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CONTRIBUTIONS ON TREMATODA-SNAIL INTERACTIONS

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Abstract

With increasing the incidence of human *Fasciola* infection in Egypt, there had been speculations about an adoption of *Schistosoma* snail to transmit *Fasciola* infection, but the previous knowledge demonstrated more data about snail-trematode specification. For this reason the present study was designed to follow up the reaction of domestic and foreign snails for exposure to adapt or non-adapted miracidia on the tissue and cellular level.

The data revealed failure of *Fasciola gigantica* and *Schistosoma mansoni* miracidia to complete their development in hosts other than *Lymnaea natalensis* (*L. cailliaudi*) or *Biomphalaria alexandrina* snails after exposure to low or high dose of miracidia. The foreign miracidia were able to penetrate the snail but the sporocysts were unable to migrate deeply in the sub-epithelial tissue of the foot. As marked specific tissue reaction was develop by the snail, trying to capsulate them. The degree and thickness of tissue reaction was increased with the increase in time post exposure till complete disappearance of the invading sporocysts. Besides, no tissue reaction and successful infection was recorded when the miracidia penetrate their specific snail host. On the cellular level, *B. alexandrina* snail haemolymph contained two types of cells as hyalinocytes (H) which related mainly to humeral type of defense and phagocytic cell called granulocyte (G), The cells were in different forms and structures related to time elapsed post exposure to infection. G-3 was actively divided cell detected in infected snail only. *L. natalensis* and *Physa acuta* contained granulocytes (amoebocyte) in HL and 3 connective tissue related cells as C.T. amebocytes, pore cell and granular cell. Amoebocyte is phagocytic cell showing morphological and numeral changes in relation to exposure of the snail for infection by different miracidia. Granulocytes in *P. acuta* were trapped close to foot epithelium and between C.T. matrix and playing a role in early destruction of the foreign invading miracidia.

Key word: *L. natalensis* (*cailliaudi*), *B. alexandrina*, Foreign miracidium, Haemolymph, Tissue reaction

Introduction

The snail is an obligatory intermediate host for all trematode species. With the enormous medical and economic losses produced from infection by trematode such as *Schistosoma* and *Fasciola* spp. especially with the difficulty in eradication of the mature flukes inside their human or animal hosts, the same parasites considered to be severely weak inside their snail I.M.H. Egypt is one of the fascioliasis endemic areas worldwide (Dietrich *et al*, 2015). Schistosomiasis is the second most common parasitic infection globally after malaria (WHO, 2017). There had been speculations about an adoption of *Schistosoma* snail to transmit *Fasciola* infection (Farag and El-Sayed, 1995). On the vice versa, El-Bahy *et al*. (2014) cleared that *Fasciola* develop in *Lymnaea natalensis* did not develop in *L. trun-catula*. Moreover, there was little data about types of tissue and cellular reactions that occurred inside the snails after miracidial invasion, which determined the snail role as suitable or unsuitable intermediate host for development of the invading miracidia. *B. alexandrina* snail haemolymph contains an important anti-parasite molecule that resists the *S. mansoni* infection by. Three types of cell in both haemolymph and tissue were identified and classified according to shape and granular contents as granulocytes, amoebocytes and

hyalinocytes. Electron microscope study also revealed the important role of granulocytes and amoebocytes as defense mechanism against snail infection (Helal *et al*, 2014).

Saldanha *et al*. (2009) in New Zealand reported that among the more than 1500 metacercariae recovered and individually measured, there was no relationship between the mean diameter of metacercarial cysts per isopod and isopod body length. However, intensity of infection correlated negatively with the mean diameter of cysts within an isopod, i.e. metacercariae in crowded infections attained smaller sizes on average. In contrast, the variability in cyst sizes per isopod, measured as the coefficient of variation, was independent of both isopod body length and infection intensity. They added that the results showed a disproportionate number of relatively small metacercariae come from the relatively few hosts in which large fraction of all metacercariae were aggregated. The combination of aggregation and intensity-dependent growth generates inequalities in sizes among metacercariae that will be passed on to adult worm populations in definitive hosts The resistance of some types of *Biomphalaria* versus *Schistosoma* infection was depends on presence of snail humoral factors called phenoloxidase (PO). PO enzymes play a key role in wound healing, tissue pigmentation and also in innate immune defense of the snail against invading pathogens. The pathogen molecules are detected and stimulate the activation of PO enzymes. The active PO has cytotoxic activities able to damaging the invading pathogen (Le Clec'h *et al*, 2016).

The present study aimed to to follow up the reaction of domestic and foreign snails for exposure to adapt or non-adapted miracidia on the tissue and cellular level.

Materials and Methods

B. alexandrina, *L. natalensis* and *P. acuta* snails were collected from water-sources, Abou-Rawash Giza and identified (El-Bahy *et al*, 2014). The snails were reared in a suitable aquarium in dechlorinated tap-water for deposition of egg-masses in Department of Parasitology, Faculty of Veterinary-Medicine, Giza. The mother field collected snails were, then, removed from the aquaria, their egg masses of different stages of growth were kept to develop and hatch in the original aquarium under the level of water surface. The aquarium water was partially renewed. The obtained laboratory breed snails (4-6 weeks old) were used for the experiments after this.

Collection of *F. gigantica* miracidia: *Fasciola* eggs were collected from the gall bladders of naturally infected buffaloes fresh slaughtered in Cairo abattoir. The eggs were cleaned by sieving followed by several washing and sedimentation in suitable cylinder. The eggs were stored in the refrigerator in distilled water till use after addition of penicillin G Sodium (100U) and dihydrostreptomycin (100 lg) (Sigma) per ml. Miracidia were developed in the eggs after their incubation as thin layer in Petri-dish in dark in the incubator at 28ºC (Hussein *et al*, 2010).

Collection of *S. mansoni* miracidia: Eggs of *S. mansoni* were purchased from Theodor Bilharz Research Institute, Giza. Eggs were hatched in a darkened 1000 ml flask containing warm (30ºc) tap water; the narrow top part of the flask was exposed to bright light where the actively swimming miracidia were collected in this top layer (Xu and Dresden, 1989).

Artificial infection of snails: Laboratory breed snails (4-6 weeks old) were used. Enough number of each snail species were exposed over night to selected dose of both parasites separately in relation to the selected time of fixation (Tab. 1) using special tissue culture plate (12mm diameter). The miracidia were pipetted in small drop of water, counted using a hand counter and a stereomicroscope. One snail was transferred to each well; the wells were filled by declorinated tap water and covered with a perforated

plastic sheet to prevent escape of the snail during exposure (over night). At the end of exposure time, the snails were transferred to new aquarium, reared in the laboratory (20-26ºC) and fed green lettuce. Infection of snail by high dose was for easily following up the penetrating miracidia in tissue section and to destruct the possible snail resistance versus foreign miracidia. Infection by low dose was select to facilitate development of the stage inside the snail without causing early snail mortalities (El-Bahy *et al*, 2014).

Table 1: Fixation and inspection of snails exposed to low and high dose of miracidia at selected time post exposure (pe)

Exposed snail	Used miracidia	Dose of miracidia	Time of snail fixation and haemolymph examination	Inspected snail organ
L. natalensis *B. alexandrina* *P. acuta*	*F. gigantica*	2-4 /snail	3 weeks post infection	Liver
		40-50 /snail	6, 12, 24, 48, 72 hours pe	Tissue
	S.mansoni	2-4 /snail	3 weeks post infection	Liver
		40-50 /snail	6, 12, 24, 48, 72 hours pe	Tissue

Snails inspection: At each selected time (Tab. 1), 2-4 snails from each group were fixed in Bouin's solution for 3-5 days before processing; moreover another 2 snails were crushed over clean glass slide, using a forceps and two anatomical needles to see the development of parthenitae in snail liver (El-Bahy *et al*, 2014).

Haemolymph collection: Water adhering to the snail was removed and the head foot was cleaned with tissue paper. Collection of haemolymph of *B. alexandrina* was performed by touching the foot with the point of the micropipette, the snail was forced to retract deeply into its shell and extruded haemolymph. In case of *L. natalensis* and *P. acuta*, haemolymph of cleaned snails were extracted by capillary tube from the spaces between the organs of snail (haemocoel of foot-and-mouth part) and its shell (Negm *et al*, 1995). So, as much as 30 µl of haemolymph were obtained from each individual snail. The collected samples were spread as thin film on clean glass slide fixed by methyl alcohol and stained by Giemsa as blood film.

Histopathological sections: To follow up early migration of invading miracidia and reaction of the snail against them on the tissue level, the shell of Bouin's fixed snails were removed, then the whole snail tissue was dehydrated through ascending grades of ethanol then transferred to xylene, then transferred to mixture of melted paraffin and xylene (each for 2 hours). The sample was embedded after this in metal template filled with melted paraffin, solidified the paraffin blocks and removed. Histopathological sections (4µm thickness) were cut and stained with Hematoxylin and Eosin (Bancroft and Stevens, 1996).

Results

Exposure of *L. natalensis* to 2-4 *F. gigantica* miracidia and *B. alexandrina* to infection by same dose of *S. mansoni* miracidia led to infection in all exposed snails as *F. gigantica* redia and *S. mansoni* sporocysts were recovered in the liver of still alive snails until 3 weeks post exposure. But, no parthenitae was diagnosed in *L. natalensis* exposed to low dose of *S. mansoni* miracidia and no mortalities nor infection was diagnosed in *B. alexandrina* exposed to *F. gigantica* miracidia. With increasing miracidial dose to 40-50 miracidia/snail, all *L. natalensis* exposed to *F. gigantica* miracidia died at the end of 1st week. Also, all *B. alexandrina* exposed to high dose of *S. ma-nsoni* miracidia died at the end of 2nd week. Also, no infection was recorded in *L. natalensis* exposed to high dose of *S. mansoni* or in *B. alexandrina* exposed to high dose of *F. gigantica* miracidia. No parthenitae was diagnosed in dissection of *P. acuta* previously exposed to 2-4 or 40-50 miracidia of *F. gigantica* or *S. mansoni* without snail species mortalities to the experimental (Tab. 2).

The changes occurred in the snail after exposure to their compatible or non compatible trematoda miracidia were investigated on tissue and cellular level. Histopathological

inspection for tissue reaction in snail foot 6 hours post exposure (hpe) of *L. natalensis* to *S. mansoni* miracidia revealed that successful penetration to different miracidia into tissue of the foot of the exposed *L. natalensis* and *B. alexandrina*. Sporocysts were found near the foot epithelial surface in its foreign snail, but barrowed deeper and recorded away from the surface epithelial in their domestic snails. Dissection of tissue at 12, 24 & 72 hpe revealed more fibrous reaction by the snail tissue led to capsulation of the invading sporocysts in foreign snails (Pl. 1, Fig.1d). The degree and thickness of tissue reaction increased with the increase in time post exposure till complete disappearance of the invading sporocysts. Also, no sporocyst was recorded in the subepithelial C.T. of *P. acuta* after exposure to *F. gigantica* or *S. mansoni* miracidia at 6 hpe. More interested phenomenon was recorded concerning this snail species in the form of appearance of special distribution to basophilic cells at the junction between the epithelial layer and their underling C.T. as well as in the sub-epithelial C.T. (Pl.1, Fig. 1a). With increasing the time post exposing *P. acuta* to both miracidia, marked cellular aggregations were recorded in the sub-epithelial layer of snail foot, which were able to destruct and overcome on the invading sporocysts (Pl.1, Fig.1b). Besides, no tissue reaction was recorded in *F. gigantica* sporocysts invading *L. natalensis* or around *S. mansoni* miracidia invading *B. alexandrina* snail (Pl. 1, Fig.1c). Parthenitae migrated faster in the snail tissue as they did not recorded in snail tissue after 24 hpe, The stages was recorded in snail liver after this. Dissection of all snails exposed to low dose of miracidia revealed infection by *F. gigantica* redia in liver of *L. cailliaudi* and *S.mansoni* sporocysts in liver of *B. alexandrina* only (Pl. 1, Fig.1e &f). Besides, no parthenitae could be detected in *L. cailliaudi* exposed to *S.mansoni* or *B. alexandrina* exposed to *F. gigantica* miracidia and in the same time none of both miracidia able to develop in *P.acuta* snails.

Changes in the haemolymph cells of *B. alexandrina* snails: On the cellular level, investigation of HL smears collected from different *B. alexandrina* revealed two main types of cells identified as hyalinocytes (H) and granulocytes (G) (amoebocytes). Most of these cells were in infected and non-infected snails but their number and role in defense against the invading miracidium differed as the follows: H- type one (H-1) was small in size (7.3x6.5µ) with pale acidophilic cytoplasm and small dark stained basophilic nucleus. H-2 was similar to the first type but larger (12.3x12µ) with slightly large nucleus. H-3 was more large (20.5x20.3µ) cell contained 2 nuclei seen only in snails infected by foreign miracidia after at 12-24 hpe. H-4 was morphologically similar to H-2 but contained large number of acidophilic granules and recorded in infected and non-infected snails.

The second cell in *B. alexandrina* was granulocyte appeared in different forms and structures related to time elapsed post exposure as follows: Granulocyte type 1 (G1) was small in size, ovoid (10.6x13.4µ) with thin cytoplasmic rim and small blue nucleus with large spherical acidophilic body in the cytoplasm, found in infected and non-infected snail. G2 was similar to G1 but was larger (22.2µ) in diameter. G3 was larger than the previous one with 2 nuclei and 2 cytoplasmic bodies, which actively divided cell detected in infected snail only. G4 & G5 cells were similar to G2 but larger in size with cytoplasm with retractile granular particles in infected and non-infected snails. Exposure of *B. alexandrina* snail to *F. gigantica* miracidia led to marked shifting in the average number of granulocyte in relation to hyalinocytes, moreover G-3 type was predominate over the number of G-2 form. The variations were not record in snail exposed to *S. mansoni* or in control non-infected snails. With infection duration granulocytes type 3 increased in size and engulfed varia-

ble particles in its cytoplasm. By this way this granulocyte change into other forms (G-4). These particles start to disappear with the increase in the cell size changing to G-5 that disappeared within certain periods.

L. natalensis defense related cells: Four types of cells were recognized in *L. natalensis* and *P.acuta* smears showed granulocytes (amoebocyte) in HL and 3 C.T. related cells include C.T. amebocytes, pore cell and granular cell. Amoebocyte was the main cell showing morphological and numeral changes in relation to exposure snail for infection by different miracidia.

Amoebocyte was 50-60x40-50µ with large nucleus and granular cytoplasm. The cells were few in number (one/3field) in non-infected snail. At 12-24hpe, cell appeared small in size, active proliferation increased to 3/one field. At 48 hpe to *S. mansoni* miracidia, cells were highly active; cytoplasm contained more refractile engulfed granules with few cytoplasmic vacuoles. At 72 hpe cells started to degenerate, architecture was detected with remnant of its content, and then disappeared. Changes in HL amebocytes were not detect in non-infected or snails exposed to *F. gigantica* miracidia as domestic parasite (Pl. 2, Fig.2)

P. acuta defense related cells: Two types of cells were recognized; C.T. granulocyte and HL amoebocyte. C.T. granulocyte was ovoid (15-20µ) with rounded open-phase nucleus (Pl. 2, Fig.3.A), cytoplasm contained large number of eosinophilic granules, trapped close to foot epithelium and between C.T. body matrix. The number markedly increased in snails exposed to *F. gigantica* or *S.mansoni* miracidia. The second cell was HL amoebocytes and similar in structure, distribution and function in relation to time post exposure to miracidia as in *L. natalensis* HL (Pl. 2, Fig.3b).

Table 2: Rate of successful infection in different exposed snails at 3 weeks pe

Exposed snail		Miracidia used for infection (type and dose)			
		F. gigantica miracidia		*S. mansoni* miracidia	
		2-4 / snail	40-50/snail	2-4 / snail	40-50/snail
L. natalensis	No. Exposed	50	75	50	75
	No. infected	50	All died after one week pe	0.0	0.0
	Infection %	100		0.0	0.0
B. alexandrina	No. Exposed	50	75	50	75
	No. infected	0.0	0.0	50	All died after two weeks pe
	Infection %	0.0	0.0	100	
P. acuta	No. Exposed	50	75	50	75
	No. infected	0.0	0.0	0.0	0.0
	Infection %	0.0	0.0	0.0	0.0

Discussion

Fasciola and *Schistosoma* are the two important zoonotic parasites causing severe economic and health problem in Egypt and worldwide. Both parasites were obliged to develop in intermediate snail hosts. With the difficulty in eradication of the mature flukes inside their human or animal hosts, the same parasites considered to be very weak inside their snail I.M.H. **(**Helmy *et al*, 2017). With increasing the incidence of infection by fascioliasis among human in Egypt, speculations about an adoption of *Schistosoma* snail to transmit *Fasciola* infection was reported (Farag and El-Sayed, 1995). Nevertheless, El-Bahy *et al*. (2014) reported that *Fasciola* developed in *L. natalensis* were unable to develop in *Lymnaea truncatula* for example. Also, little data about the tissue types and cellular reactions that occurred inside the snails after miracidial invasion that interfered with the suitability of this snail as suitable or unsuitable I.M.H. for development of the invading miracidia was available **(Saldanha *et al*, 2009)**. Moreover, Le Clec'h *et al*. (2016) identified some constituent and cells in *B. alexandrina* haemolymph consid-

ered to be an important anti parasite molecule involved in innate immune defense of the snails against invading pathogens.

Aiming to found an answer about the possibility of occurrence of an adaption between snails' species to act as intermediate host for other non-specific trematode, in the present study the laboratory breed *L. natalensis*, *B. alexandrina* and *P. acuta* snails were exposed separately to *F. gigantica* and *S. mansoni* miracidia in low and high dose.

In the present study, *F. gigantica* miracidia and *S. mansoni* miracidia did not develop to cercariae except in their specific snails *L. natalensis* or *B. alexandrina* respectively. Moreover exposure of both types of snails to high dose of their domestic miracidia cause mortality in the exposed snails due to dangerous development of the produced parthenitae after one week in *L. natalensis* exposed to *F. gigantica* and after 2 weeks in *B. alexandrina* exposed to high dose of *S.mansoni*. This meaning successful infection and the difference in time of death post infection was related to the nature of each parasite parthenitae as development of *F. gigantica* inside the snail including redial stage which characterized by mouth feed on the snail liver, while no redial stages during development of *S.mansoni* parthenitae as the sporocysts has no mouth and did not considered vigorous against the snail liver. In the same time no infection or mortalities could be detected in *L. natalensis* exposed to high dose of *S. mansoni* or in *B. alexandrina* exposed to high dose of *F. gigantica* miracidia or in *P.acuta* exposed to both types of miracidia. Failure of foreign miracidia to develop in snail other than their domestic snail host was accepted related to presence of type of innate resistance in the snails. This agreed with Sadaka *et al*. (2016) who reported that the genetic background in snails plays a major role in the determination of their compatibility to infection between *B. alexandrina* and *S. mansoni.*

The study showed that failure of infection in the exposed snail was associated with closely related reaction by the snail on the tissue and cellular level. Histopathological inspection revealed occurrence of successful penetration to different miracidia into tissue of the foot of the exposed snail. This followed by marked tissue reaction from the snail aiming to capsulate and destruct the invading foreign parasite prevented their further migration in the snail tissue. The degree and thickness of tissue reaction was increased with the increase in time post exposure till complete capsulation or disappearance of the invading sporocysts. In the author's opinions, the process was occurs closed up to the foot surface or little deeper in its tissue according to the nature of the snail and its cellular structure especially under their tegument layer. In the same time no tissue reaction was recorded in snail infected by its domestic parasite. This reaction introduced by the snail tissue was combined by markedly changes in the number and function of different types of cells present in the snail HL and tissue. In *B. alexandrina* there was marked increase in the number of hyalinocytes cells with detection of H-type 3 (El-Bahy, 2016). The content of granulocyte marked increase in the number and content of G-type 3 which had the ability to engulf foreign particles. This agreed with Helal *et al*. (2014) who determined that haemocytes cells in both haemolymph and tissue of *B. alexandrina* and described three types of cells classified according to their shape and granular contents. These cells were granulocytes, amoebocytes and hyalinocytes. They added that electron microscope study revealed the important role of granulocytes and amoebocytes as defense mechanism against snail infection.

In the present study, as to the size and distribution of hyalinocytes which increased in number only without detection to foreign particles in its cytoplasm, this type of cell was responsible for humeral defense mechanism of the snails. This agreed with Sadaka

et al. (2016), they mentioned that the internal defense system has the upper hand in determining the level of adult compatibility in the determination of *B. alexandrina /S.mansoni* compatibility. By the same way, marked increase in the cellular structure of HL and C.T. cells of *L. cailliaudi* and *P. acuta* was associated with the reaction of the snail versus the invading foreign miracidia.

The defense related cells in these snails were HL and amoebocytes as they appeared as phagocytic cells while other cells such as pore and granular cells was related to humeral mode of defense. This humeral mode was expressing its role via production of special component able to kill the invaders. Helal *et al*. (2014) mentioned that some constituent in *B. alexandrina* haemolymph such as Nitric oxide (NO) considered an important anti parasite molecule able to resist the infection. The level (NO) in haemolymph of *S.mansoni* infected snails was significantly increased than in non-infected snail groups. Also, Vorontsova *et al*. (2015) reported that haemocytes and haemolymph of *L. stagnalis* had PO activity that might be important in the formation of cytotoxic molecules, during snail defense immune reactions. Le Clec'h *et al*. (2016) reported that resistance of some types of snails versus infection was depends on presence of snail humoral factors (PO). PO enzymes played a key role in wound healing, tissue pigmentation. They are also involved in innate immune defense against introducing pathogens. Pathogens molecules were stimulate and activates PO enzymes. The active PO has cytotoxic activities damaging pathogen cells. Correa *et al*. (2010) stated that he most important intermediate host for *F. gigantica* is *Radix auricularia*. But, other species are also known to harbor the fluke including *Lymnaea rufescens* and *L. acuminata* in the Indian Subcontinent; *R. rubiginosa* and *R. natalensis* in Malaysia and in Africa respectively; and synonymous *L. cailliaudi* in east Africa. Phalee *et al*. (2015) in Thailand stated that adult fasciolids expelled eggs evacuated in the feces of the definitive host, usually cows or buffaloes. Miracidia hatched from the eggs in water, and penetrated only into several lymnaeid snails (intermediate hosts), such as *Lymnaea viridis*, *L. columella*, *L. cousin*, *L. ollula*, *L. natalensis*, and *L. auricularia rubiginosa*

In Egypt, Haridy *et al*. (1999) reported that during the years 1994 to 1997 the overall slaughtered animals in Egyptian abattoirs were 2,003,200 sheep and goats, 2,624,239 cattle and 3,536,744 buffaloes. The overall rates of fascioliasis were 2.02% for sheep and goats, 3.54% for cattle and 1.58% for buffaloes. Macroscopically sheep liver gave up to 100 flukes per liver in the largely dilated thick walled bile ducts. Cattle liver gave up to 275 flukes/liver inside thickened dilated and calcareous bile ducts with offensive yellowish brown bile. Buffaloes' liver gave up to 330 flukes per liver. Haseeb *et al*. (2002) reported that rabbits, donkeys and camels as well, were hosts for *F. gigantica*. Human infection causes serious hepatic pathological sequences that add to known threats to the liver of the Egyptian people. Two clinical stages are recognized in human fascioliasis. An acute stage coincides with larval migration and worm maturation in the hepatic tissue, and a chronic stage coincides with persistence of *Fasciola* worms in bile ducts. Human fascioliasis was very sporadic until last three decades where cases and outbreaks were reported. Dar *et al*. (2005) reported that *Radix natalensis* was the essential intermediate host for *F. gigantica* based on field and experimental studies. Cercarial production from *R. natalensis* experimentally infected with *F. gigantica* was affected by the definitive host species from which eggs were obtained and laboratory conditions. Another lymnaeid, *Galba truncatula* played a role in transmitting this parasite in Egypt, as it was found naturally infected with *F. gigantica*. Latter snail species, originated from France, was susceptible to experiment-

al infections with Egyptian miracidia of *F. gigantica* and it had a cercarial production close to that of local *R. natalensis*. They added that *Pseudosuccinea columella* and *Biomphalaria alexandrina*, were naturally found harboring larvae of *Fasciola* sp. but not proved as intermediate host. Soliman (2008) reported that the Australian *L. tomentosa* (host of *F. hepatica*) was shown to be receptive to miracidia of *F. gigantica* from East Africa, Malaysia and Indonesia.

Conclusion

Each snail species has its own humeral and cellular immune defense mechanism. This mechanism was able to early attack of the invading foreign miracidium and capsulate them before complete destruction of them. This snail reaction, preventing the foreign invaders migration to the snail liver and destroying them as early as it penetrates the snail tissue. Future works is ongoing based on characterization of shared protein bands between snails and their parasites. Determination of snails' genome clarifies host-parasite relationship at a molecular level.

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Plate 1: Cross section (C.S.) in domestic and foreign snails exposed to infection by *S. mansoni* and *F. gigantica* miracidia at different time post exposure.
Fig. 1a: C.S. in *P. acuta* snail showing marked distribution of basophilic cells (C.T. G) at sub-epithelial layers of foot tegument.
Fig. 1b: C.S. in *P.acuta* foot showing severe aggregation of basophilic cells in site of foreign miracidium penetration (arrow).
Fig. 1c: *F. gigantica* sporocysts (24 hpe) migrate in sub-epithelial C.T. of foot of *L. natalensis* without tissue reaction.
Fig. 1d: Sever tissue reaction closed to subepithelial tissue of *B. alexandrina* against migration of penetrating *F. gigantica* sporocysts 72 h post exposure.
Fig. 1e: *S.mansoni* sporocysts filled with cercaria developed without tissue reaction in liver of *B. alexandrina* 30 days post exposure.
Fig. 1f: *F. gigantica* **redia f**illed with cercaria developed without tissue reaction in liver of *L. natalensis* 30 days post exposure

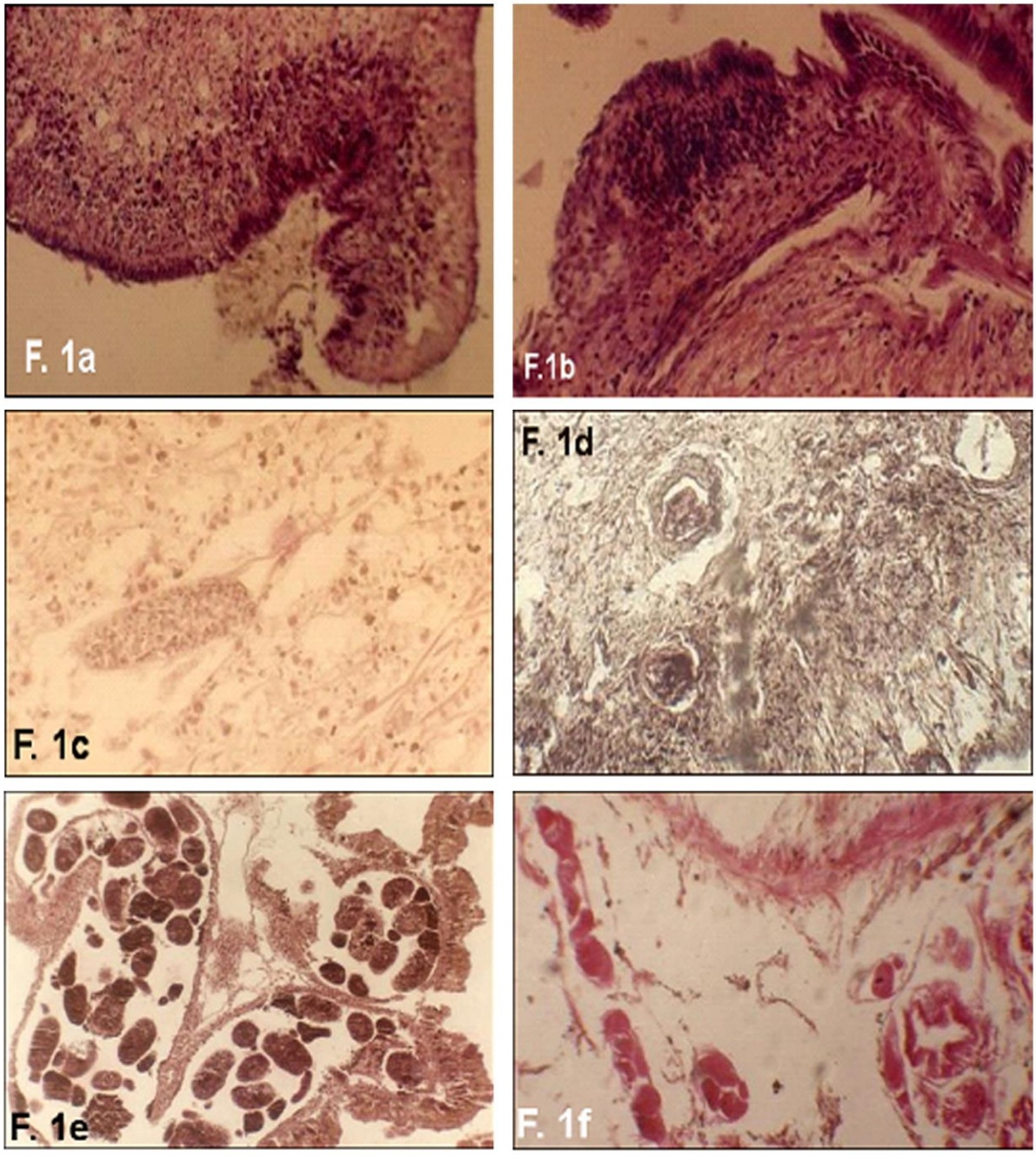

Plate 2: Changes in cells of snails exposed to high dose of foreign miracidia.

1- Changes in cells of *B. alexandrina* exposed to high dose of *F. gigantica* miracidia.

Fig.1a: Three stages of granulocyte (G-1, 2 &3) and two of hyalinocytes (H-1 &2).

Fig.1b: Rabid division of H-1 at 24 hpe.

Fig.1c: Increase in production of H-1 with increasing in size and activity of G-3 at 48hpe.

Fig.1d: Massive production of hyalinocytes and granulocyte after 48 hpe.

Fig.1e: Architectures of old snail granulocyte (G-4) in C.T. at 72 hpe.

2- Changes in cells of *L. natalensis* exposed to high dose of *S. mansoni* miracidia.

Fig.2a: Amoebocyte of non-infected snail.

Fig.2b: C.T. amoebocyte started to increase 24 h post infection.

Fig.2c: Three H-1 and amoebocytes engulfing particles at 24 hpe.

Fig.2d: Cell increase in size with increasing amount of engulfed particles at 48hpe.

Fig.2e: H-1 and G-4 engulfing more particles at 48 hpe.

Fig.2f: Snail amoebocytes after 72 hpe started to degenerate containing amount of engulfed particles.

3- Changes in cells of *P. acuta* exposed to high dose of *S. mansoni* or *F. gigantica* miracidia.

Fig.3a: C.T. granulocyte. Fig.3b: Haemolymph amoebocyte.