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MORPHOLOGICAL AND STRUCTURAL ASPECTS OF LIVER PARASITE TREMATODES IN SHEEP

BY

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A light microscopical examination was made of the liver tissues în fluke infestation (*Fasciola hepatica*) in sheep. We oberved orientation and details from the ovary and the uterus, essential for the determination of the parasite, yolk glands (indicating mature individuals), shell gland, irregular shapes of the eggs in the uterus, the parenchyma.
We took liver samples from adult sheep (between one and four years old), forced sacrifices in the abatory of Valea Adîncă. The provenience of these sheep is around the city of Jassy of Romania. For the condition of this geographic area the literature provides the existence of *Fasciola hepatica* L.,1758 and *Dicrocoelium lanceolatum* together (Olteanu,1973). At the time of our research, October 2002 -June 2003, we determined only *Fasciola hepatica.*

Introduction

The first description of the liver fluke was made by Jehan de Brie in 1379. In Romania the first infestation in human was observed by Nicolae Leon in 1908 (Olteanu, 1996). The favorable environment factors for fluke infestation were studied by C. B. Ollerenshau (Olteanu, 1993). In Romania the first infestation in human was observed by Nicolae Leon in 1908 (Olteanu, 1973) who studied also the copulation and Laurer´s canal importance (Leon, 1927). Important studies on the fasciolosis impact in Romania were written by Gh. Olteanu who discovered the first case of infestation with *Fasciola gigantica* Cubbold 1855, other agent of fasciolosis in Romania (Olteanu, 1973).

There are numerous methods for coproovoscopic diagnosis (investigation during the life of animals) but they are used with immune-biological diagnosis (sometime false positive) (Olteanu, 1973). The diagnosis of the parasite presence in a geographic area needs the investigation method after the death of parasited mammals (Dulceanu, 1996, Oneţ, 1998 and others).

Materials and Methods

The pieces of liver with flukes were fixed for light microscopy in 10 % formaldehyde and Bouin mixture, dehydrated in ethanol and amylic alcohol, embedded

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in paraffin and sectioned at 5 microns. They were stained with hemalaun-eosine and examined with a NOVEX microscope and photographed with a Canon EOS 1V.

Results and Discussions

The longitudinal (Fig.1) and cross sections (Fig. 2) revealed dorsoventrally flattened Trematodes (flukes).

In these sections we were able to locate the spines buried in the tegument. The tegument of *Fasciola hepatica* and other flukes has been studied at electronic microscope by Threadgold and Burton (1963), Lee quoted by Cheng (1974) who found mitochondria in the cuticle and that is why they considered the cuticle part of the tegument as a living structure proving a high metabolic zone. Below the tegument we found longitudinal and also circular muscle layer (Fig 3.c) passing through the parenchyma (Fig.3.d).

Details from longitudinal section reveals the oral sucker (fig.4.a), the pharynx (4.b) and the acetabulum (a large ventral sucker, without any pore, fixing the worm) with their longitudinal and circular muscle (Fig.5).

Observing the biramus intestine (intestinal ceca) (Grassé, 1961, Wallace, 1997 and others) and intestine diverticula (Fig. 2.d) we found one layer of intestinal cells (Fig.6) as the literature provides (Dawes, 1968, quoted by Cheng 1974)

On the right side of the fluke, in its superior half lies the branched ovary; a detail (Fig. 8) reveals an ovary diverticula with germinative epithelial cells.

The longitudinal section also reveals the uterus (Fig.1, Fig.7) and the branched ovary of which we provided a detail (Fig. 9).

The highly branched testes are in the second half of the worm (Radu, 1958, Olteanu, 1973, Wallace, Oneț, 1988, and others).

In the cross section we also observed the yolk gland (vitellaria) (Fig. 2, Fig 8) all along both lateral sides of the worm.

We observed the spines and the cuticle on the epithelial cells layer and eggs showing the terminal part of the uterus approaching the genital papilla with the genital pore. Around the ootip (genital intersection- the place of the fecundation of eggs) (Leon, 1927, Radu, 1958 and others) the section reveals the shell (Mehlis) gland. So, the eggs we can see in the ovary must be matures. Fasciola eliminates not embryonate eggs. The embryo develops outside the body of the worm and the host (Radu, 1957, Olteanu, 1973, Dulceanu, 1982, and others).

The mature eggs of *Fasciola hepatica* have operculum, thin shell, granular yellowish-brown contents that fills the whole egg, no blastemeres. They are large worm eggs (130 – 145 µm in leight, 70 - 90 µm in width), a nearly regular ellipse and nearly similar poles, symmetrical, strongly barrel-shaped sidewalls. The fertilized egg is surrounded by a great mass of yolk cells (Thienpont, 1986).

On longitudinal section passed through the uterus and genital intersection revealed irregular shapes (Fig. 8) of what we considered immature eggs seeing their location (Fig. 1.e, Fig. 7).

Conclusions:

In our samples of liver histologically processed and stained HE we were able to identify some aspects of internal structure in liver fluke (*Fasciola hepatica*).

We have identified mature individuals of Fasciola hepatica in biliary radicles of which longitudinal and cross section we provided to observe: the cuticle, the spines of tegument with their typical shape; the disposition of the ovary in the front part of the fluke and by the presence of the testis in the second half (both sides of the fluke body) and the presence of yolk glands have indicated only mature individuals.

The result of host-parasite relationship is, in chronic fasciolosis, the helminthoma, the host isolating the fluke by fibrosis, trying unsuccessfully to reduce the hemorrhage and toxic actions of the parasite (Nițulescu, 1964, Paul,1987, end others).

Fig. 1. Helminthoma – longitudinal section with *Fasciola hepatica* fixed in a biliar radicle; a)oral sucker, b) acetabulum (ventral sucker), c) pharynx, d) uterus, f) shell gland

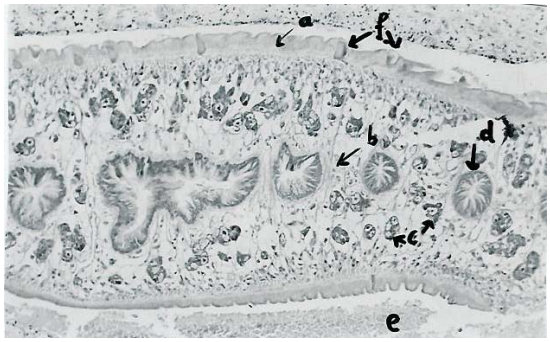

Fig. 2. Cross section through *Fasciola hepatica*: a) cuticle covering the spines, b) parenchyma, c) vitellaria, d) intestine diverticula, e) hemorrhage, f) spines (3,3 X 40)

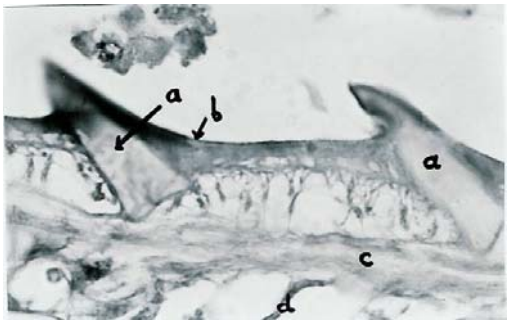

Fig. 3. Detail from longitudinal section presenting: a) spines, b) cuticle, c) muscle layer, d) parenchyma (3,3 X 100) HE

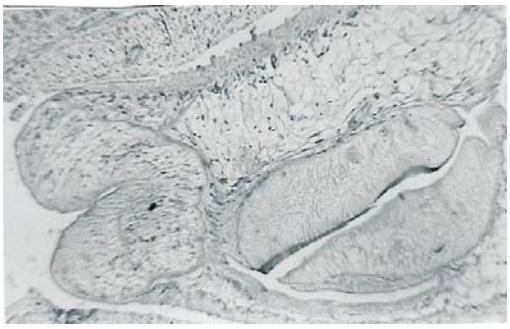

Fig. 4. Detail presenting the oral sucker and the pharynx (3,3X60) HE

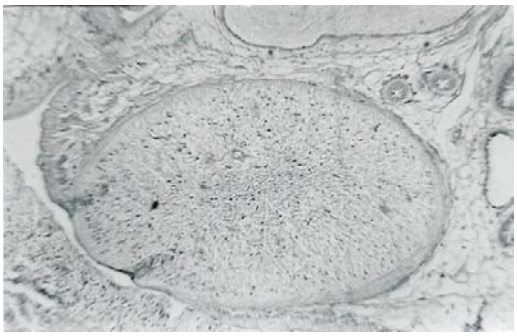

Fig. 5. Detail with ventral sucker (3,3X60) HE

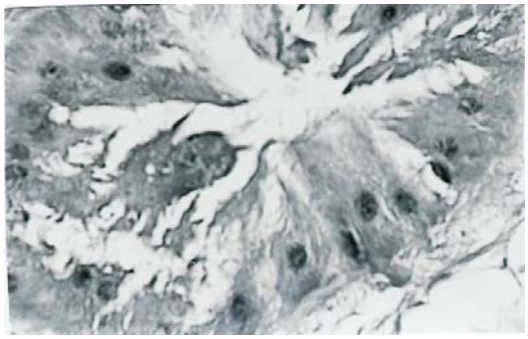

Fig. 6. Detail from intestinal diverticle (3,3X100)

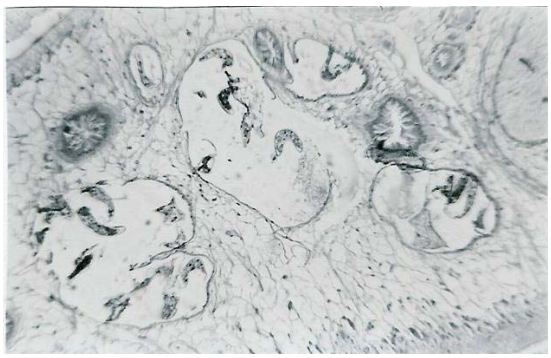

Fig. 7. Uterus with eggs (3,3X40) HE

Fig. 8. Detail with eggs with irregular shapes from uterus (3,3X100) HE

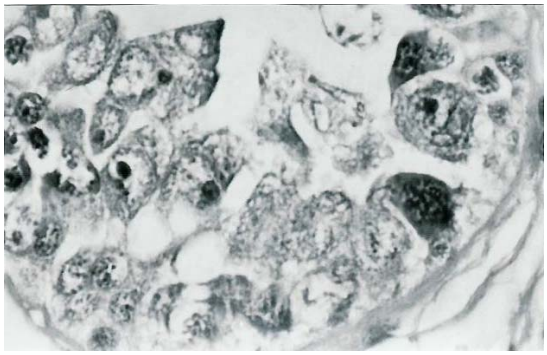

Fig. 9. Detail from ovary with irregular shaped eggs (3,3x100) HE

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