Species specificity between the trematoda *Fasciola hepatica* and the molluscan host

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Abstract

Larval development of *Fasciola hepatica* in abnormal snail intermediate host *Lymnaea palustris* was studied. This incompatible host-parasite relationship was investigated under Transmission Electron Microscopy (TEM) to determine the fate of penetrated miracidia.

It is believed that no larval development beyond the sporocyst was allowed to proceed by the defense system of *L. palustris*. This reaction was in the form of defense cell (haemocytes) aggregation around sporocyst. These haemocytes vary in appearance under TEM, some have a vary electron dense cytoplasm, others are much more electron lucid. These appeared to correspond to granulocytes and amoebocytes respectively, as described by other workers. Intermediate cells XXZAAAre were found in close contact with sporocyst and pore cells were also reported.

In *L. palustris* 18 days old, the location of sporocyst proved to be difficult. This could be due to the very efficient defense mechanism of the snail at this age and its capability to destroy and remove the sporocyst within three days post exposure, thus snails 0 day old was used.

Keywords: Sporocyst; granulocytes; amoebocytes; redia; cercaria

المناوعة بين طفيلي دودة حلزون الكبد مع المضيف من القواقع

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مخلص

تم دراسة عملية التطور اليرقي لدودة حلزون الكبد في المضيف الوسطي غير الطبيعي . ان هذه العلاقة غير المتوافقة بين الطفيلي والمضيف قد تم دراستها باستخدام المجهر الالكتروني Transmission Electron Microscopy (TEM) وذلك لتحديد مصير اليرقة المهدبة (Miracidium) المخترقة للمضيف. يعتقد انه لايوجد تطور يرقي الى مابعد الكيس البوغي وذلك بفعل الجهاز المناعي للقوقع غير التوافقي (*Lymnea palusttris*) وهذا التفاعل ظهر بشكل خلايا دفاعية (haemocytes) متجمعة حول الكيس البوغي .

هذه الخلايا ظهرت باشكال مختلفة تحت المجهر الالكتروني، فمنها ذات سايتوبلازم كثيف واخرى ذات سايتوبلاز شفاف فالاولى تتمثل بالخلايا الحبيبة والثانية الخلايا المتحولة على التعاقب .

ظهر هناك خلايا وسطية في تماس كبير مع الكيس البوغي، كما ظهرت الخلايا الثاقبة ايضا . وجدت صعوبة في تحديد مكان الكيس البوغي في القواقع غير المتوافقة (*L.palustris*) ذات عمر 18 يوما، قد يكون هذا بسبب النشاط الكفوء لجهاز المناعة في هذه القواقع وفي هذا العمر بالتحديد، لكونة قادر على تحطيم الكيس البوغي وازالتة من النسيج خلال ثلاثة ايام بعد التعرض للاصابة لذا استخدمت في هذه التجارب قواقع حديثة التفقيس (عمر صفر) .

1. Introduction

In this study the trematod *Fasciola hepatica* was chosen. Adult fluke causes a disease called "fascioliasis" which is responsible for great loss in stock rearing, resulting in great economic importance.

Recently, human cases occurred occasionally but are now increasingly reported from Europe, WHO estimates that at least 2.4 million people are infected in about 70 countries worldwide, and several millions are at risk. No continent is free from fascioliasis, and it is likely that where animal cases are reported, human cases also exist [1].

The harm done by *F. hepatica* to its mammalian host is chiefly due to the effect of the parasite on host′s liver. In human, fascioliasis is usually appears as an infection of bile ducts and liver. During the acute stage of infection symptoms result from the migration of parasite from the intestine which include gastrointestinal problems e.g. nausea, vomiting and abdominal pain. Fever and rash may occur. While, inflammation of liver gallbladder and blockage of bile ducts can occur when the parasite settles in bile duct in chronic infection [2].

The parasite has a complex life cycle requiring two hosts; a mammalian host and specific species of snail molluscan host (Fig 1). Within the mollusc the parasite develop to reach cercaria, which encysted outside to be the infective stage (metacercaria).

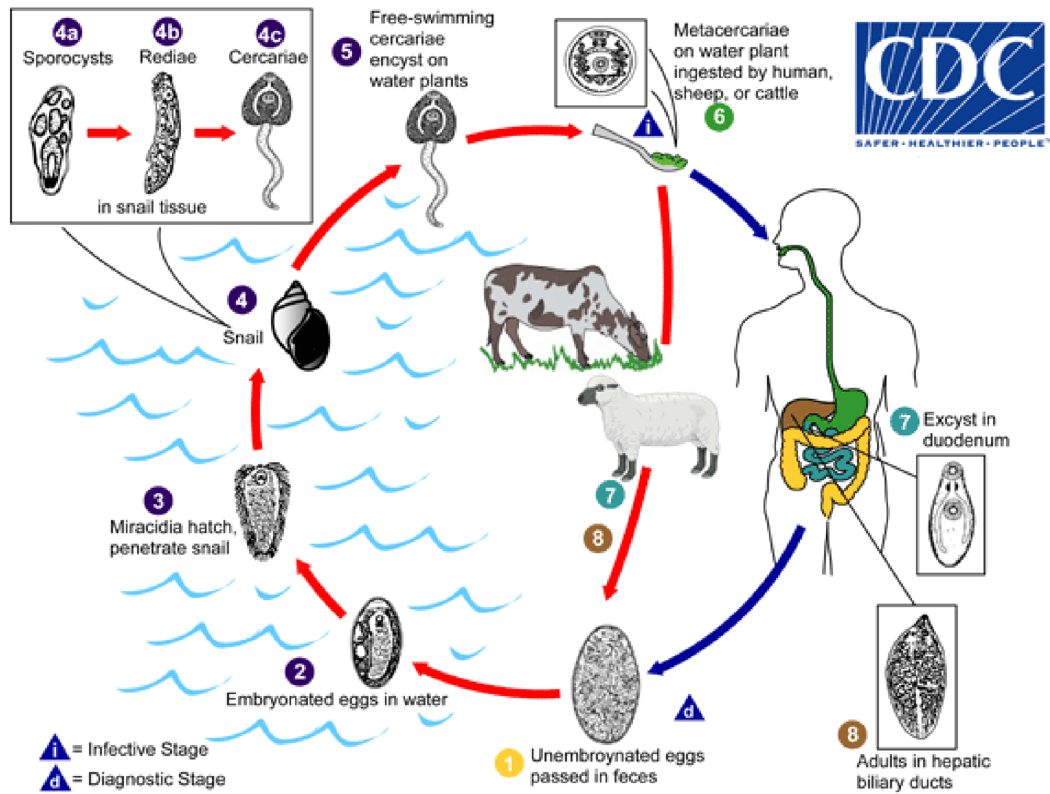

Fig. 1: Life cycle of *Fasciola hepatica* (2)

The snail *Lymnaea truncatula* is the normal intermediate host of this parasite which permits the development of larval stages and the release of cercaria. Such host- parasite relationship can be referred as compatible. On the other hand, the life cycle ceased when the miracidium penetrate different species of the genus Lymanea, *Lymanae palustris which* was used in this study, and this host-parasite relationship referred to as incompatible (Fig.2, 3).

The aim of this study was to follow the life cycle of *F. hepatica* in abnormal snail of genus *Lymnaea in-vitro* using Transmission Electron Microscope (TEM).

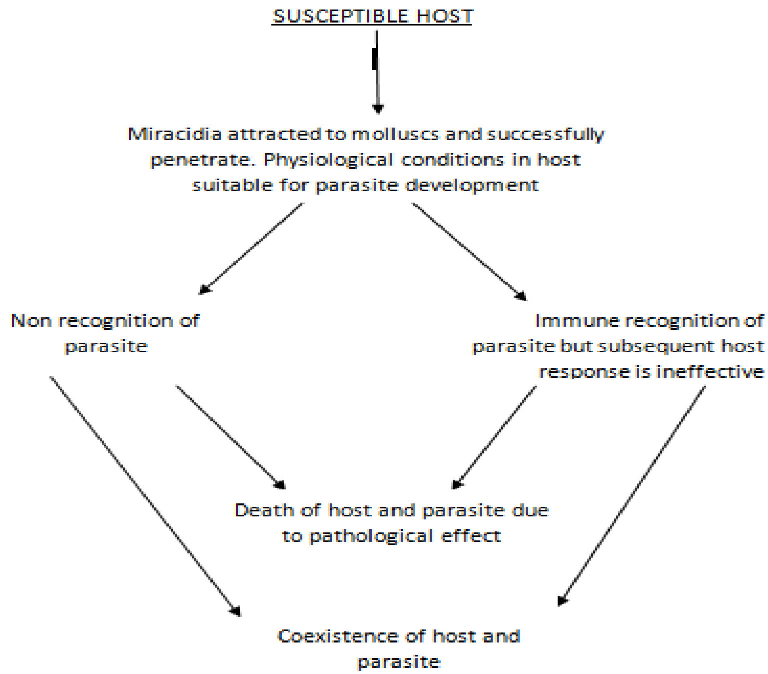

Fig.2: The compatibility in the normal host

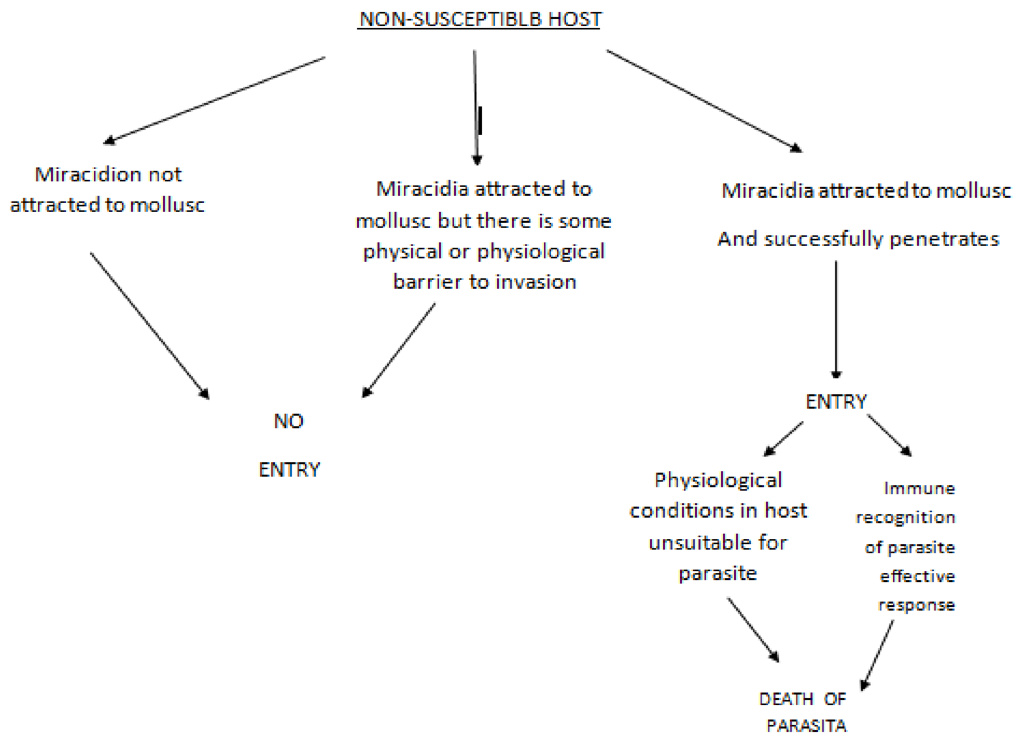

Fig.3: Some possible reasons why a mollusc is not susceptible to Digenean infection.

2. Materials and methods

2.1 Maintenance of snails in the laboratory

This was carried out in the laboratory and according to the habitat of snail species where the aquatic *Lymnaea palustris* was maintained in special trays with dechlorinated water well aerated.

A) Maintenance of *Fasciola hepatica* in the lab.

Adult flukes were obtained from experimentally infected rats to collect the eggs from the teased uterus. The eggs were rinsed several times with aerated distilled water to remove all flukes debris then placed in specimen tubes with clean distilled water for storage at 4ºC in the dark.

F. hepatica eggs at 4ºC remain unemberyonated until brought to higher tempreratures. Eggs were maintained at 25-27ºC in freshly areated water and emberyonation was completed in 14 days [3]. Mass hatching of miracidia was produced by placing them in freshly areated dechlorinated water in daylight.

2.2 Technique for Transmission Electron Microscopy

The technique was applied as follow:

1- Because of difficulties in locating the sporocysts in relatively large snails, it was decided to use newly-hatched snails. *L. palustris* 0-day old (0.d.o) were infected with 12 miracidia of *F. hepatica* (MFH) by placing snails individually in watch-glass with 1-2 ml of dechlorinated water.

2- Using a pasture pipette the exact number of miracidia were placed in each watch-glass then watch-glass was covered. The number of dead miracidia found in the watch glass the next day being the criteria to determine if successful invasion had occurred.

3- Snails were killed 120 hours post exposure (h.p.e.).

4- Infected snails were placed in the fixative solution which is 3% glutaraldehyde in 0.1 molar(M) sodium phosphate buffer solution containing 0.03 % sodium chloride, pH 7.2 at 4ºC for 3hrs.

5- The shell was completely removed from each snail in buffer after fixation. The presence of any remnant of the shell is damaging to both the edge of glass knife and snail tissues.

The schedule for fixation, dehydration and embedding was followed according to the reference [4].

The blocked-out specimens were then sectioned using ultra microtome with glass knives. Initially, 1µm sections were taken, stained with Toluidine Blue for about 30 seconds, and examined under light microscope. When a sporocyst in the snail tissue had been located, the block face was re-trimmed to the precise area required, and using new knife, ultrathin sections of between 60-70 nm were cut.

Sections with silver or grey interface color were thin sections; therefore only these sections were collected on copper grids and allowed to dry.

These were stained for 15 minutes each in saturated uranyl acetate in 50% ethanol and lead citrate. The sections were examined in TEM

3. Results and Discussion

Studies on TEM level were carried out on preparations as described in the materials and methods section. Observations revealed that an incompatible relationship exists between the parasite *F. hepatica* and the intermediate host *L. palustris* which expressed by cellular reaction in the form of encapsulation leading to destruction of the parasite within abnormal host.

Quite clearly the parasite will invade this host but no radiate and cercaria produced. In this work the preparations for ultrastructure examination was time consuming process, particularly the sectioning of snail in resin blocks to find the location of encapsulated sporocysts. Therefore, newly-hatched snails were used for their small size. This facilitated finding sporocysts prior to ultramicrotomy. It has been known for some time that the normal intermediate snail host for *F. hepatica* is *Lymnaea truncatula* [5] and it must be emphasized here that in such compatible host-parasite relationship, there is no encapsulation around the larval stages, or there is aggregation of haemocytes which are harmless to the parasite.

All digeneans have no cuticle, but expose to their host a cytoplasmic syncytium (or tegument) which is limited by plasma membrane. This tegument represents the outermost layer of the body wall and is extremely important because it is the first layer attacked by host defense cells.

It is well known that the associations between digeneans and their snail hosts are specific, and digenesis are said to be host specific with respect to their snail hosts. The relationship between *S. mansoni* and *Biomphalaria* provides further insight regarding host specificity

Not surprisingly, the degree of host specificity exhibited by different digenean species is variable, even including among closely related species. The liver fluke *Fasciola hepatica*is able to develop in some, but not all, representatives of the snail genera [6].

Penetration of miracidia into non-host snail species occurs in some cases and is often followed by encapsulation of the miracidia [7], strongly suggestive of the direct involvement of the gastropod immune system in recognizing and eliminating maladapted parasites.

This was the fate of *F. hepatica* miracidium which penetrate the snail *L. palustris* in this study and appear clearly in the plates of TEM sections.

The sporocyst′s tegument varies in thickness considerably. The presence of membranous whorls in the cytoplasm of the syncytical tegument, may suggest that sporocyst is suffering degenerative change (Plate 1).

Plate (1): Sporocyst of *F.hepatica* in *L.palustris*: electron microscopy
am: amoebocyte; gr: granulocyte; m: mitochondria; sn: snail tissue;
sp: sporocyst tissue; t: tegument; w: membranous whorls

The sporocysts examined here (120 h.p.e.) showed that surface plasma membrane exhibits many narrow folds (Plates 2). This could be the first steps of sporocyst destruction by host defense cells.

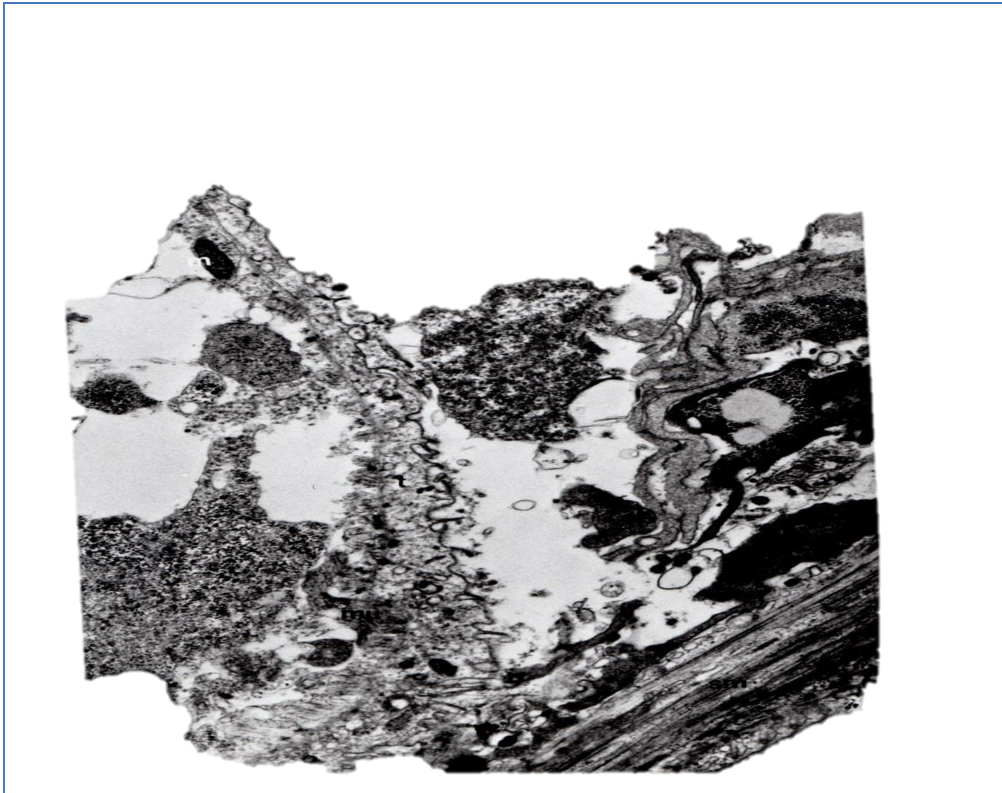

Plate (2): Sporocyst *of F.hepatica* in *L.palustris*: Electron Microscopy
f: folds; m: mitochondria; mu: muscle of sporocyst; sm: snail muscle; t: tegument

The Plates 3, 4 showed the close contact between the amoebocyte of snail and the penetrated sporocyst of *F. hepatica* tegument. The tegument appears degenerated in these plates. Another type of host defense cell, pore cell also nearby the sporocyst, although the exact function of this cell is uncertain (Plate 3).

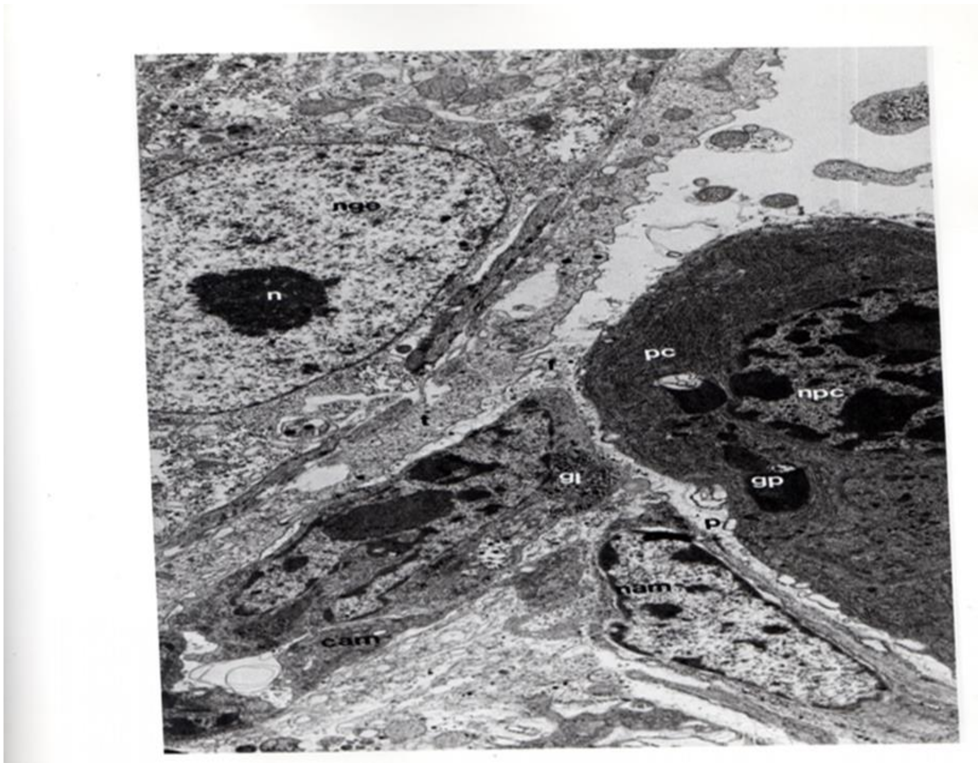

Plate (3): Sporocyst of *F.hepatica* in *L.palustris*

am: amoebocyte; nge: nucleus of germ cell; gl: glycogen deposits; f: folds; pc: pore cell; cam: cytoplasmic extension of amoebocyte; p: pigment granules; npc: nucleus of pore cell; t: tegument

Plate (4): Sporocyst of *F.hepatica* in *L.palustris*

am: amoebocyte; nge: nucleus of germ cell; gl: glycogen deposits; f: folds; pc: pore cell; cam: cytoplasmic extension of amoebocyte; p: pigment granules; npc: nucleus of pore cell; t: tegument

In the Plate (5) a large cell with short cytoplasmic extensions, the intermediate cell, appears as flattened and spread over sporocyst tegument.

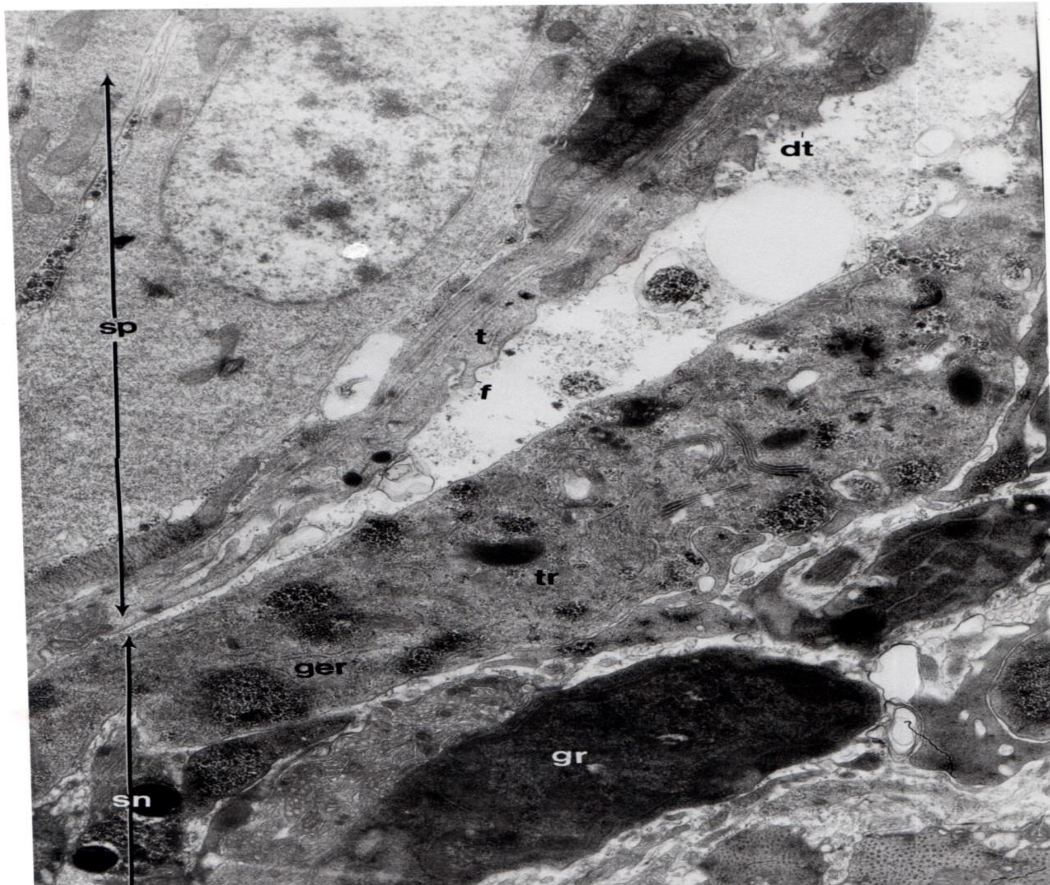

Plate (5): Sporocyst of *F.hepatica* in *L.palustris*

dt: damaged tegument; f: folds in tegument; ger: granular endoplasmic reticulum; gl: glycogen deposit; gr: granulocyte; sn: snail tissue;sp: sporocyst tissue; t: tegument; tr: intermediate cell It was postulated that the difference was ascribed to greater similarity between the surface carbohydrates of the sporocysts and haemocytes of the susceptible host than with sporocysts and haemocytes of the incompatible host species [8].

The same result was reported by Sullivan and Yeung, 2011 [9]. They found that miracidia experimentally injected into snails are often encapsulated in incompatible hosts, but can survive and develop in compatible hosts, implying that recognition and destruction by immune system components can occur once within the internal milieu of the snail.

Nunez and DeJong-Brink (1997) [10] noted that secretory-excretory products derived from sporocysts of *Trichobilharzia ocellata* had a suppressive effect on bacterial clearance by haemocytes of the host snail *Lymnaea stagnalis*, but had no comparable effect on the haemocytes of the non-host species *Planorbis corneus*. This indicates that, at least in this host-parasite pair,

suppressive effects of digenean larvae are specific in their action on haemocytes of compatible host species.

The exact mechanism basis of digenean specificity for snails remains elusive, but emerging from the detailed study of the *S. mansoni - B. glabrata* system are converging insights regarding gastropod immunology that seem to provide a compelling new way to study the phenomenon of host specificity.

Several recent studies [11, 12, 13] have shown that *S. mansoni* miracidia and sporocyst surface proteins and sporocyst transformation products bear glycan structures that are similar to those found expressed on plasma proteins or haemocytes of *B. glabrata*. This similarity adds significant additional evidence to a long-standing hypothesis [14] that parasites may escape immunological detection in their hosts by molecular mimicry. Further support for this idea comes from the observation that plasma proteins from susceptible strains of *B. glabrata* have a greater distribution and abundance of sharing of some glycans than plasma proteins from a schistosome resistant strain [14].

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