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Original Research Article

ULTRASTRUCTURAL SURFACE TOPOGRAPHY OF *FASCIOLA GIGANTICA* (TREMATODA: DIGENEA) FROM INFECTED LIVER OF BUFFALOES (*BUBALUS BUBALIS*) IN UDAIPUR, INDIA

G. SWARNAKAR* AND R. N. DAMOR

Parasitology Lab, P.G. Department of Zoology, Government Meera Girls College, M. L. S. University, Udaipur (Raj.), India

Author for Correspondence: gswarn9@yahoo.co.in

ABSTRACT

Adult liver flukes *Fasciola gigantica* have a flat body and leaf-like in shape with knife-like anterior and blunt posterior ends. *Fasciola gigantica* are more elongated than *Fasciola hepatica.* The tegumental ultrastructural surface topography of liver fluke *Fasciola gigantica* trematode parasite was studied by scanning electron microscopy (SEM) first time in Udaipur. Live liver flukes *Fasciola gigantica* were collected from liver of naturally infected buffaloes from abattoir in Udaipur. SEM observations showed few basic ultrastructural differences. *F. gigantica* have dorso-ventraly flat body, leaf-like in with sharp pointed anterior end and blunt posterior end, body of the worm divided into three regions such as anterior, middle and posterior regions, its tegumental surface appears rough due to occurrence of many different size, shape and arrangement of spines, sensory papillaes, transverse folds and grooves. Oral sucker (anterior sucker), genital pore and ventral sucker (Acetabulum) are present on ventral side of the body. Both oral and ventral suckers are spineless and covered thick rims of transverse folds. Small sized, closely-packed pointed spines are present on the anterior part of the ventral and dorsal surface of the worm. Large sized spines with increasing in number are observed in ventral and dorsal side in the middle region of the liver fluke. Short size, few in number and scattered spines, covered with tegumental surface are present in the dorsal and ventral side of posterior region of worm.The aim of the present study was to identify of the species of Fasciola present in Udaipur. The present research work would be significant because it will provide knowledge for morphological and ultrastructural characteristics of *F. gigantica* from infected liver of buffaloes (*Bubalus bubalis*) in Udaipur for fascioliasis treatment and chemotherapeutic measures.

Key Words: Ultrastructure, *Fasciola gigantica,* sensory papillae, spine, oral sucker and ventral sucker.

INTRODUCTION

Fascioliasis has been recognized as an important helminthic disease of livestock causing significant loses to livestock owners, on account of poor growth and lower productivity of domestic ruminants. Liver flukes *Fasciola gigantica* and *Fasciola hepatica* are very dangerous because they are causes various mechanical and biochemical damages to buffaloes. *Fasciola gigantica* is trematode parasites, it is one of the most abundant and damaging flat worm of buffaloes. *F. gigantica* causes fascioliasis disease, this disease shows some external and internal symptoms such as; traumatic hepatitis, hepatic fibrosis, hyperplasic cholangitis, jaundice, anaemia and oedema (bottle jaw) in domestic buffaloes. Fascioliasis is a significant live Stock problem; yearly an estimated US $ 2-3 billion are forgone due to weight loss, severe reductions in milk and meat yield as well as losses due to decreased fertility in production animals [1-8]. Due to the high level of prevalence and intensity of natural infection, Fasciola appears to be endemic in this geographical region and probably represent one of the most important animal health problems.

F. gigantica and *F. hepatica* have been reported to present in the worldwide. In India the treatment of Fasciolosis is very costly and unaffordable to owner of livestock. Some researchers were reported ultrastructural surface topography in liver flukes *F. gigantica* and *F. hepatica* [9-26, 28-30] but not in Udaipur, Rajasthan and India.

Fascioliasis disease due to *F. gigantica* were observed in Udaipur [27], Rajasthan and in India but none of scientist paid attention to study the surface topography at ultrastructural level in liver flukes *F. gigantica.*

The present study is to determine the various detail structure of surface tegument of *F. gigantica* by scanning electron microscope (SEM). The present research work would be significant because it will provide knowledge for morphological and ultrastructural characteristics of *F. gigantica* for fascioliasis treatment and chemotherapeutic as well as phytothereputic measures. Findings of the study will improve socio-economic condition of the cattle farmers of Udaipur by removing pathogenic liver fluke *Fasciola gigantica* parasites.

MATERIALS AND METHODS

1. Collection of live liver flukes:

Live liver flukes were collected from the infected liver of freshly slaughtered domestic buffaloes from local zoo abattoir and meet markets of Udaipur. The infected part of liver from time to time were brought to the laboratory and washed several times in the tap water and then they were transferred into 0.9% physiological saline. After removing the parasites carefully, from the liver, they were again washed several times in the physiological saline before fixation to remove debris and mucous etc. Such worm then fixed in different fixatives for whole mount preparation of *Fasciola gigantica* & *Fasciola hepatica* and ultrastructural study of *Fasciola gigantica* by scanning electron microscope (SEM).

2. Whole Mount Preparation of *Fasciola gigantica* and *Fasciola hepatica*:

Since different *Fasciola gigantica* and *Fasciola hepatica* species infect the liver of buffalo at a time, it is therefore necessary to make them whole mounts in order to identify them by light microscopy, based on their morphological characters. *Fasciola gigantic* and *Fasciola hepatica* have their distinct features like shape, size, topography of the various organs and other structures distinction. The whole mounts of the present *Fasciola gigantica* were identified according to the above mentioned characters. Live liver flukes were relaxation and fixed in hot AFA (Alcohol 85 ml. formalin 10 ml. and acetic acid 5ml.) at (80˚ to 85˚C) was then gradually poured in to the beaker, which not only fixed them but also made them completely relaxed. Such *Fasciola gigantica* were then pressed between two sides (to make them flat) and left in cold AFA till use. Then fixed *Fasciola gigantica* and *Fasciola hepatica* were removed from fixative, washed several times in distilled water and transferred in to chlorinated alcohol for bleaching for twelve hours.

Bleached *Fasciola gigantica* and *Fasciola hepatica* were washed in 70% alcohol, stained with alcoholic borax carmine for 5 min. and

differentiated in acid for a minute, dehydrated, cleared in clove oil for twelve hours, cleared *Fasciola gigantica* and *Fasciola hepatica* were mounted in DPX and examined both in dissecting and compound microscope to finally identify them.

3. Ultrastructural study by scanning electron microscope (SEM):

Live *Fasciola gigantica* were washed three to five times, fixed in with saline solution (0.9 percent, NaCl), then fixed overnight at 4°C.

The fixative was 4 percent glutaraldehyde in 0.1M cacodylate containing 3 percent sucrose and 0.5 mM $CaCl_2$. The *F. gigantica* were then washed for 24 hours in buffer (pH 7.2) containing 3 percent sucrose and 0.5mM $CaCl_2$ post fixed for 1 hr with 1 percent osmium tetroxide (aqueous) and dehydrated in an ethanol series. Drying and coated with gold using sputter and then observed with resolution scanning electronic microscope (SEM). Ultra-microphotographs were taken under ZEISS scanning electronic microscope at Regional Electron Microscopy Facilities, AIIMS, New Delhi

RESULTS

The tegumental ultrastructural surface topography of liver fluke *Fasciola gigantica* trematode parasite was studied by scanning electron microscopy (SEM) first time in Udaipur. *Fasciola gigantica* are commonly occurring in the bill ducts and liver of buffaloes in Udaipur (Fig. 1).

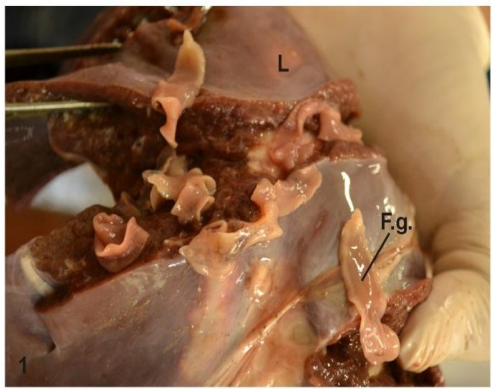

Figure 1: *F. gigantica* (F. g.) are present in the liver (L) of buffalo.

Adult liver flukes *Fasciola gigantica* have a flat body and leaf-like in shape with knife-like and tapering anterior and blunt posterior ends. *Fasciola gigantica* are more elongated 6-7 cm than *Fasciola hepatica* 3.5 cm in length and width of *F. gigantic* 1-1.5 cm where as in the *F. hepatica* width is larger 1.5 – 1.8 cm in the middle region.

F. gigantica have dorso-ventraly flat body, leaf-like in shape with sharp pointed anterior end and blunt posterior end, body of the worm divided into three regions such as anterior, middle and posterior regions (Fig. 2), its tegumental surface appears rough due to occurrence of many different size, shape and arrangement of spines, sensory papillaes, transverse folds and grooves.

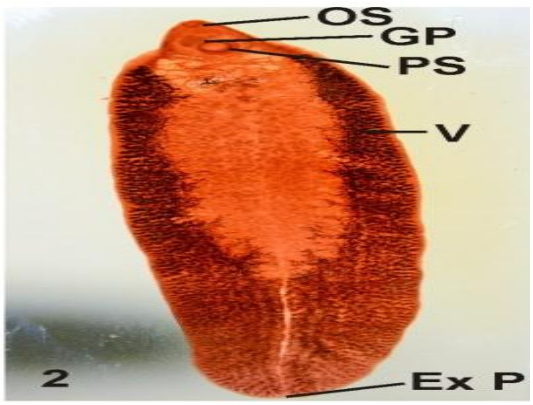

Figure 2: Full whole mount liver fluke *F. gigantica* with oral sucker (OS), genital pore, posterior sucker (PS), vitelline gland cells (V) and excretory pore (Ex P).

Oral sucker (anterior sucker), genital pore and ventral sucker (Acetabulum) are present on ventral side of the body. Both oral and ventral suckers are spineless and covered thick rims of transverse folds. Oral sucker open into the pharynx. The genital pore is located between oral and ventral suckers and near to the ventral sucker (acetabulum) some time it is also known as posterior sucker. The genital pore is a common opening of male and female reproductive system of the liver fluke because *F. gigantica* is hermaphrodites. Sometimes genital pore shows everted cirrus with small scattered spines, this type of genital structure also known as genital apparatus (3). Three types of sensory papillaes are observered such as: type 1. Papillaes are bulbous and smaller in size with smooth surface, type 2. Papillaes are bulbous in shape with nipple like tips without cilia and type 3. Papillaes are bulbous shape have nipple like tips with short cilia. On the basis of arrangement of the spines, sensory papillae and tegumental transverse folds the body of *F. gigantica* can be divided into three regions viz., anterior, middle and posterior regions (Figs. 3, 4 and 5).

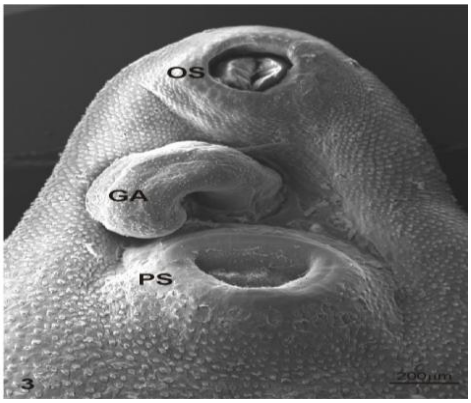

Figure 3: Ultra-micrograph by scanning electron microscope is showing anterior region of *F. gigantica* with oral sucker (OS), genital apparatus containing everted cirrus (GA) and posterior sucker (PS) X 200 µm.

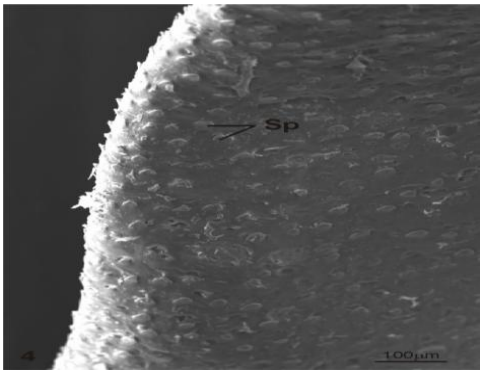

Figure 4: Ultra-micrograph by scanning electron microscope is showing middle region of *F. gigantica* with spines (Sp) X 100 µm.

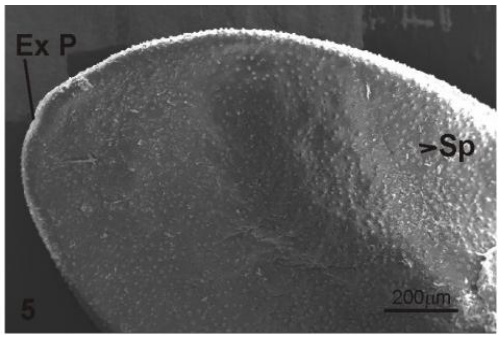

Figure 5: Ultra-micrograph by scanning electron microscope is showing posterior region of *F. gigantica* contains scattered spines (Sp) and excretory pore (Ex P) X 200 µm.

Anterior region: Spines are small sized, increasing in numbers and closely-packed present on the anterior part of the ventral and dorsal

surface of the worm. The surface of spines appears pointed and comb-like edges. Between the spines the surface area of the shows alternate groove and transverse folds. Type 1. Papillaes are observed around the oral and ventral suckers but they are bulbous, numerous, large in size with smooth surface and present in the cluster form. Type 2 and 3 Papillaes are bulbous in shape with nipple like tips without cilia and without cilia observed in clustered form in anterior region and ventral side but dorsal have few papillaes (Figs.3 and 6).

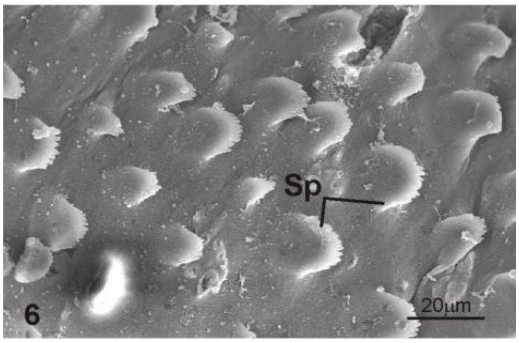

Figure 6: High power magnified ultra-micrograph by scanning electron microscope is showing comb-like spines (Sp) on *F. gigantica* with spines X 20 µm.

Middle region: Large sized spines with increasing in number are observed in ventral and dorsal side in the middle region of the liver fluke. The large size spines with sharp comb-like edges are examined on middle region of the body. type 1 sensory papillaes are in clustered form, larger in numer and short size sensory papillaes and spins are revealed on dorsal side of middle region of the body (Fig. 4).

Posterior region: The spines are gradually decreased in size and number. Spins are observed in scattered form, small in size, covered with tegumental surface, they are not comb-like and pointed. Dome shaped type 1 sensory papillaes but they are not in clustered form are present in the dorsal and ventral side of posterior region of worm. Prominent excretory pore present in on the blunt posterior tip and few spines are also observed posterior end of the body of *F. gigantica* (Fig. 5).

DISCUSSION

Fascioliasis disease has been observed in the domestic buffaloes due to infection of liver fluke *Fasciola gigantica* in the Udaipur. Whereas, fascioliasis diseases widely prevalent in domestic ruminant in all over the world, due to presence of common liver fluke *Fasciola gigantica* and *Fasciola hepatica* [1-7, 27] .Ultrastructural study of surface topography of *F. gigantica* showed same general patterns as that described in the *F. gigantica* and *F. hepatica* by other scantiest in all over the world. Whereas, few different ultrastructural variation were observed in the present study that all three types of sensory papillae such as; type 1. Papillaes are bulbous and smaller in size with smooth surface, type 2. Papillaes are bulbous in shape with nipple like tips without cilia and type 3. Papillaes are bulbous shape have nipple like tips with short cilia were present in the anterior region of the body of *F. gigantica* found in the buffaloes of Udaipur. The presence of three types sensory papillaes were indicated specialization of the tegumental functions like improve excretion, absorptive capacity and increase ionic and osmoregulation in the tegumental surface. This type of structural characters also reported in some other digenean trematodes and helminths[13-21]. Also spines are small and more in numbers in same region were indicted that this spines helpful in movement and attachment to the liver of buffaloes. Whereas, spines are absent on the oral and ventral suckers but type 1. Papillaes are observed around the oral and ventral suckers but they are bulbous, numerous, large in size with smooth surface and present in the cluster form. These papillaes provide smooth sealing with mucous of liver and bile duct of buffaloes [8-11, 28-30]. Large and numerous sensory papillae were present on oral and ventral suckers which are responsible and make pressure receptors whereas other types of sensory papillae were distributed all over the body surface they act as tango receptors[12, 15].Type 1 papillae bulbous, Comb-like and pointed spines of the tegument and ventral sucker may help to make strong connection and attachment with the bile ducts during flow of bile juice and helpful in the movement in the liver of host. Tegumental transverse fold present groves and ridges form in all over the body of the worm. These surface folds helpful for increase of surface area and improve the absorption of nutrition, important chemicals and exchange of micromolecules from liver of host. Similar structure also recognised by other researchers in some trematodes[15-26]. The presence of spines and sensory papillaes type 2 bulbous in shape with nipple like tips without cilia and type 3 papillaes are bulbous shape have nipple like tips with short cilia were present on genital apparatus everted cirrus of *F. gigantica*. These papillaes and spines may help in successful transport of sperms and cross fertilization.

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CONFLICT OF INTREST

Authors declare no Conflict of Interest.

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