), Prayagraj, Uttar Pradesh, India. The isolation, and processing of recovered nematode parasites and subsequent fixation in hot alcohol and glycerine was same as reported earlier (Malhotra et al., 2012). A sum of 42 Swiss albino mice were used in experiment during investigations after the approval of doctoral research and ethics committee, University of Allahabad (Ref. No. 216/Res/08/2096 dated 11.07.2007). These rodents were acclimatized at room temperature for 15 days, and dehelminthized after Maki and Yanagisawa (1986). The infected fish, *X. cancila* were subjected to pepsin digestion method (Chai et al., 2005) for extraction of developing larvae of zoonotic potential from muscles and viscera of host fish in laboratory (Upadhyay, 2012). The larvae were counted under stereomicroscope. The density of larvae and developing stages was expressed as Larvae per Gram (LpG). The multiple binary fission stages were washed 5 times with 0.9% normal saline and maintained in 0.85% normal saline at 25°C for 7 days. The swiss albino mice were randomly maintained in 14 groups and each group comprising 3 mice. Out of the 42 swiss albino mice, a sum of 6 mice of two random groups were taken separately and treated as control. The other 36 swiss albino mice of 12 groups were treated as experimental mice model during investigation. The experimental mice were dehelminthised by oral administration of anthelmintic drug mebendazole (Maki and Yanagisawa, 1986). The multiple binary fission stages were orally fed separately to mice and maintained at 28–30°C until examined, and later sacrificed 6-9 days post infection. The dissected liver was carefully examined for EaL_3 and $AdvL_3$ stages. The L_2 and earlier developing stages with multiple binary fission stages were recovered from muscles and viscera of experimentally infected mice by pepsin digestion method. Microphotographs were taken by image analyzer unit "MOTIC" using Biovis image plus software and Nikon trinocular computerized photomicrography unit. All the measurements of roundworms were worked out in millimeter (mm) given as range followed by mean±SE (standard error) in parentheses (Upadhyay, 2019). It was further substantiated, evaluated and validated by morphotaxometry and polythetic divisive classificatory system (≥5%) after Malhotra et al., (1981); Upadhyay et al., (2009, 2013, 2016).

Figure 1: *In situ* advanced 3rd stage larva (AdvL$_3$) of *S. multiembryonata* in *X. cancila*.

RESULTS

a. Morphology of larvae of *S. multiembryonata* (Upadhyay, 2017): The second stages (L$_2$) and advanced 3rd stage larvae (AdvL$_3$) were recovered from the hepatic cavity of fish. The tissues of liver were severely damaged due to larval pathogenicity at its advanced stages (Figure 1). The body of AdvL$_3$ was as sturdy and cuticularized as adult worms with a cephalic bulb devoid of spines, however, set off to rest of the body (Figure 2). The detailed morphometric measurements of worms were summarized in Table 1. The spination commenced from a little behind the basal muscular ridge at the base of head. The mouth was guarded by 3 lips with interlabia and 2 paired cephalic papillae as well as single larger amphid. The interlabia distinctly extended to the base of lips, where its extended arm merged into basal ridge. The typical single spines of uneven size were observed on whole body of larva. A valvular apparatus was present at the oesophageo-intestinal junction. The precocious development into paratenic forms was possessing structure of tail very similar to hinder extremity of female worms. The typical tail process of adult worms was also seen in developing larvae with caudal alae covered with spines all over. The AdvL$_3$ had all characters of a typical adult, in terms of fully developed caudal papillae. The emblematic ungulate and sessile papillae, encountered in adult female, were present in the pre-anal region of developing larvae. Extensive spinous rings were observed on caudal alae surrounding extremity of the tail and a shorter tail process in AdvL$_3$. The unique 4 pairs ventro-lateral pre-anal papillae at the distant body end of AdvL$_3$ comprised paired unequal muscular flaps, arranged longitudinal to body plane. These were emerged from the base of the elevated muscular papillated part and directed towards the proximal end of larvae. The rest of the basal portion of such terminal pre-anal papillae was encircled by irregularly alternate longer, sharp spines with a broader lower half, and simple smaller spines.

Figure 2: Caudal region of advanced 3rd stage larvae (AdvL$_3$) of *S. multiembryonata* (not to scale bar); a. Ungulate and sessile papillae, b. Typical curved tail process with caudal alae, c. Extensive spinous ring in tail process region, d. Posterior extremity with ale in dorsal view.

Table 1: Morphometric measurements (mm) of advanced 3rd stage larvae ($AdvL_3$) of *S. multiembryonata* (Upadhyay 2017).

Character	Part	Range (Mean±S.E.)
Body	L	15.354–16.758 (15.972±0.414)
	W	0.630–1.467 (0.984±0.051)
Head	L	0.027–0.054 (0.041±0.006)
	W	0.216–0.261 (0.239±0.007)
Buccal cavity	L	0.018–0.036 (0.027±0.005)
	W	0.144–0.198 (0.171±0.008)
Glandular oesophagus	L	0.630–0.864 (0.729±0.038)
	W	0.198–0.387 (0.298±0.017)
Muscular oesophagus	L	0.774–0.846 (0.799±0.016)
	W	0.360–0.594 (0.472±0.017)
Total oesophagus	L	1.404–1.710 (1.542±0.090)
	W	0.198–0.594 (0.389±0.021)
Oesophageo-intestinal process	L	0.135–0.198 (0.163±0.008)
	W	0.252–0.306 (0.273±0.009)
Cervical sac	L	1.404–1.494 (1.446±0.014)
	W	0.108–0.333 (0.208±0.016)
Distance between tail tip to anus	L	0.252–0.423 (0.346±0.030)
Tail process	L	0.063–0.090 (0.077±0.006)
	W	0.018–0.036 (0.027±0.005)

(*L* length, *W* width, *S.E.* standard error)

b. Life cycle: The investigations revealed occurrence of $AdvL_3$ stages and adult worms at the same microhabitat, *i.e.* within liver of fish, *X. cancila*. Thereby indicating, fish to be intermediate and definitive host, meanwhile these fish also served as paratenic host. The completion of life cycle of these roundworms within same fish host was thus an unique feature. The multiple binary fission stage of developing worms could be established in experimental rodent hosts. Thereby, a strong possibility of growth of this nematode in mice to apparently demonstrate a strong homeothermic affinity could not be ruled out also.

i. Under natural conditions: The adult females laid eggs (Figure 3a) with a polar plug and polar filament at one end (Figure 3b) in the lumen within tissues of liver, where eggs were released in hepatic fluid. The eggs in the stage of early cleavage (Figure 3c) migrated through venules and capillaries of hepatic-portal system of the infected fish. These stages arrived and get entangled into muscle tissues of practically every part in the body of infected fish. The stages including cleaved eggs (Figure 3d) and moulting L_2 stages (Figure 3e, f, g, h) were extracted from muscle tissues of fish during examination by tissue digestion. The elongation occurred in L_2 to attained larger size with coiled body and migration accompanied by development of a distinct tooth-like structure (Figure 3e). They rapidly grew in size to moult into early 3rd stage larvae (Figure 3i, j) within the tissues.

Simultaneously, certain eggs that encountered unfavorable developing conditions in the intrinsic environment of the body tissues of fish had undergone asexual reproduction and multiple binary fission stages were developed (Figure 3). On return of favorable developmental conditions, initially the developing embryo grew up in size within the egg, and in meanwhile progression of development in segregated eggs began. These larvae continued migration further within the muscles of body. Simultaneously at a stage, reduction in size occurred in the body of developing roundworms and final $AdvL_3$ was attained. These worms then entered into venules and capillaries to find passage to specially formed cavities with hepatic fluid within liver tissues of the same host. The $AdvL_3$ were then transformed into enormous-sized adults that attained maturity in liver. These were lodged in a cavity within liver and worms were drowned in the extracellular fluid. The mature worms could be

extracted from the cavity for collections and further study. The presence of 1 to 3 nuclei in the sections of intestinal cells of adults and larvae from liver tissues of the infected fish under natural conditions (Figure 4) illustrated their morphological features.

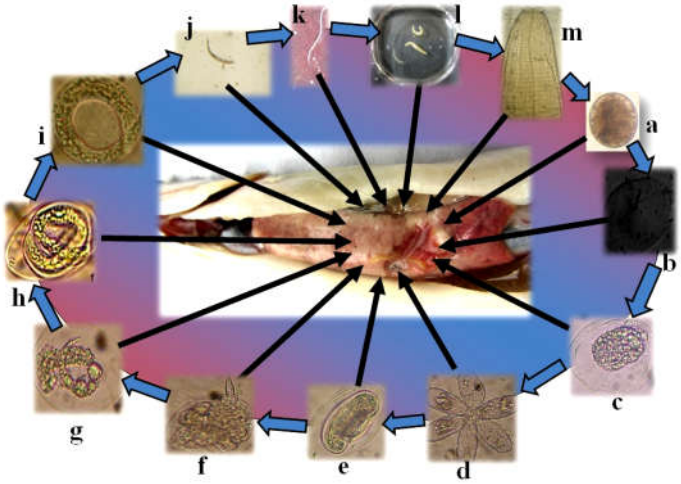

Figure 3: Developmental precocious and autogenic life cycle stages of *S. multiembryonata* in definitive *X. cancila* during natural infections (not to scale bar); a. Egg, b. Polar plug on egg, c. Early development of egg showing cleavage, d. Multiple binary fission, e. 1st stage larva (L_1), f. Tooth-like process on developing 2nd stage larva (L_2), g.h.i. Moulting second stage larvae (L_2), j. Early 3rd stage larva (EL_3), k. Advanced 3rd stage larva ($AdvL_3$), l. Freshly isolated adults, m. Anterior end of adults with peculiar spination (magnified).

ii. Under experimental conditions: ***Experimental infection of Swiss albino mice:*** The attempts were made on 36 experimental Swiss albino mice (2 groups of 12 sets, each with 3 mice). The one group of 6 sets was infected with live eggs collected from uterus of mature female nematodes infesting liver of naturally infected crow fish. The second group of other 6 sets was with multiple binary fission stages, after extracting these stages from the muscles and viscera of naturally infected garfish. The developing stages, *viz.* multiple binary fission stage, L_2 and $AdvL_3$ were recovered post infection at varied time intervals. Thus infections were successful in all the experimental mice. The rate of recovery after the experiment was ranged between 4.0–29.16% (11.76±1%). The encapsulation of larvae was not recorded up to 25th day post infection. The occurrence of encapsulated early larval stages commenced on 45th days post infection in muscle tissues and viscera. The wall of cyst was thin and encapsulations of larvae in fully developed cysts were recorded on 90th day post infection onwards. The manifestation was diagnosed within striated muscles of tongue of experimental mice due to which deshaped extremity of the tongue was visible.

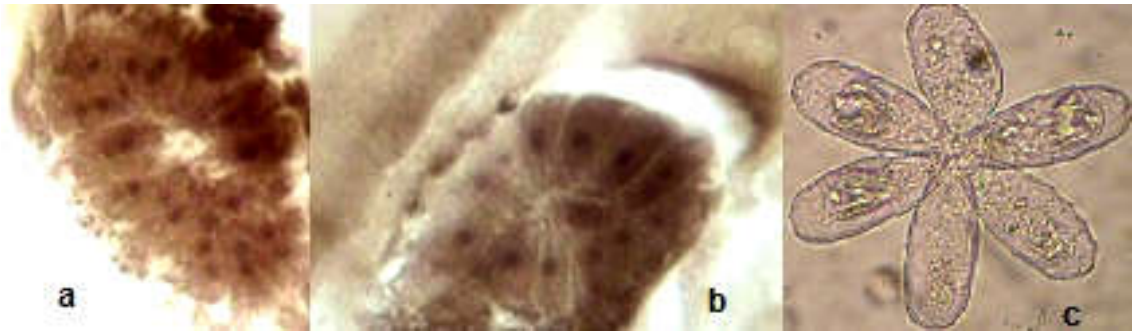

Figure 4: *S. multiembryonata* (not to scale bar). a. Transverse section (T.S.) of intestinal epithelial cells with 3 nuclei, b. Intestinal epithelial cells with single nucleus, c. Multiple binary fission stage extracted from muscles of fish.

a. Egg: The uterus contained one-celled stage of rounded to oval fertilized brownish grey colored eggs. The size of eggs were measured, 0.035–0.180(0.107±0.010) x 0.031–0.171(0.089±0.011). The egg shell surface was devoid of pits of any kind, and it had a cap-like thickening at one end that facilitated release of L_2 during hatching. A fibrillar polar filament was heavily coiled encircling polar cap at one end of the egg. The hatching commenced between 9th to 14th days.

b. Second stage larva (L_2): Sluggish movements with periodical rapid jerks were frequently observed in the newly hatched L_2 within the outer, quite capacious covering that was of much larger in size than that of moving L_2. The wriggles were visible under the transparent, smooth and delicate sheath around larva. A pertinent sharp chitinized spine-like structure, often called, "larval tooth" was typically placed at the rounded anterior extremity of larva. The body of larva measured, 10.26–13.49(14.71±0.029) x 0.29–0.43(0.35±0.002), and encircling envelope measured, 17.69–19.02(18.16±0.104) x 0.87-1.29(1.18±0.015). The functional use of larval tooth encompassed piercing to pave way for dispersal of sheath around egg, and assistance in penetration into muscles of host body during its migration within body of experimental mice.

c. Development from L_2 to early third stage larva (EL_3): The metamorphosis necessitates shortening of body of L_2 within a short span of time, accompanied by morphological changes beginning with appearance of three short minute transparent lips to replace larval tooth at anterior extremity. The larva at this stage of development attained a size of 12.04–14.28(13.21±0.003) x 0.35–1.09(0.95±0.01) at the end of 17th day. The larger granules representing growing oesophagus and intestine were visible showing marked growth during this period. The tapering of tail took shape and a sub-terminal anus appeared. The transverse rows of minute rudimentary spines started appearing all over the body behind anterior end of developing worm by 21st day, and a pair of cervical sacs reached up to the level of nerve ring. By this time moulting to EL_3 commenced. The movements in larval body ceased perceptibly at this stage except occasional movement of the anterior end (Figure 3i, j).

d. Advanced third stage larva ($AdvL_3$): Gradually EL_3 transformed rapidly into $AdvL_3$ after moulting, particularly with widening of body with further growth and development, engulfed in yellowish brown fluid, within hepatic cavity on liver. $AdvL_3$ attained maturity with distinct genital organs. No further change in morphological dimensions of body organs were encountered even after 25th day of development, though the development was observed till 95th day in experimental mice. These larvae were invariably encountered with adult worms in hepatic cavity formed over the surface of liver, which contained yellowish fluid, although coelomic fluid was transparent. The detailed measurements of various organs of the body of $AdvL_3$ are compiled in Table 1.

DISCUSSION

The remarkably giant size body of $AdvL_3$, at least 4 times greater in the size of body of any gnathostomatoid worms reported so far. The occurrence of caudal papillae resembling roundish bosses corroborated as described in *G. lamothei* and *G. miyazaki* with peculiar single-spined ornamentation all over body (Bertoni-Ruiz *et al*., 2011). The evidence of single-host life cycle was proved though the experiments and findings reflected that larvae reported here, may not enter into 4th stage of development, and $AdvL_3$ might transform into mature forms in a newer habitat, *i.e.* liver, within body of newly reported definitive hosts, *X. cancila*. The mode of infestation, establishment of nemic larvae, and development of 3rd stage larvae to adult through $AdvL_3$ without further moulting in liver of experimental rodent hosts, corroborated the findings in natural fish definitive hosts and showing precocious development (Mosqueda-Cabrera *et al*., 2009). The autogenic life cycle of worms reported herein was distinctly different from all other gnathostomatoid worms (Miyazaki, 1960; Davengsvang, 1980). The successful attainment of life cycle of the said roundworms *S. multiembryonata* was recorded as a unique new report in a fresh water garfish, *X. cancila*. The pattern of life cycle and stages were closely related to echinocephalids essentially comprised single intermediate host; however, at least two intermediate hosts along a definitive host, were required to complete the life cycle of other closely related genera *Gnathostoma*. The $AdvL_3$ of *G. doloresi* were recovered from rodent paratenic hosts under natural conditions (Imai and Hasegawa, 2001). They

also suggested that $AdvL_3$ of *G. doloresi* moulted only once to attain adult stage in final host. Therefore, the adults were 4th stage of life cycle and consequently there was no fourth stage larva in *G. doloresi* corroborated to present findings (Imai and Hasegawa 2001). It was emphasized that several fish, amphibians, reptilians and birds from Mexico served as intermediate and paratenic hosts of $AdvL_3$ stages of *Gnathostoma* (Mosqueda-Cabrera et al., 2009). The arrested development of $AdvL_3$ in the cysts embedded in muscles of second intermediate host, *viz.* tadpole, after consumption of first intermediate host like copepod was recorded (Mosqueda-Cabrera *et al*., 2009). The occurrence of monkeys as second intermediate as well as paratenic hosts of *Gnathostoma* was also on record (Soesatya, 1985). The migration of parasitic larvae of *G. turgidum* to liver, for longer time to attain juvenile phase were similar to conclusions of present investigations (Mosqueda-Cabrera *et al*., 2009). The juveniles of *G. turgidum* taken path to reached stomach for further stages of their life cycle (Mosqueda-Cabrera *et al.,* 2009) were contradicted in present study. Autogenicity in life cycle of present worms was a striking reason in contrast to depict completion of their life cycle within same water body, where $AdvL_3$ developed into adults within liver itself and no further migration took place. However, the larval stages prior to $AdvL_3$ were encountered in muscle tissues of same fish, at different locations.

Interesting variants in precocity were discussed with special emphasis on the presence of transverse rows of tiny single-pointed spines only on the anterior fourth rows of body surface of $AdvL_3$ of *G. turgidum* (Ash, 1962). The findings of the earlier workers proved a point that the two larvae collected by them did not exactly fit into the typical $AdvL_3$. The second important point, in their view, was five times larger size of larvae than expected, but without moulting. The literature cited (Anderson and Bartlett, 1993) highlighted comparable reasons of gnathostomatoid life cycle from the spirurin life history. No noticeable growth of L_3 was recorded in definitive host. The larva of former grew substantially in body size, often more than 3 times, before the moult, essentially to compensate for the absence of L_4 in *Gnathostoma*. Precocity thus could be a very valid point of discussion. It was strongly suggested that the predisposition of single moult after $AdvL_3$ to attain maturity in worms of *S. multiembryonata* (Upadhyay, 2017) could realistically be true. Thus it provided evidence of 4 stages (after 3 moults, and absence of 4th moult) in the life cycle of gnathostomatoid nematodes, instead of 5 stages (Imai and Hasegawa, 2001). The typical 4 pairs of ventro-lateral pre-anal papillae on the body of $AdvL_3$ with specialized muscular flaps were comparable with roundish bosses reported in pre-anal area of male nematodes of *G. miyazakii* and *G. lamothei* (Bertoni-Ruiz et al., 2011). The distinction of paired unequal muscular flaps around pre-anal papillae, arranged longitudinal to body plane, was a remarkable feature.

The life cycle of *S. multiembryonata* (Upadhyay, 2017) was further comparable with the species of *Trichinella*. On this account, the former resembled with genus *Trichinella* in which multiple binary fission was being reported here. However, the differentiating features in latter were polar eggs (*vs* single polar plug in present worms), aspinous body (*vs* single-spine distribution all over the body) and single spicule in male worms (*vs* 2 sub-equal spicules) (Sadaow *et al*., 2015; Gomez-Morales et al., 2018). The eggs with bipolar plugs were also recorded in *G. americanum,* and *G. malaysiae* while unipolar plugs on eggs of *G. binucleatum*, *G. lamothei* and *G. spinigerum* have been on record. The egg shell of the Mexican species of *Gnathostoma* was not pitted, but those of *G. spinigerum* and other gnathostome species had many pits on the surface, on the contrary, the present worms did not have pits on the surface of egg shell (Miyajaki, 1960, Soesatya, 1985; Koga *et al*., 2003).

Besides absence of 4th moult stage from the life cycle of gnathostomatoids, that has been identical with similar feature in present worms, the presence of a cuticularized spine-like tooth, called larval tooth, in L_2 has shown remarkable similarity. Under natural conditions this spine-like larval tooth supposed to used for exsheathment and penetration to the wall of digestive tract of copepod intermediate hosts. In present case, larval tooth presumably helped in migration of larvae to reach up to its final destination, *i.e.* liver *via* hepatic-portal venules. Resultantly, as the larva grew predominantly in muscles of host body, and undergone 3rd moulting to attain EL_3 stage (Mosqueda-Cabrera et al., 2009; Bertoni-Ruiz *et al*., 2011).

The role of number of nuclei in intestinal cells was significant in differentiation and species identification of gnathostomatoids (Sohn and Lee, 1998; Camacho *et al*., 2002). But the features of nuclear patterns in intestinal cells of *S. mutiembryonata* (Upadhyay, 2017) were not compatible with those of *G. binucleatum, G. doloresi, G. hispidum* and *G. nipponicum* (Miyajaki, 1960). The other species under genus *Gnathostoma* were closer to present worms in this regard. However, an average of 2 and 3–4 nuclei per intestinal cell in *G. binucleatum* and *G. spinigerum* respectively (Camacho *et al*., 2002); mainly 2 in *G. doloresi*, and 0–2 nuclei in *G. hispidum* have been reported. The specimens of *G. nipponicum* showed 1 cell with no nucleus, 12 cells with 1 nucleus, 6 cells with 2 nuclei, and 3 cells with 4 nuclei (Ando *et al*., 1988).

The larval stages recovered from smaller cavities in liver of fish resembled more closely to the EL_3. It reflected post-moult development of larvae into $AdvL_3$ stage. The subsequent enlargement of body along with growth and development of genital organs within liver was investigated. The advancement of L_3 took place as simultaneous events, and the absence of L_4 appeared to be a stronger likelihood of precocious development. The substantial growth in size of $AdvL_3$ of *Gnathostoma* was often three times more before the moult (Imai and Hasegawa 2001). In an opinion such unusual preparatory growth occurred to compensate the absence of L_4, and developmental changes demonstrated a close relationship with the absence of L_4 (Anderson and Bartlett 1993; Cho et al., 2007). The precocious development though, occurring during L_3 to transform it to mature worms, after the final moult, with identical pattern of spination and musculature, as seen in caudal papillae of adult male worms in cavity within liver tissues of fish, was a distinct possibility (Ash, 1962). The apprehension of an association of phylogenetic affinity between a primitive paratenic host and the natural definitive host can further be tested on the basis of detailed search for experimental hosts from a wide variety of groups that have already been the subject of experiments in current study. The findings of this study were, further in agreement with *Gnathostoma*, *Spiroxys*, and presumably, all other gnathostomatoids had only four stages, *i.e.* three larval stages and one adult stage, in their life cycles (Imai and Hasegawa, 2001).

The migration of $AdvL_3$ to reach up to the striated muscles of tongue of experimental mice and potential of development of these worms in a homeotherm during present study received support from yesteryears findings where cysts of *Trichinella* were recorded from the striated muscles of tongue of rats (Fankhauser, 2005). The migration and development of *G. procyonis*, a nematode of zoonotic significance has been described in mammalian hosts, particularly raccoons that were experimentally infected with $AdvL_3$ (Anderson, 1988). The edible fresh water fish have played an important role in transmission of a variety of gnathostomatoids to human beings, because of the fish being their second intermediate host. The finding of infective $AdvL_3$ of *G. nipponicum* in leftover largemouth bass, *Micropterus salmonoides* meat was recently associated as source of infection in humans to explain indirect zoonoses (Ishida *et al*., 2003). The experimental evidences reflected the causes of zoonoses due to infection of present roundworms. The infestation caused poor development, morbidity and sometimes death by compromising nutritional status, affecting cognitive processes, including tissue reactions, such as granuloma, histolysis and necrosis. Through the current investigation author supposed to recommend the maintenance of healthy and disease free society based upon aquaculture through education about host parasite interactions and their bioecology.

CONCLUSION

The study reflected that larvae of *Sturdynema multiembryonata* may not reach to the fourth larval stage (L_4) of development, and the advanced 3rd stage larvae ($AdvL_3$) might transformed into mature forms in a newer microhabitat, *i.e.* liver, within body of newly reported definitive host, *Xenentodon cancila*. The pattern of infestation and establishment of the target nematode larvae and attaining of maturity from AdvL3 larvae without moulting into 4th stage larva in experimental rodent host corroborated the findings in natural fish definitive hosts. The autogenic lifecycle and precocious development of present finding was unique and substantiated *in vitro* successfully. Since a larger human population in Indian continent is dependent on fish as staple food, therefore, utmost care should be taken to educate people regarding ill effects of such parasitic infections that have zoonotic efficiency to harm them. This study is particularly significant because the association of currently investigated

gnathostomatoids of pisces to homeotherms assessed. The implications and causes have also been worked out that may provide a new mile stone in the maintenance of healthy and disease free society based upon aquaculture.

SIGNIFICANCE' STATEMENT

The autogenic life cycle and precocious development in present findings were substantiated and proved *in vitro*. The utmost care should be taken to educate people regarding the ill effect of such parasitic infections with zoonotic potential and health problems.

CONFLICTS OF INTEREST

The author claims no conflicts of interest because none financial support was received from any government, non-government agency or organization to conduct this research work.

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