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ULTRASTRUCTURAL STUDY OF HOST-PARASITE RELATIONSHIP AND PATHOGENICITY OF *EIMERIA SP*. INFECTING LIBYAN JIRD (*MERIONES LIBYCUS*) *(LICHTENSTEIN,* 1828)

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Abstract

The relationship of *Eimeria sp.* and its host fat LibyanJird (*Meriones libycus*) was studied on an ultrastructural level. The host cellular organelles (nucleus, mitochondria, endoplasmic reticulum and golgi apparatus) and the changes of its infected intestinal epithelial cells during the development of parasitic stages (schizogony and gamogony) were studied and compared with those non-infected cells. The ultrastructures of intravacuolar tubules and folds in the parasitophorous vacuole (P.V.) were described. These fine structures may involve in the transport-tation of materials from the host cell to across the parasitovorous vacuole (P.V.).

Key words: Libya, Ultrastructure, *Eimeria* sp., Pathogenicity, *Psammomys obesus obesus,* Host-Parasite Relationship.

Introduction

The study of coccidian parasites in general, has greatly advanced. The taxonomy, developmental stages and host-parasite relationship were extensively studied (Landers, 1960; Reid, 1973; Scholtyseck, 1975; Millard and Lawn, 1982; Abdel-Ghaffar, 1990; Ahmed *et al*, 1995).

Parasite develop-ment, theinvasion, and the infection course depended upon this reciprocal relationship that influences the defense system of the host and pathogenesis (Bertolino and Canestri-Trotti, 2005).

The pathological effects and pathogenicity of coccidian led to great loss among domestic animals (Hein, 1976; Mesfin *et al*, 1978; Ernst, 1987; Danforth *et al*, 1992;Toulah *et al*, 2010). Although studies turned more to the fine structural analysis of life cycle and changes associated with development of different stages (Scholtyseck, 1979; Melhorn, 1988), yet, more attention has been given to the pathogenicity and the host-parasite interaction.

In the present study, *Eimeria* sp. naturally infecting *Meriones libycus* was used experimentally as a pathogen to its host and relationship was critically studied on the ultrastructural level.

Materials and Methods

The *Eimeria* sp. infecting the Libyan Jird (*Meriones libycus*), Family Muridae, order Rodentia was chosen. The fourteen *M. libycus* were isolated and tested to be coccidian- free by daily examination of faecal samples for at least 10 days before inoculation. Strict precautions were taken to prevent adventitious infection with coccidian. Coccidian-free rats were inoculated orally with approximately $1x10^5$ viable sporulated *Eimeria* oocysts collected and identified from naturally infected rats. The surface of these oocysts were sterilized in 10% potassium dichromate for 10 minutes then washed three times in distilled water, and finally re-suspended in glucosesolution before use.

Two infected rats were sacrificed every 24 hours post-infection (P.I) intervals. Two control animals were kept under the same conditions as infected ones and the feces was daily examined. The control rats were coccidian-free indicated precautions taken were adequate. The tissues of infected cecum and colon were fixed immediately in 3% gluteraldehyde buffered in 0.1 M sodium cacodylate (PH=7.3-7.4) for at least 4 hours a 4^oC.

Fixed tissues were passed 4-5 times in the same buffer (cacodylate) for 10-15 minutes for each, to remove the excess of the fixative. Treatment was carried out using 2% osmium tetroxide (O_5O_4) buffered also in sodium cacodylate for 2-3 hours, followed by 4-5 washings in the same buffer 20 minutes for each. Dehydration was done in ascending series of ethanol, staining with a mixture of uranyl acetate and phosphotungestic acid in 70% alcohol overnight. Dehydrated materials were treated with a mixture of absolute ethanol and propylene oxide (15 min.) and finally embedded in Araldire mixture.

Ultrathin sections were mounted on copper grids and stained with saturated alcoholic uranyl acetate for minutes and followed by alkaline lead citrate for 1-2 minutes (Reynolds, 1963). Microscopical examination was carried out by Philips (400 T) Transmission electron microscope.

Results

Results are shown in figures (1 to 8).

Discussion

In the present study, post-mortem examination showed that pathological changes were restricted to the cecum and colon. These sites were swollen, filled with yellow-white mucuid materials composed of amounts of cellular debris and blood, with inflamed bloody mucosa, necrosis and multifocal hemorrhage with moderate odema.

Electron microscopic study revealed that normal epithelial cells (Figs 1&2) had central nuclei, clear nucleoli and systemic distribution of chromatin. On the other hand, the infected epithelial cells were hypertrophied and assumed a rounded form (Figs 3-8). The parasitic stages were not in direct contact with the host cell contents, all parasitic stages were enclosed in the parasitophorous vacuole (P.V).Each vacuole was limited by a unit mem-brane (Figs 3-8).

Regarding host-parasite relationship

at the cellular level, the stages of parasite were occupied nearly whole cytoplasm of the infected cells. Each parasitic stage was enclosed in parastophorous vacuole that separated the parasite from cytoplasm of host cell (Fig.6). The development of schizonts and gametes were accompanied by some changes in the cytoplasm and the nucleus of the infected host cell.

Many previous studied revealed that the main site of host-parasite interaction was the parasitophorus vacuole which usually surrounded all intracellular parasitic stage (Muller and Scholtseck, 1974). The exact origin of P.V. and its limiting membrane were a matter of speculation for a long time, but become established in its host origin.

Nuclei of the infected host cells were usually hypertrophied, deformed, and displaced from its central position (Figs 3, 4 &6). Some nuclei partially surrounded the parasite parasitophorus vacuole and its chromatin was not distributed (Fig. 4).

During the maturation of parasitic stages, mitochondria of the infected host cell showed changes due to the parasitism infected epithelial cells contained a great number of mitochondria which appeared swollen when compared to with those in normal cells (Figs 4,6). The extension of the inner membrane, the mitochondrian cristae appear more shortened and not reaching the mitochondria other side. In many infected cells, the cristae of mitochondria were fragmented or completely dissolved (Figs. 5 &8).

In addition, endoplasmic reticulum (ERO showed several changes when compared with normal one of infected cells (Fig. 5). The changes in endoplasmic reticulum depend on prepatent period of the parasite.

During the parasite development, intra-vacuolar tubules (IT) and folds (IF) were observed only during microgamont maturation. The intra-vacular tubules (IT) occupies the whole space of P.V. (Fig. 8). The intravacular folds were distributed around the limiting membrane of P.V. and never observed in a direct contact with the parasite surface. Usually these folds were extended up to mid-way of the parasitophorous vacuole towards the parasite surface (Fig.8).

The parasitophorous vacuole and its contents form the immediate environment for the parasite including intravacuolar tubules and folds, suggested that they might be involved in transport of materials from the host cell to parasite (Hammond, 1973; Michael, 1975; Varghese, 1975; Abdel-Ghaffar, 1990; Bashtar *et al*, 1992; Wiedmer *et al*, 2011; Kurth *et al*, 2012).

The present study showed two different way of transportation, the intravacuolar tubules and folds associated with development of gamonts, only intravacuolar tubules distributed over the P.V. Ahmed *et al*. (1995) reported that intravacuolar tubules associated with the first generation merozoites and intravacuolar folds associated with second generation merozoites presence or absence of these structures seems to be not only species-specific but also a

developmental stage specific (Hammond *et al,* 1967; Abdel-Ghaffar, 1990).

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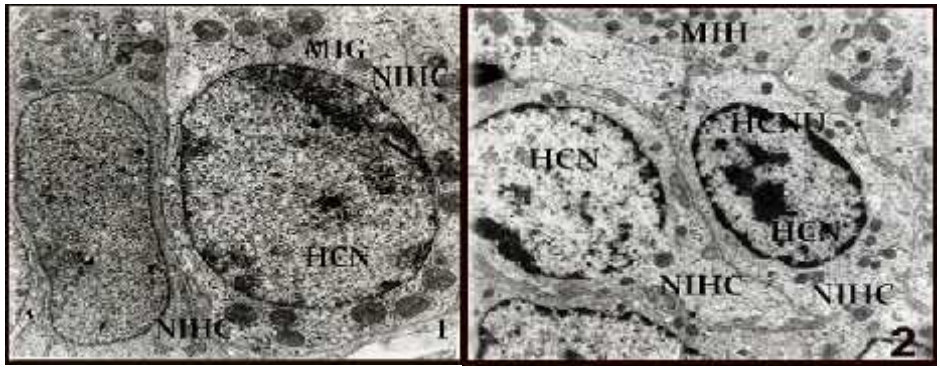
List of Abbreviations

CR: Cristae.
ERH: Host cell endoplasmic reticulum.
GB: Gologi bodies.
HCN: Host cell nucleous.
HCNU: Host cell nucleous.
IF: Intravacuolar folds.
IHC: Infected host cell.
IV: Intravacuolar tubules.
M: Merozoites.
MIG: Microgamont.
MI: Mitochondria.
MG: Microgament.
MIH: Host cell mitochondria.
N: Nucleus.
NIHC: Non-infected host cell.
NU: Nucleolus.
PV: Parasitophorous vacuole.
RB: Residual body.

Explanation of TEM Figures

Fig.1&2: Non-infected *Psammomys O. obesus* intestinal epithelial cells with coccidian. Central position nucleus (HCN) and shape of mitochondria and (MIH) endoplasmic reticulum (ERH) X 15000
Fig. 3: Early schizont within parasitophorous vacuole (P.V), nucleus of infected epithelial cells displaced from its position. X45000
Fig. 4: Second generation merozoites (M) of *Eimeria* within parasitophorousvacuole (P.V.). Infected epithelial cell with nucleus displaced from position and partially surrounding parasite P.V. and dilated cristae of endoplasmic reticulum (ERH). X1600
Fig. 5: Second generation merozoites (M), hypertrophoid hosts mitochondria (MIH). X16800
Fig. 6: Developing microgamont (MIG) with attached and detached microgametes (MG). Nucleus of infected epithelial cell displaced from position. X38200.
Fig. 7: Microgamont of *Eimeria*. Intravacuolar tubules (IT) providing a mode of connection between host cell and parasite. X35800.
Fig. 8: Intravacuolar folds (IF) passing across P.V. of developing microgamete. X 45000


MIG
NIHC
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1
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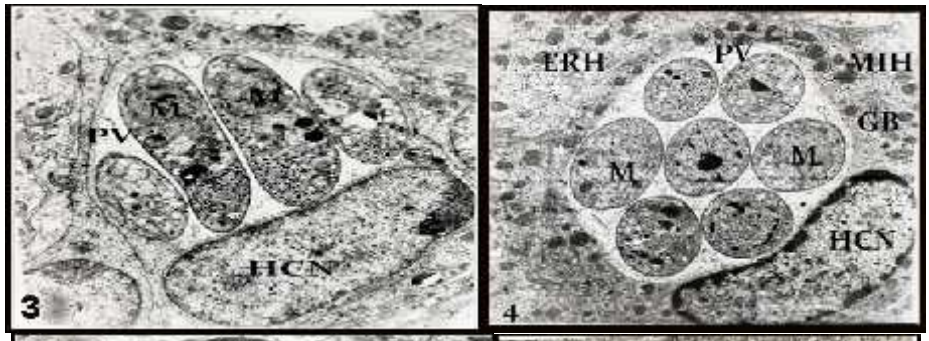
PV
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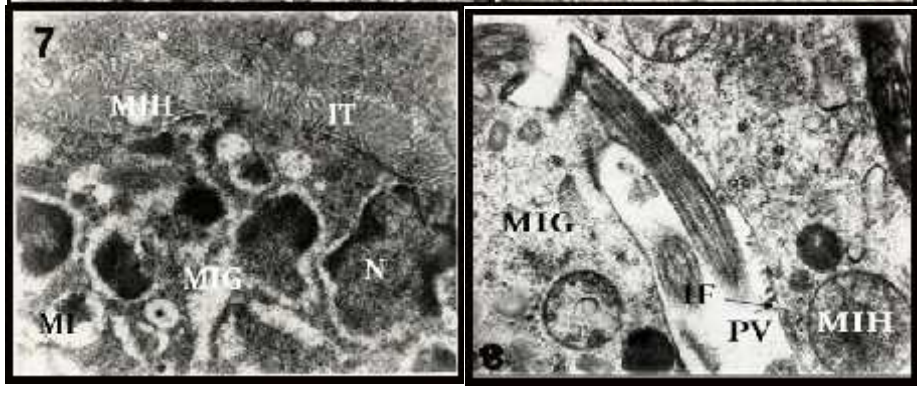
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MIH
IT
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