

A STUDY OF THE SUCKERS OF SCHISTOSOMA SPINDALE

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Abstract: *Schistosomiasis in Bubalus bubalis is caused by the trematode, Schistosoma spindale. It inhabits the portal and mesenteric veins of the primary host, Bubalus bubalis. S.spindale is a dioecious species. Females live most of the time wrapped in the gynaecophoric canal of the males. The oral sucker surrounds the mouth and ventral sucker is located posterior to the bifurcation of the gut. The significance of the suckers in the helminth parasite can be suggested by its ability to adhere to the blood vessels. So also to engulf contents of blood through the suckers. The suckers play a definite role in the schistosome life history. An indepth study of the suckers of S.spindale have been undertaken in the present study. The oral sucker was found to be present at the terminal region. The Ventral sucker was found to be round and pedunculated. The inner surface of sucker is found to have numerous spines by Scanning Electron Microscopy. The study of whole mount and Scanning Electron Microscopic work of S.spindale have been carried out in the present investigation.*

Key Words: *Schistosoma spindale, Oral sucker, Ventral Sucker, Scanning Electron Microscopy (SEM).*

1. INTRODUCTION:

Schistosoma spindale is the parasite which causes hepato-intestinal Schistosomiasis in buffaloes. Schistosomiasis is a fifth major helminthiasis of domestic animals in the Indian sub-continent (Sumanth et al.2004). *Indoplanorbis exustus* is the intermediate host of *S.spindale*. Schistosomes show considerable sexual dimorphism. The adult female resides within the adult male's gynaecophoric canal. It is a modification of the ventral surface of the male forming a groove. The paired worms move against the flow of the host's blood. They find their niche in the mesenteric circulation where they begin egg production. The eggs move into the lumen of the host's intestine and are released into the environment with the faeces. The characteristic of the parasite to survive in the host's blood lies in the sucker, which engulfs contents of the blood. It also aids the parasite in adhering to the surface of the blood vessel. The suckers play a vital role in the adaptation of Schistosome in the host's blood. The present investigation is an attempt to study sucker regions of *S.spindale*.

2. LITERATURE REVIEW:

Schistosoma spindale was discovered by Montgomery in Muktesar, India (Montgomery, 1906 b). Vrijberg (1907) recorded *S.spindale* in Sumatra. Soparkar published a detailed description of the cercaria of *S.spindalis* in 1921. Fairly and Jasudasan (1930) dealt with the seasonal infection of the snails, *Indoplanorbis exustus* with the cercaria of *S.spindale*. Fairley and Mackie (1930) dealt with the pathological changes with the infection of *S.spindale* brought about in the host tissues. A comparative study of *S.spindale* and *S.nasale* was made by Rao (1934). The nervous system and esterase distribution in *S.spindalis* were demonstrated by Rao et al. (1982). The acetylcholinesterase activity was prominent in the nervous tissue, oral sucker, pharynx, ventral sucker and excretory bladder of *S.spindale*. He compared the two schistosomes from different stand points, e.g. the shape of the egg and the morphology of the miracidium, cercaria and adults and concluded that the two forms were distinct species. Studies on the morphological, biochemical and histochemical aspects of the trematode, *S. spindale* was done by Vanita (2007). Comparison of the oral sucker, ventral suckers and the genital pores of female *S.japonicum* worms derived from water buffalo and goats by SEM was done by Yang et al (2013). He studied the microarray analysis of gene expression profiles of *S. japonicum* derived from less susceptible host water buffalo and susceptible host goat (Yang, et al., 2013). Studies of *S.spindale*, Montgomery, 1906 was done by Vanita et al. (2017). Ultrastructural study on morphology of *S. spindale* was studied by Sudhakar Et al. (2018). They investigated the morphological features along with morphometry of different structures of *S.spindale* which were visualized by Scanning Electron Microscopy (SEM). Morphological observations of Adult *S.spindale* was done by Vanita et al (2018). Histochemical localizations of malate dehydrogenase in *S.spindale* was done by Vanita et al (2019). Studies done by various researchers reveal significant aspects of the parasite, *S.spindale*. The present study is an added dimension to the existing lacuna of the structural characteristics of suckers of *S.spindale*.

3. MATERIALS AND METHOD:

Collection of parasites: The intestine of buffalo infected with *S.spindale* were collected from the local slaughter house in Hyderabad. Mesenteric portal veins were observed and localization of the parasites in blood vessel of the intestines was done. The parasites were collected by dissecting and then with the help of needle and brush.

Histology: The histology of the parasite's anatomical structure was done by paraffin embedding process of the parasite to make blocks which were sectioned and stained using Grenacher's Borax Carmine and Ehrlich's Haematoxylin-Eosin. (Brancoft, 1975).

Light Microscopy: The morphology was studied by flattening between two slides and fixed in 4 % formalin for 24 hours. These worms were used for morphological studies after staining them using stains like Borax Carmine and Haematoxylin Eosin stain.

Scanning Electron Microscopy: Adult male and female *S. spindale* were obtained from the mesenteric veins of naturally infected *Bubalus bubalis* in Hyderabad, India. The parasites were fixed in 2.5% Gluteraldehyde in 0.05M phosphate buffer (pH 7.2) for 24 hr at 4⁰C and post fixed 2% aqueous Osmium tetroxide in the same buffer for alcohol and processed for 2 hr. After the post fixation samples were dehydrated in series of graded alcohol and processed for critical point drying with Electron Microscopy Science CPD Unit. The dried samples were mounted over the stubs. Finally, applied a thin layer of platinum metal over the sample using an automated sputter coater (JEOL JFC-1600) for 5 min. Then samples were observed and scanned in SEM (JOEL-JSM 5600) at various magnifications. SEM studies were carried as per the principles and techniques described by John and Lonne (1999).

4. RESULT:

Light Microscopy:

- Anterior end of the male *S. spindale* stained by Borax Carmine seen under light microscopy and highlights oral sucker, ventral sucker of *S.spindale*.(Fig.1A,2A,3A,4A,5A,6A,7A,8A).
- Sections of the anterior end of the *S.spindale* stained by haematoxylin eosin stain. (Fig. 9A,10A,11A,12A,13A,14A,15A,16A,17A,18A)
- Oral sucker is sub-terminal and is obliquely placed in both male and female. (Fig.1A,2A,3A,4A,5A,6A,7A,8A).
- Male *S.spindale* longitudinal section showing oral sucker, ventral sucker and the gynaecophoric canal.(Fig.11A)
- Section of the Oral sucker of male *S.spindale* stained by Haematoxylin eosin stain. Dark blue stained are the nucleated regions and light pink are the cytoplasmic regions stained with haematoxylin. (Fig.13A, 18A). The rim of the oral sucker of the male is muscular and thick. Its lumen is funnel shaped which terminates at the commencement of the alimentary canal.
- Section of the female *S.spindale* showing Ventral sucker. (Fig.14A).
- Longitudinal Section of the ventral sucker of the male *S.spindale* showing haematoxylin stained regions. Nucleated regions are stained dark blue and cytoplasmic regions stained light pink.(Fig.10A).
- Section of the cup shaped, pedunculated Ventral sucker of the male *S.spindale*. (Fig.15A). Section of the male *S.spindale* showing oral sucker and the ventral sucker.(Fig 9A, 12A,16A,17A).

Scanning Electron Microscopy:

- SEM of male *S.spindale* with female in the gynaecophoric canal.(Fig.1B).
- Anterior region of the male having female in the gynaecophoric canal showing dimensions of oral sucker and ventral sucker(Fig.2B)
- Anterior region of the male *S.spindale* showing oral sucker and the ventral sucker(3B)
- Oral sucker of the male *S.spindale*(Fig 1B,2B,3B). The surface of the oral sucker is completely covered with numerous blunt spines directed inwardly. SEM Studies showed spines in the oral sucker(4B,5B,6B,7B,8B). Concentric layers of ridges and grooves were seen in the oral sucker The oral sucker is surrounded by aspinose muscular wall.
- Ventral sucker of the male *S.spindale* (Fig 1B,2B,3B). The ventral sucker is a fairly large protrusible organ and is infundibular in form with its margin more or less wavy. It is cup shaped and borne on a peduncle of the male *S. Spindale*. Ventral Sucker also shows concentric ridges and grooves with spines under high magnification (Fig.9B,10B,11B,12B).

5. DISCUSSION:

The results of the present study have revealed significant information about the structure of *S.spindale*. Oral sucker is sub-terminal. Ventral sucker is pedunculated and cup-shaped organ. The surface of the suckers are smooth when seen through Light microscopy. But when seen through Scanning Electron Microscopy (SEM), it shows minute spines in a circular manner in the oral sucker and the ventral sucker. Ultrastructure of the ventral sucker of *S. mansoni* cercaria was studied by Carolyn Cousin et al(1995). He found ventral sucker to be cup-shaped structure consisting of complex of circular and longitudinal muscles in the *S.mansoni* cercaria. Sudhakar et al. (2018) investigated the morphological features along with morphometry of different structures of *S.spindale* which were visualized by Scanning Electron Microscopy(SEM) and found the oral sucker and ventral sucker to be 2.6 μ m and 2.5 μ m. The oral sucker is surrounded by an aspinose muscular wall. The pedunculated ventral sucker was spinulate(Narain and Mahanta,1990). The present study showed concentric layers of ridges and grooves having spines on the oral sucker and the ventral suckers of *S.spindale*. The spines in the oral sucker and ventral sucker measured from 1.5 μ m to 2.5 μ m. The results of the present investigation was analogous to results of the work done by the researchers in *S.spindale*. These studies on the sucker of Schistosomes portray a significant aspect of the sanguivorous life.

6. CONCLUSION:

Adaptation to parasitic mode of life entails many modification in the organisms. To understand the phenomenon of parasitism various studies have been carried out. The present work on the study of sucker of *S.spindale* have been made to investigate the structural characteristics of suckers. The significance of suckers can be accounted by the fact that it aids in engulfing nutritional contents of the blood and also to attach firmly to the surface of the blood vessels. These parasites are unacceptable threat to domesticated animals in many parts of the world. Intestinal Schistosomiasis due to *S. spindale* is an economically important blood fluke infection widespread in India and other developing countries. It is manifested as a chronic diarrhoeic disease if a large number

of worm pairs inhabit the mesentery as per the studies done by Agarwal and Southgate, 2000. According to Degheidty and Shalaby, 2010 an understanding of micro-morphological features plays an important role in the development of vaccines. El-Shabasy et al., 2015 had revealed that alterations in the ultrastructure of schistosome worms were useful for evaluation of antischistosomal drugs for which there is necessity of understanding of normal ultrastructural morphology. Thus, the present study gives an insight about the structure of suckers of *S.spindale*, for future research.

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Annexure: Figures



1 B



2 B



3 B



4 B



5 B



6 B



7 B



8 B



9 B



10 B



11 B

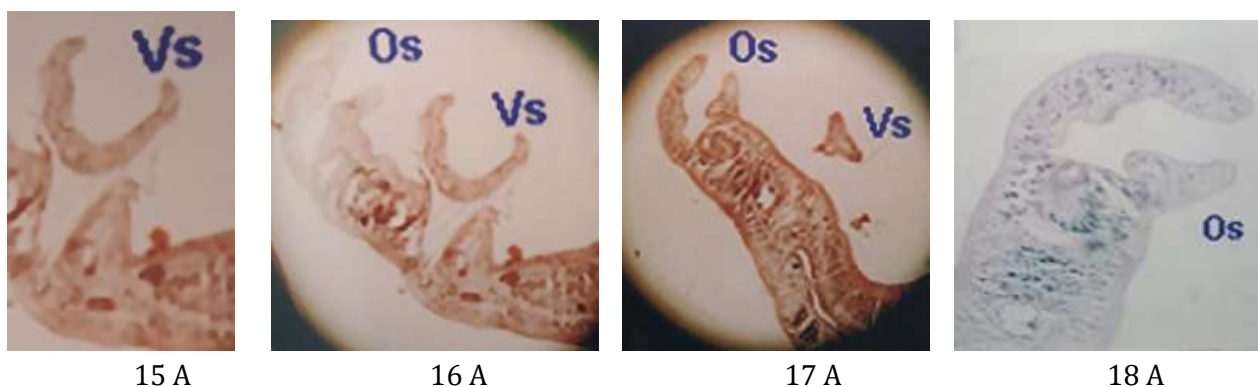
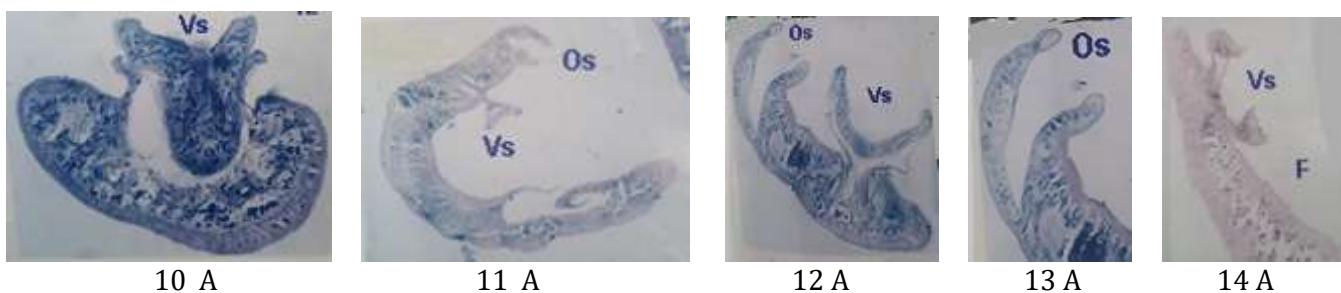
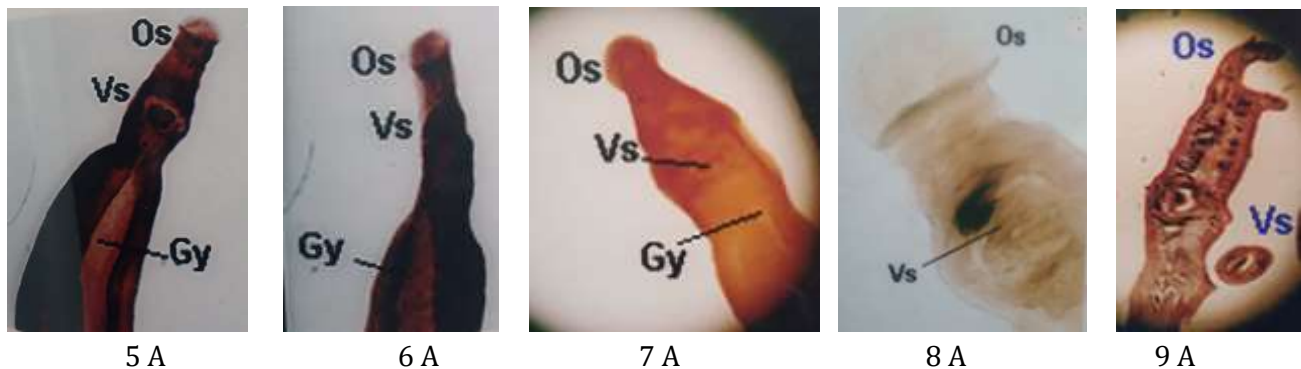
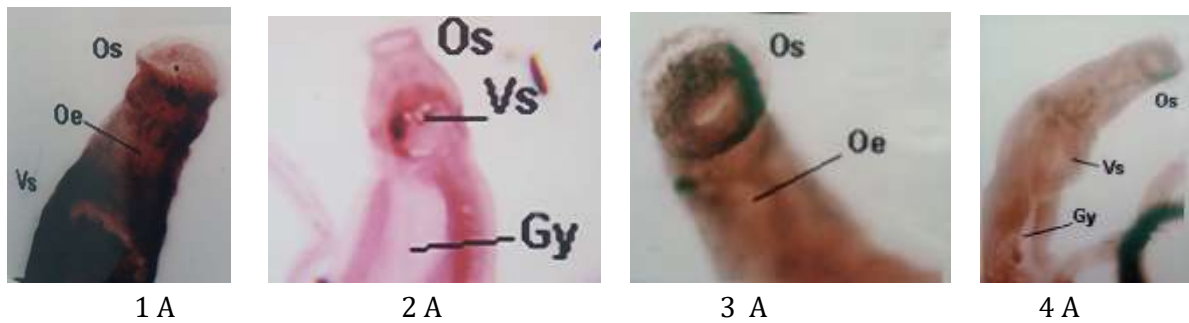


12 B

Scanning Electron Microscopic photographs of *S.spindale*:

Fig.1 B. SEM of Male *Schistosoma spindale* with female in the gynaecophoric canal $\times 50$. Fig.2 B. Anterior region of male having female in the gynaecophoric canal showing dimensions of oral sucker and ventral sucker $\times 85$. Fig.3B. Anterior region showing oral and ventral sucker $\times 200$. Fig.4. Oral sucker of the male *S.spindale* $\times 270$. Fig.5B. Magnified view of the oral sucker showing circular regions having spines $\times 750$. Fig. 6 B. Enlarged view of the oral sucker $\times 1800$. Fig.7 B. Spines of the oral sucker $\times 3700$. Fig.8 B. Spines of oral sucker under higher magnification. Fig. 9 B. Ventral sucker of the male *S.spindale* . $\times 1000$. Fig.10 B. Magnified view of the ventral sucker $\times 2200$. Fig. 11B. Enlarged view of part of ventral sucker $\times 4300$. Fig.12 B. Spines of the ventral sucker under high magnification

Annexure: Figures



Suckers of *Schistosoma spindale* as seen through Light Microscopy: Anterior end of *S. spindale* showing oral sucker and ventral sucker stained with Borax carmine (Fig.1A,2A,3A,4A,5A,6A,7A,8A) and haematoxylin eosin stained sections of the anterior regions of *S. spindale* (Fig.9A,10A,11A,12A,13A,14A,15A,16A,17A,18A). Fig.10A. Longitudinal Section of the ventral sucker of the male *S. spindale* showing haematoxylin stained regions. Nucleated regions are stained dark blue and cytoplasmic regions stained light pink. Fig.11A. Male *S. spindale* longitudinal section showing oral sucker, ventral sucker and the gynaecophoric canal. Fig.14A. Section of the female *S. spindale* showing Ventral sucker. Fig.15A. Section of the Ventral sucker of the male *S. spindale*. Section of the male *S. spindale* showing oral sucker and the ventral sucker. (Fig 9A, 12A, 16A, 17A). Fig.13A, 18A. Section of the Oral sucker of male *S. spindale* stained by Haematoxylin eosin stain. Dark blue stained are the nucleated regions and light pink are the cytoplasmic regions stained with haematoxylin.