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Morphological Structure of Tegument in Fasciolahepatica Affecting Sheep in Saudi Arabia

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Abstract: ***Fasciola was one of the first infectious agents to be discovered and implicated an etiologic agent of disease . Fascioliasis is one of the most important parasitic diseases of domestic ruminants causing severe economic losses throughout the world. Additionally, it is increasingly being recognized as an important emerging/re-emerging infection of humans. In this study we used scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques to identify and characterize the species of Fasciola present in Saudi Arabia(KSA). This is the first morphological and untrastructural study on Fasciola in the country. Prior to this study ,the species of Fasciola in KSA had not been properly identified by any technique. Accurate identification of the species is the initial step for its control, as identification followed by biological and ecological characterization is provides the necessary information for field management of the species.***

Keywords: Fasciola hepatica, tegument, TEM, SEM, KSA.

1. Introduction

Fascioliasis is one of the most important and common parasitic diseases of domestic ruminants throughout the world, causing immense economic losses. In addition, it is increasingly being recognized as an important emerging/re-emerging infection of humans **(Chen and Mott, 1990; Mas-Coma et al., 2009).** There is significant potential for geographic expansion of fascioliasis due to the considerable colonization capacities of its causal agents and vector species. Of vector-borne diseases, fascioliasis has the widest known latitudinal, longitudinal, and altitudinal distribution, **(Mas-Coma et al., 2003),** recent reports suggest the epidemiology of Fasciola spp is changing with prevalence in Europe country **(Rojo-vazquez et al .,2012)** and in Saudi Arabia in Taif area their study confirmed the prevalence of Fascioliasis in slaughter cattle's and were recorded 52% **(Degheidy and Al-Malki 2012)**. The etiologic agents are various species of liver flukes belonging to the genera Fasciola and Fascioloides. The most important species of Fasciola are F. hepatica Linnaeus, 1758, and F. giganticaCobbold, 1856. Fasciola hepatica has a cosmopolitan distribution and primarily inhabits regions with temperate and subtropical climates. Fasciola gigantica predominant in the tropical and subtropical regions of Africa, Asia, and Southeast Asia (Mas-Coma and Bargues,1997 ;Spithill et al.,1999 Lotfy and Hillyer,2003 ;). Other minor Fasciola species include F. nyanzaeLeiper, 1910 reported from the hippopotamus in Uganda, F. tragelaphi (Pike and Condy,1966) found in the Sitatunga-antelope, and F. jacksoniCobbold, 1869 found in the elephant. The two formers are limited to isolated areas of East Africa, the latter is endemic to India and Pakistan (Lotfy et al.,2008). In the Kingdom of Saudi Arabia (KSA), fascioliasis was reported was reported in imported and local animals **(Abou-Zinadah and Fouad, 2005; Sanad and Al-Megrin, 2005, Al-Megrin ,2010, Degheidy and Al-Malki 2012)**. However, the species of Fasciola present in KSA was never properly identified. In any country, accurate identification of the resident species of Fasciola is critical , as identification followed by biological and ecological characterization provides information that is necessary for the field control of pathogen **(Lotfy et al., 2002).** Different parameters were used to differentiate between F. hepatica and F. gigantica. These include morphology and ecology, host-parasite relationships, karyotyping, genotyping , molecular genetics, and biochemical as markers **(Lotfy and Hillyer, 2003)**. The aim of the present study was to identify of the species of Fasciola present in KSA based on morphological and ultrastructural characteristics.

2. Materials and Methods

2.1 Collection of adult Fasciola specimens

Adult Fasciola specimens were isolated from the bile ducts of sheep slaughtered at the slaughterhouse of Dammam (Eastern Province, Saudi Arabia). After its collection from the host liver, flukes were washed in physiological saline (0.9% NaCl solution).

2.2 Morphological identification of adultFasciola specimens:

The species of Fasciola were identified by using the morphological and morphoanatomical criteria described before by others **(Watanabe, 1965;Lotfy et al., 2002).** Transverse sections of some adult flukes were stained by haematoxylin and eosin to study the layers of the tegument**. (Hanna et al ., 2013)**

2.3 Preparation for examination by SEM:

Flukes were Initially flat-fixed for 30 min at room temperature in a 3:1 mixture of 4% glutaraldehyde and 1% osmium tetroxide this procedure according **(Luna ,1968;McConville et al .,2009; Naem et al., 2012)** and viewed in a JEOL 6400 scanning electron microscope operated at voltage of 25-20 kV.

2.4 Preparation for examination by TEM:

Flukes as we do in SEM but the Specimens were dissected and we used Only the mid-body for this TEM and the

procedure according as **(Sobhoh et al., 2000)** and prepare specimens in the Central Laboratory of the University of King Saud and also viewed by JEOL 1011CX transmission electron microscope at 80 kV.

3. Results

A total of 123 Fasciola flukes were collected from 26 imported sheep. We could not succeed to find infection in endogenous animals**. (Fig. 1).** By Scanning electron microscope (SEM) examination, the outer surface of the flukes, with the exception of the rims of both suckers, was seen covered with spines direct towards the posterior. the spines were tightly-packed, were longer than they were broad, and had distinctive serrations along their tips **(Fig. 3)** .In the fluke samples collected during the present study the cuticular spines of the dorsal side of the acetabular region were small and thin which confirmed the diagnosis of F. hepatica **(Fig. 2,3,4).**

Histological and untrastructural examination during the present study revealed that the tegument of the Fasciola flukes was a syncytial epithelium. It was composed of an outer anucleate distal cytoplasm that was connected by cytoplasmic strands to nucleated cell bodies, known as tegumental cells, which were located in the parenchyma beneath the basal lamina and muscle layers. The tegument matrix contained cytoplasmic organelles, contractile vacuoles, dense secretory bodies and pinosomes **(Figs 5 & 6).,** It is a syncytial epithelium composed of an outer anucleate distal cytoplasm connected by cytoplasmic strands to nucleated cell bodies (tegumental cells) located in the parenchyma beneath the basal lamina and muscle layers. The tegument matrix contains the usual cytoplasmic organelles (mitochondria, Golgi bodies and endoplasmic reticulum), contractile vacuoles, dense secretory bodies and pinosomes. one type produce G1 granules and the other produces G2.However, only Two type of cell is present which produces G1 and G2 granules. These two criteria confirmed the diagnosis of F. hepatica **(Fig. 7, 8).**

4. Discussion

Previous studies differentiated F. gigantica from F. hepatica on account of its elongated body, less thinner body, less developed shoulders, larger ventral sucker, larger testes area, anterior positioning of the testes and the presence of numerous secondary intestinal branches. In F. gigantica, the intestinal caeca are characterized by the presence of numerous secondary as well as tertiary median branches, Conversely F. hepatica, has few secondary median branches which are similar to small pouches. Additionally , F. hepatica has a simpler system of lateral branching than that of F. gigantica. The lateral branches in F. gigantica are at approximately right angles to the main stem, whereas in F. hepatica they have a pronounced backward direction and in some specimens are almost parallel to the main stem. The posterior half of the F. hepatica body gradually narrows into a V-shaped outline, while in F. gigantica the narrowing only commences at a very short distance from the tail end **(Watanabe, 1965; Lotfy et al., 2002).**

Previous studies were differentiated F. gigantica from F. hepatica on account of the cuticular scales of the dorsal side of the acetabular region. In F. gigantica the cuticular scales are longer, stouter and broader at their roots, while in F. hepatica they are smaller and thinner. Moreover, in F. gigantica prominent fine striations was observed on the surface of the cuticular scales which are absent in F. hepatica **(Watanabe, 1965; Lotfy et al., 2002;).**Based on the presence of various organelles and the density of the cytoskeleton, the tegument of adult Fasciola flukes is divided into four layers of specialized functions **(Threadgold, 1967; Sobhon et al., 2000)**.The four layers are essentially identical in both species.

However, differences between the two species have been reported with regards to the tegumental granules and cells that produce them. Two types of tegument cells were reported in F. hepatica, one type produces G1 granules and the other produces G2 .However, only one type of cell is present in F. gigantica and it produces both G1 and G2 granules (corresponding G1 and G2 granules). Moreover, the number of G1, granules in adult tegument of F. hepatica is relatively lower than the number of G1 granules in F. gigantica **(Threadgold, 1967; Sobhon et al., 2000).**

5. Conclusion

Based on the afro mentioned results the present study confirmed the presence of F. hepatica in imported sheep in the study area. We didn't detect any F. giganticainfections. This may indicate that endogenous Fasciola infections are not common in the study area. Further studies are needed to assess Fasciola prevalence through the country.

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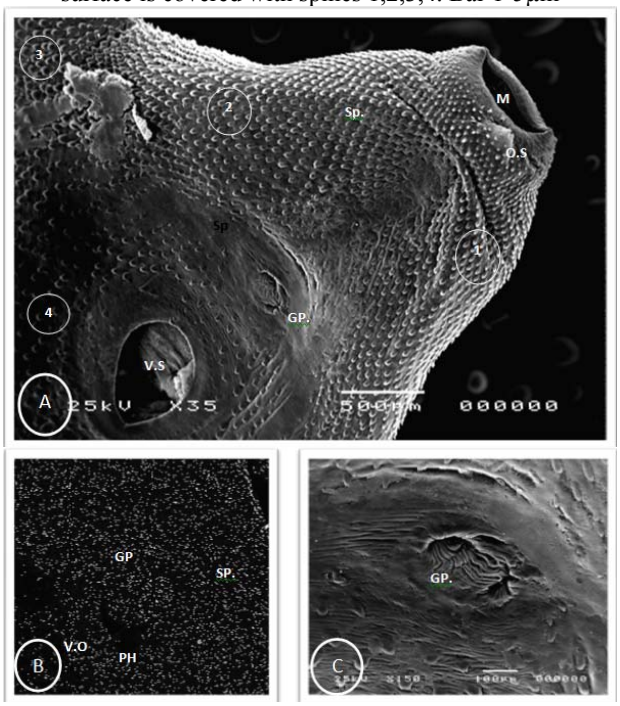
Figures

Figure 1: Whole mounts of adult Fasciola flukes

Figure 2: (A,B,C)Surface topography of the anterior portion of adult Fasciola fluke. Ventral surface of the apical cone showing the oral sucker (O.S), mouth (M.) ventral sucker (V.S) and gonopore (GP.)Pharynx (PH)Bar500µm . The surface is covered with spines 1,2,3,4. Bar 1-5µm



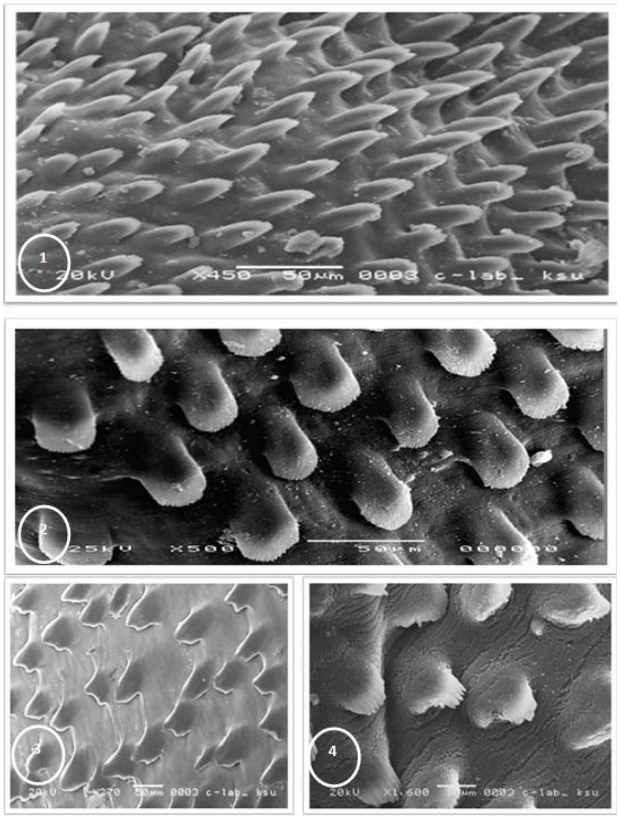


Figure 3: The Fasciola covering by spine in the dorsal surface (A) Bar 100µm, and (B) showing the spine in high magnification, Bar 10 µm , in (C) the large marginal of spine(SP.)have serration (arrows)like-comb Bar 5 µm

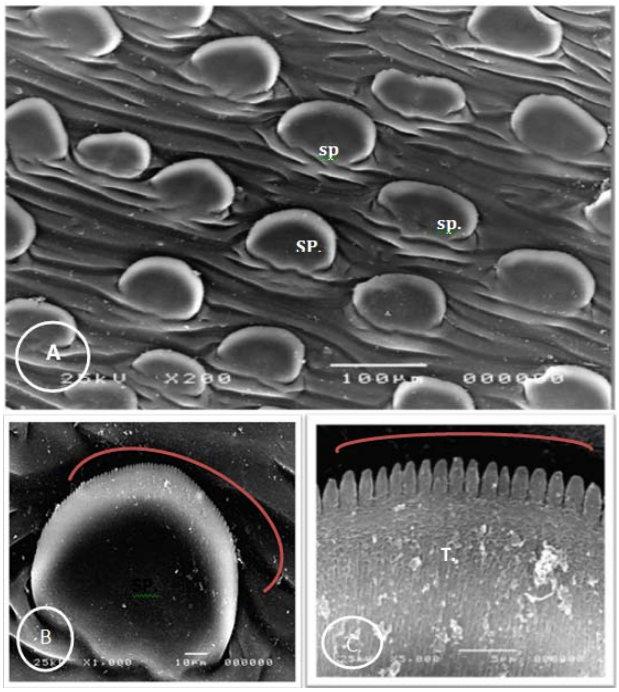


Figure 4: The surface of anterior portion in adult Fasciola fluke showing the oral sucker (O.S.) in high magnification present the opening mouth (M.) (A.) have sensory papillae (P.) (B, C) Bar10 µm

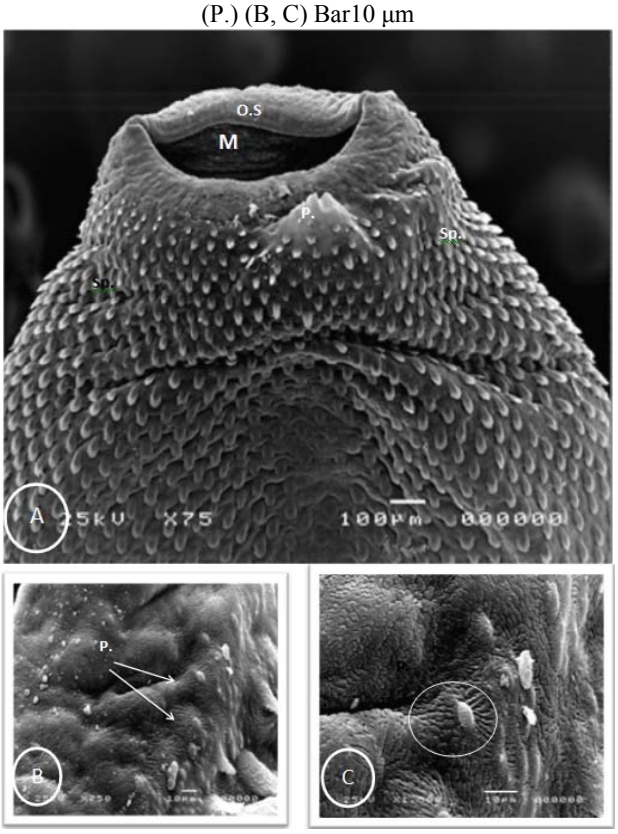
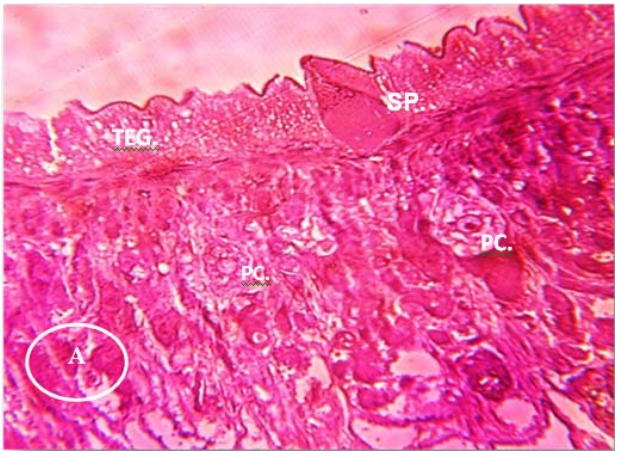


Figure 5: General view of tegument layersin the first layer present tegument (TEG.) and spine (SP.) by staining H&E (A), (B) In large magnification showing parenchyma cell (PC.) and X40



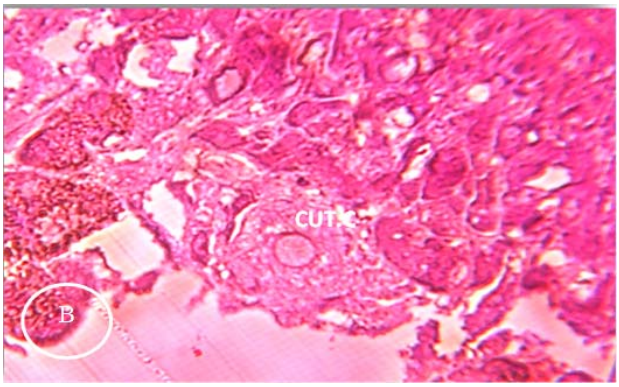


Figure 6: General view of tegument of an adult Fasciola fluke showing ridge (Ri.) and pit (Pt.),Valley (V), cuticle (CU), basement membrane (BM) ,protoplasmic tube (PT), circular muscle (CM), longitudinal muscular (LM), apical cuticle (AP), parenchyma cell (PC) ,Bar2 µm

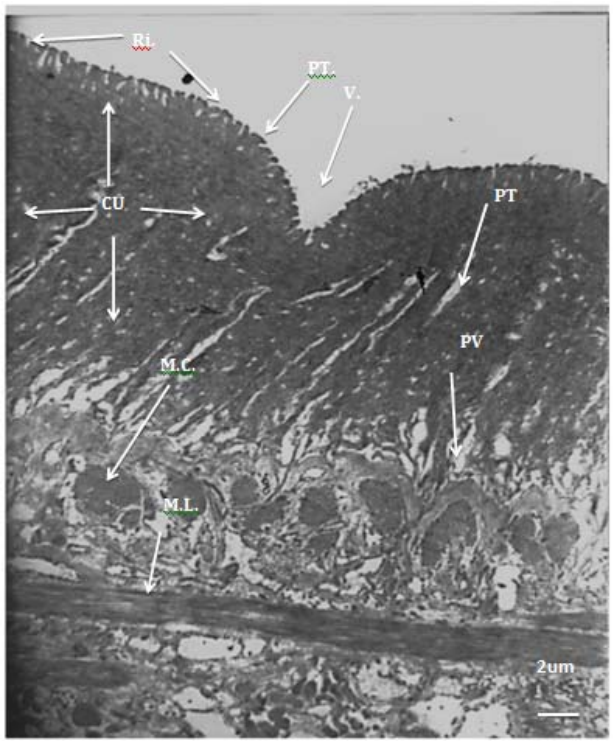
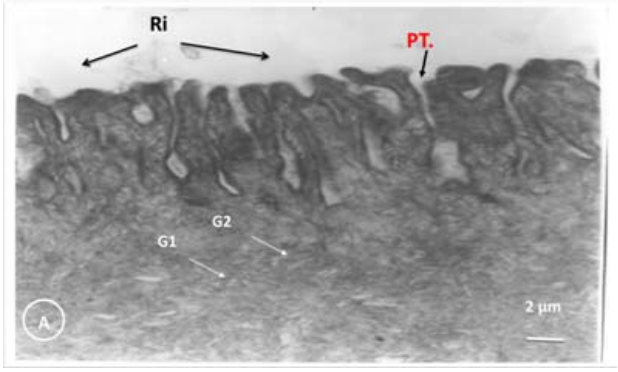


Figure 7: (A) Ultrastructure of tegument of adult Fasciola fluke showing the first layers: ridge (Ri.) and pit (PT.) (B): high power of tegument present (G1, G2) granules, Bar2 µm



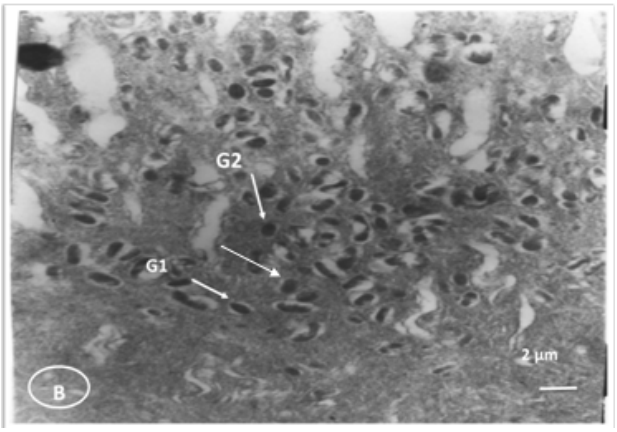


Figure 8: In high magnification of cross section in second layers present (M.) mitochondria, tegument cell (AP.). and nuclues (N.) arounded by two kind of granules (G.), vacules (V.)., Bar1 – 2 um