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Schistosomula of *Schistosoma Mansoni* and *Schistosoma Margreboweie*in the Mouse Liver; a Histological Study

I. B. KALHORO[++], H. KALHORO*, SAFIA KALHORO**, SAIKA KALHORO***

Department of Anatomy & Histology, Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University, Tando Jam.

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Abstract: The histo-morphological developmental changes in schistosomula of two groups, *Schistosoma (S) mansoni* and *S. margreboweie* inside the liver of definitive host mice was studied at Days post infection (DPI) 7, 10, 14, 21, 28, 35 and 42.In histologically divisions schistosomula groups, *S. mansoni* and *S. margreboweie* appeared on 21, 28 and 35 DPI and 10, 14, 21, 28, 35 and 42 DPI respectively in the mice liver. According to the results in branches of hepatic arteries and veinsthe number of *S. mansoni* and *S. margreboweie* was six and twelve respectively. At 14 DPI, schistosomula group *S. margreboweie was* divided into three regions, the thin anterior, wide middle and thin posterior regions. Afterwards DPI 35 schistosomula group *S. mansoni* was conflicted at middle body region, noticeable substantial musculature nearby the ventral sucker as well as an enlargement in gut caecum was observed. Both groups, *Schistosoma (S) mansoni* and *S. margreboweie* mean and standard deviation remained 128.41±22.002 µm and 91.00+-32.41µm. The variance in body diameter of both groups, *Schistosoma (S) mansoni* and *S. margreboweie* were statistically analyzed and found to be significantly different. ($P < 0.1191$). Both groups, *Schistosoma (S) mansoni* and *S. margreboweie* means and standard deviation of the cuticle thickness stood 8.50±1.20 µm and 6.70±4.10 µm respectively. Schistosomula of two groups, *Schistosoma (S) mansoni* and *S. margreboweie* resulted difference in cuticle diameter and found to be significantly same ($P > 0.4841$).

Keywords: - Histo-morphology, Schistosomulum, *Schistosoma mansoni*, *Schistosoma margreboweie* and Liver.

1. INTRODUCTION

Schistosomiasis a disease caused by the s*chistosoma* species, significant parasite of humans (Jamison *et al*., 2006). *S. margrebowiei* Le Roux, 1933 effects usually upon antelope, cattle and waterbuck in Southern and Central Africa (Ogbe, 1985). Schistosomes are known to be trematodes. Possessing dioecious character at the mature stage and located in definitive host's blood vessels. A number of hermaphroditictrematodes are positioned in the tract of intestine as well as organ, just like the definitive host liver. The lifecycle of schist some comprises of two particular hosts: a host definitive (i.e. human) and a single definitive host snail. The cercaria emerges during day light within the snail by which they impel themselves in water by the means of their diverged tail, vigorously seeking out from their host definitive. After the identification of the human dermis, they breach it within very short time. The entire process take place in three phases. An initial attachment to the dermis, followed by the cercaria which creeps over the dermis finding a appropriate assimilation site, generally a hair follicle and preceding assimilation into epidermis using proteolytic secretions beginning by the cercarial post-acetabular and ending at pre-acetabularglands. Schistosomule, an endoparasitic larva transmutes from the head of cercaria while penetration. Schistosomulum, a cercarium without tail remains few days on the dermis and afterwards within 3-4 days its allocat towards the lungs (Brindley, *et al*., 2009).After the entrance towards dermal lymphatic and venulescirculation, they survived upon blood, regurgitating "haem" in form of hemozoin (Oliveira. *et al.,* 2000). Schistosomulum migration route concerning lung and hepatic aperture system and its variance has been reported by many researchers. After leaving lungs, parasites enters the thoracic cavity, penetrating through the diaphragm, liver capsule also its tissues to extent the vein of hepatic portal (Bruce *et al***.,** 1974).The Schistosomula takes between 8 and 20 days to complete the migration route from the dermis towards the hepatic portal system which is wholly intravascular (Miller and Wilson, 1980). Parasites move back-up of pulmonary artery and up the vena cava and liver vein and momenta ring inside the right side of the heart and the hepatic sinusoid to extent the vein of hepatic portal (Gorgei, *et al***.,** 1986).By way of pulmonary vein parasites passing heart by left side meant for the distributing within the body in proportion to cardiac

[++]Corresponding author: Prof Dr. Illahi Bux Kalhoro, Email: ibkalhoro@gmail.com
*Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University, Tando Jam.
**Faculty of Crop Production, cs Engineering, University of Sindh Jamshoro, PakiSindh Agriculture University, Tando Jam.
***Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro, Pakistan

output. Those Schistosomula are found to be stuck in the liver when they enter splanchnic arteries traverse capillary beds to the liver portal structure. The remaining total traverses capillary beds systemically to the heart venous compartment and returns back towards the lungs. Inside the liver, a number of circuits arise from the vasculature before they be trapped in a site which is found to be critical for developmental process. When the stage of DPI 5-7, Schistosomules were observed moving by means of circulation starting the left side of heart towards the hepato-portal circulation (>15 days) (Beltran and Boissier, 2008). Temporary passing inside the pulmonary capillaries they moved to the systemic circulation and in the end carried to the host mesenteric vein (Brindley, *et al*., 2009). The aboral surface of mature schistosomes which had been carefully visualized in a number of scanning electron microscope (SEM) studies (McLaren, 1980), that also revealed tegumental alterations after schistosomicides treatment (MagalhãesFilho, 1987) or afterwards incubation in various media (Kalapothakis, *et al*., 1988). In recent times, the research has evaluated that the gynaecophoric canal possess spines at the anterior region, enormous volume of tubercles in comparison with the other spines in which prevailed form the right side of the canal. At the distal extremity region the excretory pore possessing a "volcano gate-like" feature was sited (Machado-Silva *et al*., 1997). Nevertheless, the aboral surface of the mature schist some is studied by scanning electron microscopy (SEM) and it was not conceivable to evaluate the inner organization (McLaren, 1980).The present paper describes the histo-morphological developmental changes under light microscope (LM) in the schistosomula of two groups, *Schistosoma (S) mansoni* and *S. margreboweie* inside the liver of ultimate host mice thru infections.

2. MATERIALS AND METHODS

A number of thirty five mice of female sex belonging to Original (BKTO) strain of Bantim and Kingman Tyler, having a weight of around 20-35g each were originally obtained from Lochinrar National Park, Zambia. Individually all mice were infested with a number 200 cercariae of *S. mansoni* (Puerto Rican strain)and *S. margreboweie.* The *S. mansoni*cercariae retained inside *Biomphalariaqlabrata* albino snails and randomly bred mice by following the procedures of Taylor *et al*., (1969). While *S. margreboweie* retained inside *Bulinusnatalensis* intermediary host snails (original stock obtained: Experimental Taxonomy Unit of the British Museum of Natural History, London). The investigational animals anaesthetized with Sodium pentobarbitone (Nembutal) earlier the cercariae application and clipped from the part of the abdominal hairs. Application of cercariae at the abdominal dermis was applied by using the ring. The total number of mouse was sacrificed at DPI 7, 10, 14, 21, 28, 35 and 42 proceeding with autopsies was done instantly afterwards by dislocating the neck region. Histologically, the preservation each mouse was performed in Heidenhain's Susa fixative followed by washing with dehydrated ethanol and infiltration and embedding in historesin. Selected thick sections of size 4 µm in polychrome method were stained. The interpretation of the selected sections was performed on Ernst Leitz Light Microscope (Model No. 786554 Germany). Further, mean values (± SD) for each parameter for both groups of parasites were calculated.

Statistical analysis performed by the statistical software Graph PadInstat was subjected to ascertain the variation in these mean values among schistosomula of both groups.

3. RESULTS

1) Determination of the schistosomulum of both groups *S. mansoni* and *S. margreboweie* with in mouse liver.

Total quantity as well as determination of **schistosomulum of both groups** *S. mansoni* and *S. margreboweie* parasites are presented in **(Table 1).** According to our findings, *S. mansoni was not visible within mice liver* mice on 7, 10, 14 and 42 DPI. While S. *margreboweie* on 7 DPI was not visible. By means of sections histologically schistosomula *group S. mansoni* was perceived at 21, 28 and 35 DPI while the *S. margreboweie* were observed perceived in the liver of mice at 10, 14, 21, 28, 35 and 42 DPI. It is clearly indicated in the Table no.1 that *S. margreboweie* appeared earlier as well as remained longer within the liver in comparison with the second group of parasite.

In branches of hepatic arteries and veins, number less six and highest twelve of *S. mansoni* and *S. margreboweie* were observed respectively. Furthermore schistosomula existed in highest proportions in the interior branches of hepatic vein and arteries in mouse.

Table 1. Location and quantity of schistosomula both groups *S. mansoni* and *S. margreboweie* in the mouse Liver

DPI	Species	Quantity	Location
7	*S. mansoni*	-	-
	S.margreboweie	-	-
10	*S. mansoni*	-	-
	S.margreboweie	2	Hepatic portal vein Branch
14	*S. mansoni*	-	-
	S.margreboweie	3	Hepatic portal vein Branch
21	*S. mansoni*	2	
	S.margreboweie	5+1	Hepatic portal vein Branch + hepatic artery
28	*S. mansoni*	1	Hepatic artery Branch
	S.margreboweie	1	Hepatic portal vein Branch
35	*S. mansoni*	3	Hepatic portal vein Branch
	S.margreboweie	1	Hepatic portal vein Branch
42	*S. mansoni*	-	-
	S.margreboweie	1	Hepatic artery Branch

2) Histology of schistosomulum of Both Groups *S. mansoni* and *S. margrebowiei*:

Both parasites of schistosomula were determined through transverse and longitudinal sections in mice liver. Variable stained red light with a darker outward circular, middle level and muscle fibers possessing longitudinal and noticeable ridges were identified on the schistosomulum cuticle. PAS positive material having limited granules were also detected on the cuticle. Enormous numbers of nuclei having inconstant shape displaying particular spaces in the midpoint were seen inside the schistosomulum body.

Furthermost nuclei cells presenting uniformly stained chromatin lined at nuclear membrane also within insufficient nucleoli as well as swelling musculature known as the PAS positive material are visible. On day 10, stained yellow and red pigments inside the gut caecae were detected. On 14 DPI *S. margreboweie* body was screening anteriorly, mid and posteriorly regions. In the anteriorly thin region oral sucker packed with stained dark nuclei, unoccupied space along within few areas fiber muscles were visible. While inside the wide mid region, noticeable nuclei layer surrounding the gut caeca was visible. Uterus possessing a muscular thick wall was seen by right side of the gut caecum. Gynaecophoric canal owning stained dark nuclei were visible surrounded by a prominent muscular layer from the lateral position of schistosomula. **(Fig.1).**

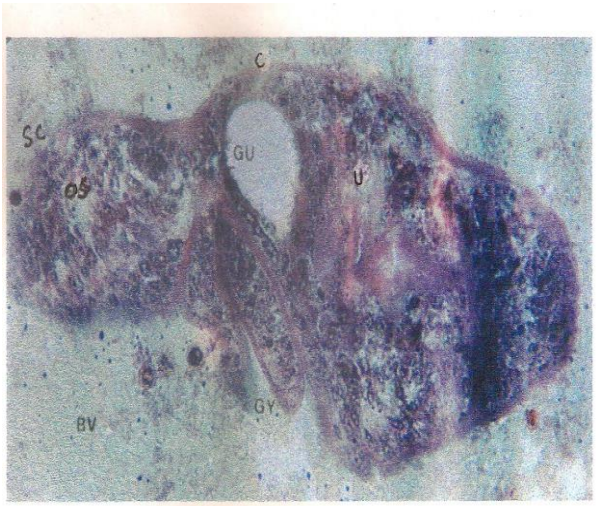

FIG. 1 Schistosomula of *S. margrebowiei* developed in the liver of mouse after 14 dpi. 25µm

Abbreviation: - BV = Blood Vessel, C = Cuticle, DPI = Days post-infection, GC = Gut Caecum, GY = Gynaecophoric canal, OS = Oral sucker, PA = Parenchymal cells, S= Schistosoma, SC = Schistosomula, U = Uterus.

Afterwards DPI 21 and 28, in the gut of schistosomulum an increment in thickness of muscular wall and diameter was observed. Besides this a widening of uterus and muscular wall thickness observed on DPI 35. Afterwards DPI 42 late

schistosomula consisted of parenchymal cells in enormous quantity along with body musculature and gut caecum. Subsequently DPI 21, gynaecophoric canal within gut caecum body and testis in transverse section appeared in *S. mansoni*. which on further at DPI 28 a thick muscular wall at the gut caecum as well as PAS positive granules were noticed schistosomula of both groups. After DPI 35 *S. mansoni* comprised a narrowing at mid region of body, heavy conspicuous musculature surrounding the areas of ventral sucker by enlarged gut caecum.

Ventral sucker forming an oval shape enclosed with thick muscular layer in addition stained heavily dark nuclei in middle region of the body **(Fig. 2).** By means of histological sections, the gut caecum, uterus and gynaecophoric canal measurement and thickness of diameter are shown in **(Table–2).** An greater than before thickness and gut caeca diameter of *S. margreboweie* on days 21 and 28 can be clearly shown from the **(Table. 2)**

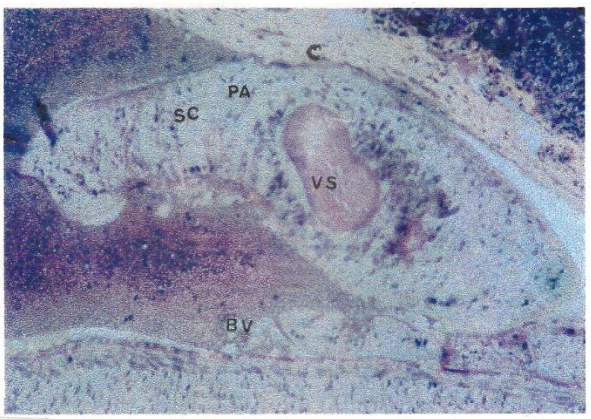

FIG. 2 Schistosomula of *S. mansoni* developed in the liver of mouse after 35 dpi.

Abbreviation:- BV = Blood Vessel, C = Cuticle, DPI = Days post-infection, PA = Parenchymal cells, S = Schistosoma, SC = Schistosomula, VS = Ventral Sucker.

Table - 2 Diameter and thickness of various organs of Both Groups

Days Post Infection	Organ	*S. mansoni*(µm)		*S. margreboweie* (µm	
		Diameter	Thickness	Diameter	Thickness
10	Gut caeca	-	-	34.27	3.16
14	Uterus	-	-	-	3.80
	Gynaecophoric canal	-	-	19.04	1.90
21	Gut	-	4.76	38.39	8.40
	Gynaecophoriccanal	15.23	2.85	-	-
28	Gut	78.06	9.99	94.24	4.76
35	Uterus	-	-	11.42	3.80
42	Gut	-	-	41.88	-

3) Size of liver schistosomulum

A type of transverse and longitudinal forms of worms appeared in total observations of the schistosomula. Afterwards a comparison of sizes to determine the maximum diameter of the schistosomula segments was performed. Our results showed that *S. mansoni* and *S. margreboweie* mean and standard deviation remained 128.41±22.002 µm 91.00±32.41µm respectively. The body diameter difference of both groups when statistically analyzed between 10 to 42 DPI remained significantly different. ($P < 0.1191$). Mean and standard deviation of cuticle thickness of *S. mansoni* and *S. margreboweie* remained 8.50±1.20 µm and 6.70±4.10 µm respectively. The results were considered significant ($P > 0.4841$) after statistically analyzing the difference in diameter of the cuticle of both groups. Form the obtained results a significant difference in the thickness of cuticle among the both species of schistosomula ranging by 10 to 42 DPI. **(Table No. 3).**

Table -3:- Body Mean diameter and cuticle thickness of *S. mansoni* and *S. margreboweie* in mouse liver.

DPI	*S. mansoni*		*S. margreboweie*	
	Body (µm)	Cuticle (µm)	Body (µm)	Cuticle (µm)
7	-	-	-	-
10	-	-	60.00	2.90
14	-	-	70.80	3.50
21	103.30	8.10	115.20	6.02
28	144.22	7.61	82.90	5.71
35	137.72	9.83	72.40	7.61
42	-	-	144.70	14.30

4. DISCUSSION

The findings of the current study of *S. margreboweie* were observed from an interval 10, 14, 21, 28, 35 and 42 DPI. Whereas, in *S. mansoni* was detected at DPI 21, 28 and 35. No any schistosomula were stated on day 7 in both groups. Similar observations were stated for *S. mansoni* by Wheater and Wilson, 1979; Bloch, 1980; Miller and Wilson, 1980; Crabtree and Wilson, 1984.Distribution of parasites to systemic organs, following exit from the lungs, paralleled the fractional distribution of cardiac output has been studied in other studies. Schistosomula in the hepatic porch scheme were accumulated by 21 DPI.

Merely 2-3 permits of bugs surrounding the vascular scheme may be in requirement for the production of hepatic porch populace (Wilson *et al*., 1986). According to Beltran and Boissier (2008), schistosomule roams on the way to the lungs 5–7 days post-penetration moving via flow by left side of the heart to hepatoportal flow at 15 days, these statements are closely related to observations with the current study for *S. margreboweie*.

In current finding, schistosomula physique of both groups were considered by the enormous facts of small nuclei variably formed and expanding gut caecum in the muscular wall consisting of nuclei. A laterally attachment of gynaecophoric channel, uterus with dense strong fence, oral and ventral saps occurred noticeable within the schistosomula body.

In the current finding the cuticle of both groups schistosomula were branded with flexible PAS-granules prominent ridges and internal longitudinal strong strands prolonging towards regions of body.

Our findings are closely similar with Crabtree and Wilson, (1980) and Voge, *et al.*, (1978) with exception in the cuticle mean wideness of the schistosomula though, their outcomes disclosed that tegument shallow endured rough depending on notch of body delay which was thrown in to sloping edges and cribs from 4 to 14 DPI (Crabtree and Wilson, 1980). The tegumental shells of the young male and females were alike, dorsal and ventral shells of the male are alike beforehand the development of the gvnaecophoral channel (Voge, *et al.*, 1978).

Recent finding regular yellow and red marked pigments were visualized in the *S. margreboweie* gut caecae on day 10. Similar explanations had been reported by Clegg, (1965) after 14 DPI *S. mansoni* gut caecae occupied with pigments, resulted from the hemoglobin digestion and maximum food was utilized of worms for the period of rapid growth later 14 DPI is probably absorbed through the caecae. This type of variance probably due to days, parasite specie and host.

Initial growth of the gynaecophoric channel on the cross delays of *S. margreboweie* body was found in current study. Though, these findings on 21 DPI in *S. mansoni* and 20 DPI in *S. margreboweie* were reported by Clegg (1965) and Ogbe (1983).

S. mansoni, histologically comprised with a constriction at mid-body section, areas of ventral sucker surrounded by prominent heavy musculature and laterally enlarged gut caecum.Although, the outcome stated by Clegg, (1965) gut caecae of *S. mansoni* is posteriorly linked to the ventral sucker, which is in agreed with the recent finding.

Recent revision the mean and standard deviation of the body diameter was 91.00±32.41µm of *S. margreboweie*. The results are similar at 16 DPI which were reported by Voge, *et al*., (1978) that dorsal and ventral shallow of *S. margreboweie* worms was 0.4 mm long.

It may be concluded on the basis of our findings that the variances in observation, number and location of both groups of schistosomula *S. Mansoni* and *S. margreboweie* at the interval of 7 to 42 DPI in the mice liver was examined. Schistosomula, in histological sections was categorized by prominent ridges of various muscle fibers, cuticle, uniformly stained nuclei and some spaces were detected within the body. At 14 DPI to onwards wide thin regions were detected in the body of schistosomula, in which various organs such as oral and ventral suckers, gut caecum and uterus in female and gynaecophoric canal was also observed in the male worm

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