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Light and Electron Microscopy of Macrogametogenesis and Oocyst Formation in *Eimeria bateri* Infecting Quails from Saudi Arabia

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Abstract. The development of macrogametes of *Eimeria bateri* was studied by light and electron microscopy in the epithelial cells of the villi at 88, 96, 106 and 120 hrs post-infection (P.I.). The appearance of young macrogamonts at 88 hrs P.I. was characterized by the loss of the characteristics of the apicomplexa (polar ring, rhoptries, micronemes, conoid, subpellicular microtubules), while the pellicle became only one unit memberane. Mature macrogametes were seen at 96 hrs P.I. and they were recognized by their large size, appearance of wall-forming bodies of type I and type II as well as the increased numbers of cell organoids and food reserves (lipids and polysaccharides). Young oocysts were recorded at 106 and 120 hrs P.I., and characterized by their 2-layered oocyst wall and the absence of the wall forming the bodies of both types. Host cell reaction to infection included the enlargement of the cell, swollen and degeneration of mitochondria and increased activities of the goblet cells and their mucoid secretions.

Introduction

Members of the family Eimeriidae are apicomplexan parasites that belong to the eucoccidian suborder Eimeriina. They have an obligatory monoxenous life cycle [1]. At the same time, many ultrastructural reports were announced upon the endogenous stages of various coccidians infecting house animals and birds, including macrogametogenesis [2-4]. Moreover, the sexual phase of the life cycle is of great significance of the coccidians [1, 3-7].

Despite the animal fauna of Saudi Arabia that was very rich with its poultry, domestic and wild animals, very few parasitological studies of their coccidian infections were known [8-11]. Therefore, the present study aims to investigate the light and ultrastructural characteristics of macrogametogenesis and oocyst wall formation of *Eimeria bateri*, in addition to some aspects of the host-parasite relationships.

Materials and Methods

Eight 7-week old coccidia free quails (*Coturnix coturnix*) were isolated singly in separate wire-mesh hanging cages. Experimental infection was carried out using sporulated oocysts of *E. bateri* previously collected, identified and allowed to sporulate from naturally infected quails. Two experimentally infected birds were sacrificed at 88, 96, 106, 120 hrs P.I. (post-infection). The site of infection and invasion was recorded before [12]. The mucosa of the infected portions was scraped, fixed in 5% v/v glutraldehyde, and processed for EM [12]. Ultrathin sections were examined with JEM-100 CX-Joel transmission electron microscope.

Results

The majority of the 2nd generation merozoites initiate developmental gamonts after the invasion of the intestinal epithelial cells. The earliest macrogamonts were first identified at 88 hrs P.I. and were occurred singly in parasitophorous vacuoles (Figs. 1-3). Macrogamonts were distinguishable from schizonts and microgamonts by their relatively large nucleus. They often contain a promenant nucleus and the wall-forming bodies (Figs. 2-4). During the gradual transformation of the female differentiated merozoites into the globular large marcrogamonts, most characteristics of the apical complex as rhoptries conoid and micronemes disappeared (Fig. 6). The developing macrogamont is limited only by a unit membrane, which was underlayed in some places by the former merozoite's inner membrane complex (Fig. 6). The most distinct cytoplasmic inclusions accumulated at this stage was the wall forming the bodies of the 2nd type (WFBII). These were spongy in their appearance, formed within the enlarged vesicles of the endoplasmic reticulum and were located centrally around the nucleus. The WFBI appeared next to WFBII, and these were smaller in size and located more peripherally (Fig. 7). As development proceeded, the macrogamont and its enclosing parasitophorous vacuole grew distinctly in size so that the cytoplasm of the infected host cell became a narrow strip surrounding them and always containing only degenerated fragmented cell organelles (Figs. 2-9). Lipid and polysaccharide inclusions were encountered throughout the developmental process of the macrogamont (Figs. 6 and 7). The mature macrogamete possessed numerous wall-forming bodies of both types as well as lipid droplets and polyscaccharide granules (Fig. 7). Mature macrogametes measured 9 x 15 μm and usually observed at 96 hrs.

When the mature macrogamete had been fertilized by a microgamete, the formation of the oocyst wall was initiated. The post fertilization indications in zygotes were the disaggregation and/or the fusion of the wall forming bodies of each type together (Figs. 5-9), as well as the remarkable increase in lipid and polysaccharide inclusions (Figs. 7-9).

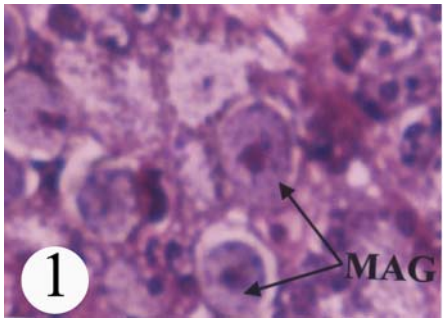

Fig. 1. Developing macrogamonts (MAG) at 88 hrs P.I. X 1300.

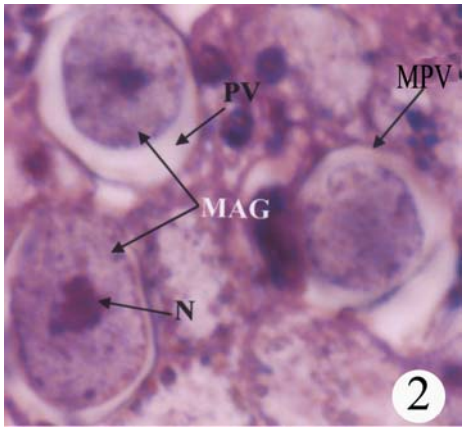

Fig. 2. Immature macrogamonts (MAG) within large parasitophorous vacuoles (PV). X 1800.

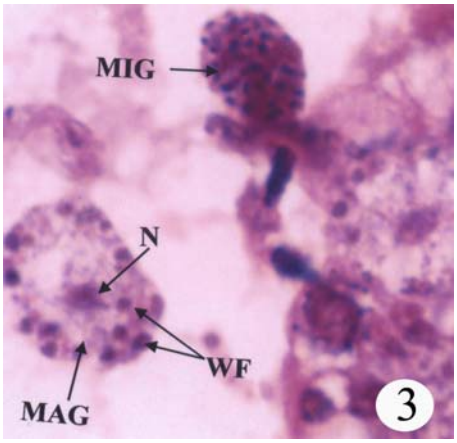

Fig. 3. Macrogametes (MAG) with wall forming bodies (WF). X 1950.

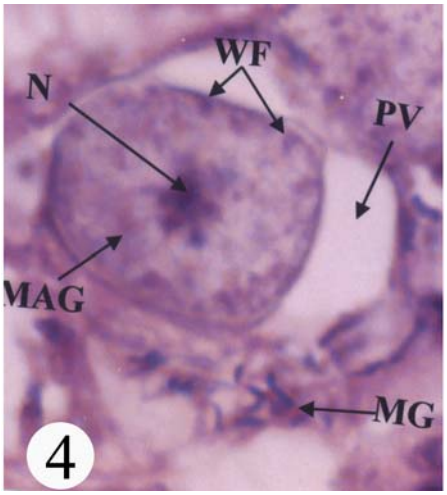

Fig. 4. Macrogametes (MAG) with wall forming bodies (WF). X 1950.

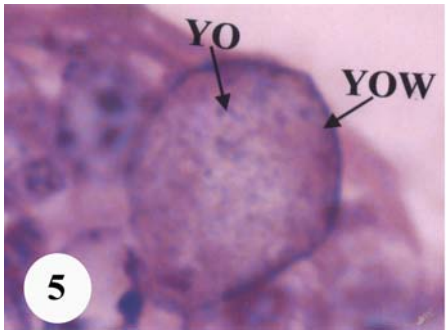

Fig. 5. Young oocyst (YO) after fertilization. X 1750.

Disintegration and gradual dissolution of WFBI were correlated to the appearance of a membrane bound layer beneath the limiting membrane of the fertilized macrogamete. This layer represents the outer layer of the future oocyst wall, while the WFBII fused together and occupied a more peripheral position which later form the inner layer of the oocyst wall (Figs. 8 and 9). It was observed that the process of oocyst wall formation did not occur symmetrically in all places, but the wall forming bodies of each type fused together in distinct places and then spread laterally. Also, after the completion of this process, the wall-forming bodies of both types I & II were no longer seen in the young oocyst (Figs. 5, 8-9).

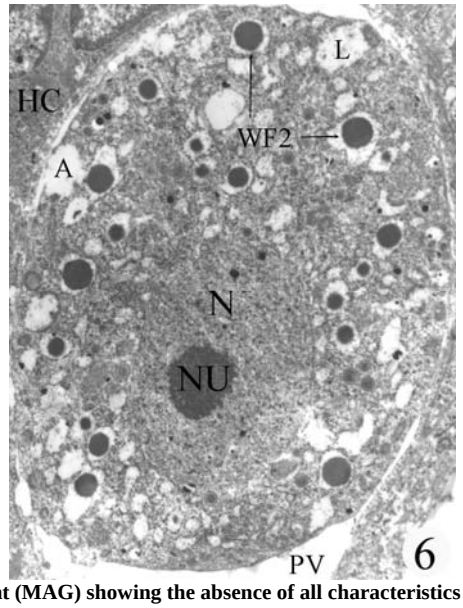

Fig. 6. Young macrogamont (MAG) showing the absence of all characteristics of the apicomplexa and appearance of the wall forming bodies (WFB II). Note also the large nucleus (N) and the promenant nucleolus (Nu). X 11500.

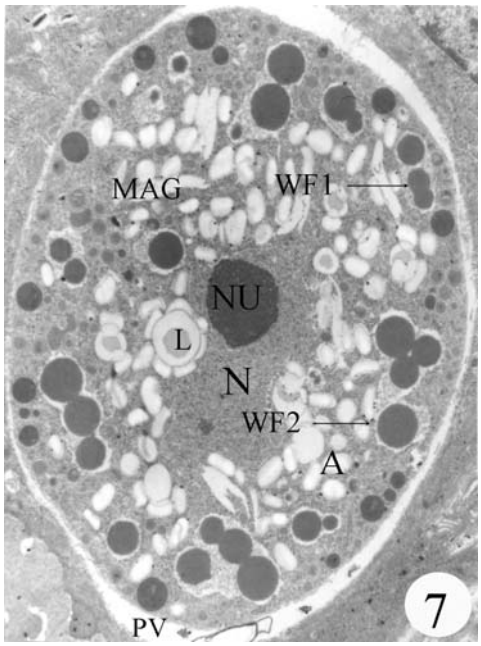

Fig. 7. Mature macrogamete (MAG) with wall-forming bodies (I & II). Note the accumulation of both lipid and polysaccharides (L & A), (PV) Parasitophorous vacuole. X 12000.

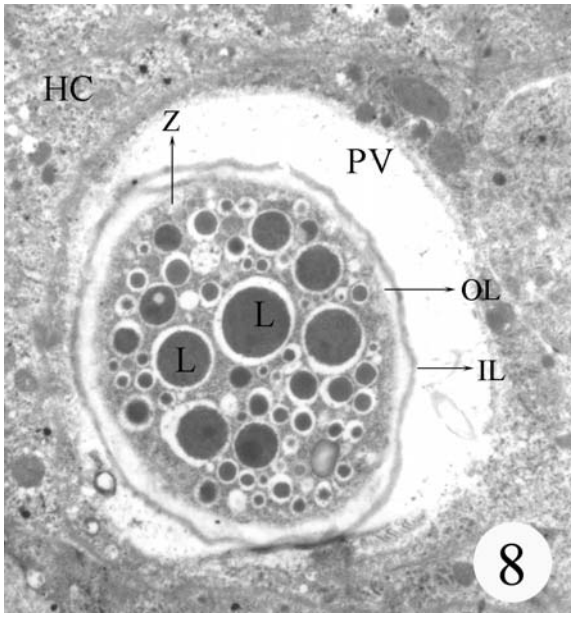

Fig. 8. Developing oocyst (YO) after fertilization, showing the appearance of the outer layer (OL) of the oocyst wall, while the inner layer is not completed. X 9350.

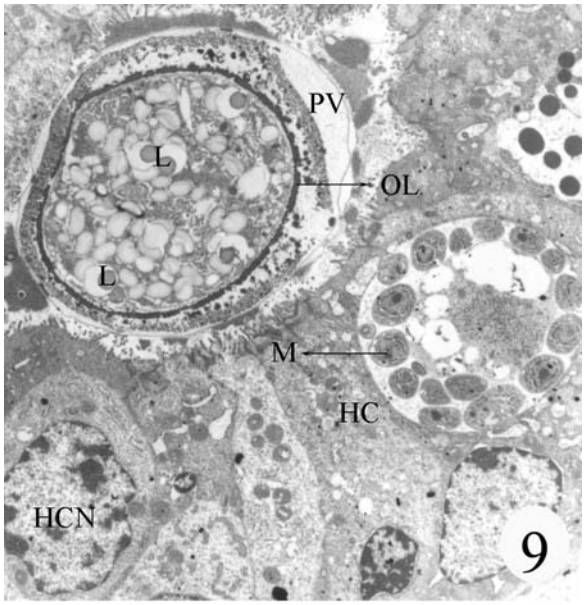

Fig. 9. An oocyst with an outer boundary of 2 layers (OL & IL). Note the accumulation of the lipid droplets (L) and the amylopectin granules (A) in the oocysts. X 8500.

Throughout the study, it was clear that parasites only harboring the columnar absorptive epithelial cells and goblet cells never parasitized. Also, the host cells were mainly affected mechanically, while the developing gamont exhausted nearly the whole contents of the host cell. However, the host cell nucleus became creasent in shape and it was located peripherally at the other side of the parasite. On the other hand, goblet cells at the sites of infection were generally activated and the mucoid secretions were increased. Although infected birds seemed to be weak, less active and excreting greenish mucoid faeces usually recover after 10-12 days P.I.

Discussion

Coccidian macrogamete development to maturation and the appearance of the wall-forming bodies I and II which always led to the formation of the oocyst wall are of high interest because the events of fertelization are still completely obscure. Moreover, this process is usually culminated in the production of a stage highly resistant to various environmental factors and sporulation initiating the subsequent infection of new hosts [1, 3, 4, 6, 13-17].

The commencement of the sexual phase in any eimerian infection takes place after a species-specific number of asexual cycles [1, 4, 6, 16]. The development of macrogaments in *E. bateri* (present study) arise mainly from the 2nd and 3rd generation merozoites at 88 and 96 hrs P.I. respectively.

The transformation from merozoite to macrogamont including some modifications in shape from elongate to round and in structure, especially the absence of all the architectures of the apical complex as well as the appearance of the wall forming bodies I and II in the macrogamonts. Similar observations were recorded before on many eimerian species [1, 3, 4, 6, 13, 14]. Furthermore, early macrogamonts in the present study were enclosed with a unit membrane which is probably more suitable for the rapid volume increase accompanied by maturation, transport of food materials and fertilization later on [1, 4, 6].

Mature macrogametes had two types of wall-forming bodies (WFBI & WFBII) which were named after their fate and function rather than the order of their appearance [1, 6, 17-19]. However, one type of wall forming bodies was reported in *E. truncata* [2]. Also, [1] added that the number of wall–forming bodies is a genus-dependent character, since they were absent in *Plasmodium*.

Regarding the origin of the wall-forming bodies, many authors supported the opinion that WFBII appeared early from the endoplasmic reticulum [6, 14, 18]. Meanwhile, WFBI developed later from Golgi complex [20]. In contrast to the previous suggestions, [17] stated that WFBI developed before WFBII in *E. tenella*. They added that only WFBII played the major role in oocyst wall formation, whereas WFBI formed the residual body.

Detailed observations of fertilization in coccidian parasites are rare and scarce [14, 21]. The remarkable disaggregation and/or disappearance of the wall forming bodies of the first type forming an irregular osmiophilic masses which subsequently fused together forming the outer bound layer. At the same time, the WFBII moves peripherally and coalesced together forming the inner layer of the oocyst wall [6, 14, 18]. In general, oocysts of *E. bateri* had 2 prominent layers similar to other *Eimeria* sp. [1, 6, 14, 17, 18, 20]. However, single oocyst wall was reported in some studies [1, 22]. The presence of reserve food materials as lipid droplets and polysaccharides in macrogametes and unsportulated oocysts of *E. bateri* was reported before in the genus *Eimeria* [3, 4, 6, 14].

The present study showed that morphological and structural changes in the infected host cells can be directly attributed to the eimerian infection, since non-infected cells seemed usually normal and also increased functions of the goblet cells was also recorded only at the sites of infection. Furthermore, hypertrophy and swollen mitochondria of the infected host cells are similar to some reports [3, 4, 6]. This may indicate the secretion of a coccidian toxic products which caused the mal-function of the intestinal cells as a general effect.

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دراسة با هر الضوئي والإلكتروني على تكوين الأمشاج المؤنثة والأكياس البيضية في أيميريا باتري التي تصيب طائر السمان في المملكة العربية السعودية

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المملكة العربية السعودية

(قدم للنشر في ١٤٢٧/١٢/٢٠هـ؛ وقبل للنشر في ١٤٢٨/١/٢٣هـ)

ملخص البحث. تم دراسة أطوار تكوين الأمشاج المؤنثة في أيميريا باتري *(Eimeria bateri)* في الخلايا الطلائية للخملات بعد ٨٨، و ٩٦، و ١٠٦، و ١٢٠ ساعة من العدوى التجريبية لطيور غير مصابة طبيعيًّا. حيث ظهرت الجامونتات المؤنثة الصغيرة بعد ٨٨ ساعة من العدوى، وقد تميزت بشكلها شبه الكروي، كما أنها فقدت جميع خصائص معقدات القمة التركيبية، أما الجاميتات المؤنثة الناضجة فقد ظهرت بعد ٩٦ ساعة من العدوى وتميزت بحجمها الكبير وظهور الأجسام البانية للجدار بنوعيها (I, II)، كما أنها احتوت على كميات كبيرة من المواد الدهنية والسكريات الغذائية بالإضافة إلى العضيات الخلوية مثل الأجسام السبحية، والشبكة الإندوبلازمية، والريبوسومات، وأجسام جولجي. ومن خلال الدراسة الحالية لم تُسجل وقائع عملية الإخصاب، لكن الأكياس البيضية الصغيرة (غير الناضجة) ظهرت لأول مرة بعد ١٠٦ ساعات من العدوى، وقد تميزت تلك الأكياس البيضية بجدار خارجي من طبقتين غير منتظمتين مع غياب أو تحلل للأجسام البانية للجدار حيث اختفت نهائيًّا.

ومن الملاحظات المسجلة على خلايا العائل انتفاخ الخلايا المصابة وتضخمها مع انتفاخ الأجسام السبحية والأنوية، أما السيتوبلازم فكان قليلاً كمًّا وغير محبب وأقل امتصاصًا للصبغات، ومن ناحية أخرى ازداد انشطار الخلايا الكأسية وإفرازاتها المخاطية التي تنزل مع البراز، وقد يكون مائلاً للاخضرار. والجدير بالذكر أن أيميريا باتري غير قاتلة وأن العوائل المصابة تُشفى بعد ١٠-١٢ يومًا من الإصابة.