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FINE STRUCTURAL STUDY ON EIMERIA SP. INFECTING THE LIBYAN JIRDS (*MERIONES LIBYCUS*) IN SAUDI ARABIA: MEROGONY, MACROGAMETOGENESIS AND HOST-PARASITE RELATIONSHIP

By

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Abstract

Ultrastructural characteristics of merogony and the development of mature merozoits and macrogametogensis of *Eimeria sp*. infecting the Libyan jird were investigated. Mono and binucleated schizonts were detected. Developed merozoites showed all the apicomplexan architecture (Pellicle, conoid, rhoptries, micronemes … etc). Transformation into macrogametes were studied. Early macrogamonts characterized by the loss of all apicomblexan characters and the appearance of wall-forming bodies were the first indication for macrogametogenesis. As development precepded, two types of wall forming bodies (I, II) were clearly detected. Cell organelles including amylopectin granules and lipid globules were greatly increased in mature macrogametes. Young oocyst (zygote) with double-layer oocyst wall were also detected. Host cell reaction due to infection included hypertrophy of the infected host cell, enlargement, deformation and displacement of the host cell nucleus. Swollen and degeneration of mitochondria, endoplasmic reticulum and cytoplasmic vacuolation of the host cell. All parasite stages were enclosed in parasitophorous vacuole limited by unit membrane. Extended damage effect appeared in some neighboring host cell indicating the secretion of some toxins by the parasite.

Key words: *Eimeria* spp, Oocyst, Ultrastructure, Libyan jird, Saudi Arabia.

Introduction

Nowadays, advent of electron microscope has paved the way to large development in the study of parasitic protozoa. Many *Eimeria* species infecting different animals were described. Most of these species were identified according their shedded oocysts, their morphological and morphometric characteristics. Many ultra-structure reports were announced upon endogenous stages of different Coccidia infecting mammals. birds and reptiles (Mehlhorn, 2006). However, the parasites of wild animals got less attention and less studies (Bashtar and Abdel-Ghaffar, 1987).

Because of such wild animals, which may play a role as reservoirs and/or intermediate hosts for many zoonotic parasites, it was importantly to pay more attention to such animals and zoonotic parasites. Some of these studies dealt with such hosts and parasites (Abdel-Ghaffar 1990; Abdel-Ghaffar *et al*, 1991a,b) who investigated the eimerian infection and ultrastructural characteristics of the first generation schizonts and merozoites of *E. arvicanthi* infecting the field rat *Arvicanthis niloticus* in Egypt.

The present study aimed to investigate the *Eimeria* infection among one of the commonest wild mammals, the Libyan jird (*Meriones libycus*) in Saudi Arabia.

Materials and Methods

The Libyan jird *Meriones libycus,* which is widely spread globally and not only restricted to Libya as their name may presume were captured from Al Nassieem, east of Riyadh City, the Capital. Samples were immediately transported to the Parasitology Research Laboratory, Faculty of Science and Humanities, Shaqra University.

The collected animals were investigated for natural coccidian infection bu using the usual techniques. Naturally infected animals were isolated and shedded oocysts were collected and identified. These oocysts were washed

and allowed to sporulate. Young jirds were isolated and tested to be coccidia free through daily examination of the fecal samples for ten days before inoculation. Strict precautions were taken to prevent zoonotic infection. Fifteen non infected animals were inoculated through a stomach tube by 10^5 sporulated eimerian oocysts previously collected. Three infected Jirds were killed at intervals of 12 hr. post infection.

The mucosa of the upper part of colon just below caecum were scratched and immediately fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.3 for at least 4h. The specimens were post fixed with 2% OsO_4 in same buffer. Samples were then dehydrated in ascending series of ethanol and stained with uranil acetate and phosphotungestic acid in 70% ethanol for about 2 hr each. Finally, materials were embedded in Araldite medium (Serva). Semi and ultrathin sections were prepared using Reichert Ultracut-microtome. Examination of ultrathin sections was carried out by Zeiss transmission (EM 952).

Results

In the present study, it was difficult to identify the free sporozites freed from the inoculated sporulated oocysts and how they invade the host cells. The first stage observed was the uninuclear rounded schizont which still had some apicomplexan characteristics after transformation. Remnants of the two membranous pellicle, some micronemes and amylopectin were observed (Fig. 1), as development precedes bi and multinuclear schizonts were detected.

Tubular type of mitochondria characteristic for such stages were very clear gathered just beneath the boundary of the parasite which surrounded by a unit membrane. Parasites were enclosed in a clear wide parasitophorous vacuole varies in size and contents. It differs from clear zone separating the parasite from its host cell to granulated one finally limited by a unit membrane representing parasitophorous vacuole membrane. Regarding the host-parasite reaction, it was clearly detected. Deformation of the host cell nucleus, swollen and degeneration of mitochondria cristae of which some come in direct contact with the parasitophorous vacuole membrane (Fig. 2).

At the end of these process, mature schizonts with fully formed merozoites were observed which varies in size and number. These stages showed all the apicomplexan characteristics. In longitudinal sections of these merozoits, conoid, micronemes and rhoprites were observed at the anterior end of the parasite, clear large nucleus was situated in the posterior half of the body. The whole body length was limited by a bilayered pellicle, some amylopectin granules reserve food materials were also observed (Fig.3).

The contents of the infected host cell seems to be exhausted. During transformation of the elongated fusiform merozoite motile stages, globular stages loosing all apicomplexan characteristics were observed (Fig.4).

These stages were singly occurred in a wide parasitophorous vacuole. Each of which contained single central large nucleus usually with prominent nucleolus. The first indication of macrogametogenesis and the formation of macrogamont as an early stage in the process was the first appearance of few large osmiophilic bodies distributed in the cytoplasm. They were called wall-forming bodies. These bodies developed within enlarged vesicles. Again the parasites are limited by a unit membrane and all apical complex characteristics were lost. The host cell cytoplasm is mostly exhausted and the parasitophorous membrane came nearly in contact to the host cell membrane (Fig. 4). As development precede, another type of wall forming bodies appeared which was smaller than others and arranged more peripherally. Therefore, two types of wall forming bodies were observed namely wall-forming bodies I and II. Wall forming bodies II was that which appeared at first while those of the first type developed later in the cytoplasm. The wall forming bodies were homogenous electron dense bodies which differs in size and location. Mature macrogametes were characterized by their rich supply of reserve food materials

including large lipid globules and the carbohydrate reserve food material which proved to be amylopectin granules which appeared as brilliant small granules distributed randomly in the cytoplasm (Fig. 5). Through this study, events of fertilization were not recorded. Young oocysts or zygotes were only seen. These stages were characterized by completely disappearance of wall-forming bodies and oocyst wall formation, composed of two membranes, an inner thin one surround by a thick layer to outside. Large central nucleus and reserve food materials were occupying the whole cytoplasm (Fig. 6).

All the previously described stages were infecting the columnar absorptive epithelial cells only of the mucosa. Goblet cells were never seen to be parasitized. Throughout the whole examination, it was clear that the host cells were mainly affected due to the parasite mechanical action. The long clyndrical host cell mitochondria were swollen and attained a spherical form, their cristae tend to decrease in number and began to degenerate. Besides, a concentration of some mitochondria in the very close contact to the parasitophorous vacuole was the most interesting record in the present study (Fig. 2). More interesting was the extended damage effect in some neighboring host cells indicating the secretion of some toxins by the parasite.

Discussion

The different stages developed throughout the whole life cycle of coccidian parasites of the phylum Apicomplexa showed a considerable changes in shape and structure. The changes led actually to a new functional state. Two distinct forms were clearly observed, a motile stages including tsporozoites and merozoite which have all the apical complex characters including conoid, rhoptries, micronemes… etc and the non-motile stages including schizonts and gamonts beside zygotes and oocysts. The present observed merozoites were similar to those previously described (Scholtyseck and Abdel-Ghaffar, 1981; Mehlhorn, 2008). During transformation of these motile stages, most of apical complex organelles were evidently gradually disappeared. Longitudinal merozoites changed to globular form, the pellicle surrounding these merozoites was lost and the new developed stages were limited by a unit membrane. In this respect, the present results are similar to those previously described (Chobotar *et al,* 1975; Bashtar *et al,* 1984; Abdel-Ghaffar, 1990). In contrast to these findings, the cyst-forming Coccidia, Frenkelia (Gobel *et al,* 1978), *Besnoitia* species (Senaud and Mehlhorn, 1978) and *Toxoplasma gondii* (Scholtyseck, 1973; 1979) maintaining the normal three-layered pellicle during schizogonic cycle.

The sexual cycle of the Coccidia usually develop from merozoites after certain asexual generations. The sexual phase of the life cycle is of great importance because it probably plays an important role in the host specificity of the Coccidia (Scholtyseck *et al,* 1971a).

The maturation of coccidian macrogamete and the formation of the oocyst wall have been always of considerable interest. This is because such phase of development culminates in the production of a stage which is highly resistant to various environmental factors and the means of subsequent infection of new host animals (Chobotar *et al,* 1980; Abdel-Ghaffar 1990; and Mehlhorn 2008).

During the transformation of sexually differentiated merozoites into macrogamonts, the first macrogamonts indication characterizing them from other ones is the appearance of the early developed wall-forming bodies (Scholtyseck *et al,* 1969a; Chobotar *et al,* 1975; Mehlhorn 2008). In the present study the transformation of merozoites into macrigamonts was similar to that reported for other *Eimeria* species (Speer *et al,* 1973; Wheat *et al,* 1976; Abdel-Aal 1991; Abdel-Aal *et al,* 2000).

Parasites which produce cyst wall such as Coccidia, Myxozoa and Microsporidia develop different kinds of the so called wall-forming bodies. In the present study, wall-forming bodies were differentiated into two types (WFI & WFII). These are defined

according to their fate and not to their appearance. These bodies fuse below the cell membrane in an inner and outer cyst wall. This finding was previously recorded for other *Eimeria* and *Isospora* (Bashtar *et al,* 1991; El Toukhy *et al,* 1997; Al-Hoot 2000). In contrast, one type of wall-forming bodies was recorded depending to the genus described. Only one type of wall-forming bodies was recorded in *Eimeria mivati* (Vrba *et al*, 2011) and some *Sarcocystis* species (Vetterling *et al,* 1973; Zaman and Colley, 1975). On the other hand, these bodies were not identified during macrogamonts maturation of other *Sarcocystis* species (Sheffield and Fayer, 1978; Entzeroth 1982).

Regarding the host-parasite relationship, it was clear that the parasite greatly affect the infected host cell through exhausting of the cytoplasm and the changes takes place in the different cell organelles which is clearly observed in the present study. This indicate that host-parasite relationship has ultimately cytological basis (Trager, 1974). However, the site of infection was restricted in the present study to the absorptive epithelial cell's and the ability of coccidian parasites to infect different types of cells was proved (Michael 1975). All changes recorded in the present study was similar to that reported in *Eimeria* and *Toxoplasma* infections (Sheppard, 1974; Ferguson *et al,* 1976; Abdel-Ghaffar *et al,* 1986; Bashtar *et al,* 1991).

Conclusion

Coccidiosis is the most prevalent disease causing widespread economic loss, especially in poultry farms and pet animals with potential risk for animal handlers.

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Abbreviations in TEM figures

A	Amylopectin
C	Conoid
HC	Host cell
HCN	Host cell nucleus
IW	inner wall
L	Lipid globules
MI	Mitochondria
MIH	Host cell mitochondria
MN	micronemes
MPV	parasitophouous vacuole membrane
MV	microvilli
N	nucleus
NU	Nucleolus
OW	outer wall
PE	Pellicle
PV	Parasitophorous vacuole
RH	Rhoptries
WFI	Wall-forming bodies of the first type
WFII	Wall-forming bodies of the second type
WF	Wall-forming bodies

Explanation of figures

Fig. 1: Uninuclear schizont infecting an epithelial cell. Note pellicle, central nucleus and amylopectin reserve food material. (X 18000)
Fig. 2: Developed binucleated schizont in large parasitophorous vacuole. Note damaged host cell mitochondria attached to parasitophorous vacuole membrane (X 21000).
Fig. 3: Developed merozoites in mature schizont which exhaust nearly whole cytoplasm of host cell. Note apical complex characteristics in each merozoite (X 25000).
Fig. 4: Developed macrogamont indicated by appearance of wall-forming bodies. Note displaced and deformed host cell nucleus, large central nucleus with prominant nucleolus of gamont (X 23000).
Fig. 5: Mature macrogamete with wall-forming bodies; rich supply of reserve food material including lipids & amylopectin (X 20000).
Fig. 6: Young oocyst (zygote). Note formation of oocyst wall consists of an outer thick membrane and inner one, disappearance of wall-forming bodies and very rich supply of reserve food materialsof lipids and amylopectin (X26000).