Life cycle of *Eimeria rousetti* sp. nov. (Alveolata: Apicomplexa: Eimeriidae) infecting the frugivorous bat, *Rousettus aegyptiacus* Geoffroy, 1810 (Mammalia: Chiroptera: Pteropodidae) in Egypt.

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Abstract: Developmental stages of the life cycle of *Eimeria rousetti* sp. nov. were described for the first time from the frugivorous bat, *Rousettus aegyptiacus* in Egypt. The infection rate was 32%. Oocysts were collected and identified from naturally infected bats. They were subspherical to ovoid in shape; measured 24.43 x 19.33 µm and limited by a smooth colourless double-layered wall; no micropyle but a polar granule was observed. Events of sporulation were described and sporulation time was found to be 85-90 hrs. at 28±3°C. An oocyst residuum was also observed. The sporocyst measured 11.34 x 6.62 µm with sporocyst residuum; Stieda and substieda bodies were also observed. Experimental inoculation of sporulated oocysts was carried out and the developmental endogenous stages (merogony and gamogony) were followed up and described. The prepatent period was 4 days, while the patent period was 12-14 days. Merogony took place in the *lamina propria* and epithelial cells of the middle third of the small intestine of the experimentally infected bats at 25-60 hrs. p.i. Only one generation of meronts was observed. Early uninuclear meronts were seen 25-30 hrs. p.i. and measured 4.40 x 3.69 µm, while the mature meronts measured 9.44 x 7.10 µm and contained 6-15 fully- differentiated merozoites. Gamogony occurred at 60-96 hrs. p.i. and took place at the same site. The microgamonts measured 8.60 x 6.62 µm and contained 7-18 small nuclei. At the same time, macrogamonts measured 9.12 x 8.22 µm, while mature macrogametes measured 10.25 x 9.68 µm and contained 2 types of wall-forming bodies (types I&II). At 90-96 hrs. p.i., newly-formed zygotes or young oocysts were observed in the epithelial cells of the experimentally infected bats. In the present study, fusion of the wall-forming bodies (types I & II) to produce the bilayered wall of the oocyst could be observed at the periphery.
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1. Introduction

Members of family Eimeriidae are alveolate apicomplexan parasites belonging to the eucoccidian suborder Eimeriina (Levine, 1988). They have an obligatory monoxenous as well as heteroxenous life cycle (Pellérdy, 1974; Mehlhorn, 2001). These parasitic coccidians are the cause of coccidiosis (eimeriosis), an economically significant disease in poultry, domestic and wild animals and considered as major causes of significant morbidity and mortality in livestock and wildlife (Pellérdy, 1974; Chapman *et al*., 2002; Chapman, 2003; Gres *et al.,* 2003; Bashtar *et al*., 2003; Taylor *et al.,* 2007; Al-Mathal, 2008; Kim *et al.,* 2010; Lee *et al.,* 2011; Saratsis *et al.,* 2011). This group of coccidians has now assumed a medical as well as veterinary importance (Long, 1990; Slapeta *et al.,* 2001, 2003; Adriano *et al*., 2003; Bashtar *et al*., 2003; Shazly *et al.,* 2005; Kim *et al.,* 2010; Fritzler *et al.,* 2011).

The genus *Eimeria*, with more than 1800 species described to date, is the largest apicomplexan genus and may be the most specious genus of all animal genera (Slapeta *et al.,* 2003). For the identification of an *Eimeria* species, many parameters are considered. These parameters include the dimensions and morphology of the oocysts, host and site specificity, prepatent and patent periods, the morphology and location of endogenous stages within the host cells and the pathogenicity of the parasite (Scholtyseck, 1979; Fayed, 1997, 2003; Duszynski, 2002; Shazly *et al.,* 2005; McAllister and Upton, 2009; Fritzler *et al.,* 2011; Hofstatter and Guaraldo, 2011; McAllister *et al.* 2011).

Chiroptera as one of the most successful orders of mammals in term of their number of species (there are nearly 1,240 species of bats around the world, making up about one quarter of all mammal species); their high powered flight nocturnal activities (night fliers) and their using sonar echos to find their way around attract our attention ((Altringham, 1996). To the best of our knowledge, the study of eimerian parasites infecting Egyptian bats is very rare, scarce and fragmented (Cerná and Rysavý, 1976; Ka-oud *et al.,* 1989). Nevertheless, most of these studies were restricted only to the prevalence of infection and the description of the oocyst morphology. Therefore, the present study aims to investigate and discuss the characteristic features of the life cycle stages of

then stained with haematoxylin and eosin. The stained sections were examined and photographed using a Zeiss research photomicroscope.

3. Results

1. Rate of natural infection:

Sixteen out of 50 (32%) frugivorous bats *Rousettus aegyptiacus* collected from Giza, Beni-Suef and Fayoum provinces in Egypt were found to be naturally infected with oocysts of the present eimerian type.

2. Exogenous stages:

The collected oocysts from the naturally infected bats were found to be subspherical to ovoid in shape (Figs. 1-6) and measured 22.75-26.11 µm in length and 17.52-21.14 µm in width (n=50), with a mean of 24.43 x 19.33 µm. The length to width ratio was 1.30-1.24 with a mean of 1.27; no micropyle but a polar granule was observed (Fig. 6). The investigated oocysts were limited by a smooth colourless double-layered wall; the outer layer was light and thin, while the inner one was dark and thick (Figs. 1-6). In freshly shedded non-sporulated oocysts, a fully-formed spherical sporont (zygote) occupied the entire volume of the oocyst. Its cytoplasm was granulated with large granules mostly around the periphery and smaller ones in the interior (Figs. 1-3). The nucleus could be seen in the freshly passed oocysts (Figs. 2 & 3). Oocysts were allowed to sporulate at 28±3°C.

At the beginning of the sporulation process, it was observed that the sporont began to condensate forming a spherical mass either shifted towards one pole of the oocyst or towards its center leaving a clear space between the sporont and the oocyst wall and then the sporont evaginated (Figs. 1-3). The cytoplasm then divided into 2, 3 and finally 4 sporoblasts with the appearance of an oocyst residuum (Figs. 4 & 5). Later on, the sporoblasts began to elongate with the formation of sporocysts and differentiation of the sporozoites (Figs. 5 & 6).

At the end of sporulation, each sporulated oocyst contained 4 ovoid sporocysts, each with 2 banana-shaped sporozoites and sporocyst residuum (Figs. 5&6). These sporocysts were observed to be tapered at one end that bears Stieda and substieda bodies (Figs. 5&6). The sporocyst measured 10.24-12.44 µm in length and 6.23-7.01 µm in width (n=50), with a mean of 11.34 x 6.62 µm. The length to width ratio was 1.64-1.77 with an average of 1.71. The sporocyst residuum appeared in the form of small and large granules throughout the entire sporocyst (Fig. 6). Sporulation time of oocysts was found to be 85-90 hrs at 28±3°C.

3. Endogenous stages:

The time that elapsed from the beginning of the experimental inoculation till the first appearance of

Eimeria rousetti sp. nov. infecting the frugivorous bat, *Rousettus aegyptiacus* in Egypt by light microscopy.

2. Materials and Methods

Experimental animals used in the present study were the frugivorous bats, *Rousettus aegyptiacus* Geoffroy, 1810 (Mammalia: Chiroptera: Megachiroptera: Pteropodidae). The forelimbs of bats are webbed and developed as wings, making them the only mammals naturally capable of true and sustained flight (Altringham, 1996). Fifty bats were trapped and collected alive with the aid of mist nets from Giza, Beni-Suef and Fayoum provinces in Egypt and then transported to the laboratory at Zoology Department, Faculty of Science, Cairo University where they were killed and dissected individually. The intestinal tract, caecum, rectum and colon of the bats were slit lengthwise and their contents were collected separately and examined for the incidence of Coccidia. At intervals of 24 hrs, microscopical examination of the collected faeces was done by the flotation technique (Long *et al.,* 1976). Oocysts collected were allowed to sporulate in 2.5 % potassium dichromate solution at 28±3°C to inhibit bacterial growth. The events of sporulation were observedat intervals of 12 hrs. by microscopical examination. The morphology of sporulated and non-sporulated oocysts was studied and photographed using a Zeiss research photomicroscope.

To study the different merogonic and gamogonic stages, 20 coccidia-free bats were experimentally inoculated orally with approximately $1x10^5$ viable sporulated oocysts of the present parasite *Eimeria rousetti* (pure strain) previously collected and identified from the naturally infected bats. The oocysts were excysted before inoculation by the method of Kowalik and Zahner (1999). Two experimentally inoculated bats were sacrificed at intervals of 12, 24, 36, 48, 60, 72, 84, 96 and 120 hrs. p.i. Tissues of small intestine, liver, rectum, caecum and duodenum were fixed immediately in 3% glutaraldehyde in 0.1 M Sodium cacodylate buffer (pH 7.3-7.4) for at least 4 hours at 4°C. Two control bats were kept under the same conditions as infected ones and the faeces were usually examined. The faeces of the control bats were usually coccidia-free, which indicated that the precautions taken were adequate.

Processing for light microscopy was done by the usual technique of dehydration in ascending series of ethyl alcohol, clearing in xylene and finally embedding in paraplast at 63°C. Sections of about 3-5 µm thickness were prepared using a rotary microtome. After deparaffination, the sections were hydrated in a descending series of ethyl alcohol and

produced merozoites invaded new epithelial cells of the middle third of the small intestine of the experimentally inoculated bats and were differentiated into gamonts. They were spherical in shape, each with a clear centric nucleus, located within a small parasitophorous vacuole, measured 5.5-7.2 x 5.6-7.4 µm (n=50), with a mean of 6.2 x 6.3 µm (Figs. 14&15) and were observed at 65 hrs. p.i.. From 65-75 hrs. p.i., some of these gamonts became microgamonts while others led to the formation of macrogamonts.

The microgamonts were characterized by the presence of a large number of small nuclei (7-18) either randomly spread in the cytoplasm (Figs. 16&17) or peripherally arranged (Fig. 16) and measured 6.84-10.36 x 5.98-7.26 µm (n=50), with a mean of 8.60 x 6.62 µm. After a process of microgametogenesis, the microgametes were developed (Fig. 17). In the present study, free and slender microgametes were obvious and present in numbers that made their light photography feasible. Their number ranged in each microgamont from 8 to 24, measured 2.88-3.35 x 1.94-2.54 µm (n=50), with a mean of 3.12 x 2.24 µm and were observed at about 75-85 hrs. p.i. (Figs. 16&17).

Meanwhile, macrogamonts observed at 70-80 hrs. p.i. were spherical in shape, each with a large centric nucleus, situated within a large parasitophorous vacuole and measured 8.38-9.86 x 7.98-8.46 µm (n=50), with a mean of 9.12 x 8.22 µm (Fig. 15). The development of each macrogamont resulted in the formation of a single macrogamete. This type of development did not include any nuclear division. Each macrogamete contained a large central nucleus and two types of wall-forming bodies (types I & II) which arranged in the peripheral cytoplasm (Figs. 15, 18--21). These macrogametes were observed at 75-85 hrs. p.i. and measured 9.18-11.32 x 8.70-10.65 µm (n=50), with a mean of 10.25 x 9.68 µm (Figs. 19-21). At 90-96 hrs. p.i., newly-formed zygotes or young oocysts were observed in the epithelial cells of the experimentally infected bats. The majority of them appeared subspherical to oval in the shape, each with a large centric nucleus, situated in a clear and large parasitophorous vacuole and measured 12.70-16.28 x 12.20-14.51 µm (n=50), with a mean of 14.49 x 13.36 µm (Figs. 21-22). In the present investigation, the fusion of the wall-forming bodies (types I & II) to produce the bilayered wall of the oocyst (outer layer and inner one) could be observed at the periphery (Figs. 22-25).

4. Discussion

1. Rate of natural infection:

Duszynski *et al.* (1988) and Duszynski (1997) recorded that the chiropteran bats were showing a oocysts in the faeces (prepatent period) was 4 days, while the entire duration of oocyst existence or production in the faeces (patent period) was 12-14 days.

a- Merogony:

The merogonic stages of the eimerian species under investigation started as its free sporozoites within the intestine of the bats invade the mucosal lining of the epithelial cells. In the present study, merogony took place in the *lamina propria* and the epithelial cells of the middle third of the small intestine of the experimentally infected bats at 25-60 hrs. p.i. and only one generation was observed. None of the merogonic stages were seen either in the liver, spleen, kidney or other parts of the intestine. Light microscopic study showed that after the invasion of the sporozoites to the epithelial cells, they became rounded or ovoid and grew in size to form developing meronts (Figs. 7&8). Meanwhile, a parasitophorous vacuole appeared enclosing the parasite and grew in size during the growth of the meronts (Figs. 7 & 8). In the present investigation, it was difficult to detect sporozoites penetrating the intestinal epithelial cells.

The early uninuclear meronts were spherical or subspherical, seen 25-30 hrs. post inoculation and measured 3.86-4.94 x 3.25-4.13 µm (n=50), with a mean of 4.40 x 3.69 µm (Fig. 7). As nuclear division progressed, these meronts increased in size, became mature and measured 8.36-10.52 x 6.76-7.44 µm (n=50), with a mean of 9.44 x 7.10 µm. These mature meronts contained 6-15 nuclei when observed at 30-50 hrs. p.i. (Figs. 9-11). Later on, nuclei of each meront were arranged at the periphery directly under its border (Fig. 13). Each peripheral nucleus represented a site of a developing merozoite (Figs. 9-11). The developing merozoites were protruded and gradually budded off from the mother meront cytoplasm by ectomerogonous manner (Figs. 9-11). After 60 hrs. p.i. mature meronts with 6-15 fully-differentiated merozoites were observed within a clear parasitophorous vacuole (Figs. 12&13). The free merozoites were banana-shaped and measured 3.26-4.16 x 2.56- 3.08 µm (n=50), with a mean of 3.71 x 2.82 µm (Figs. 12&13). No residual body could be seen in this type of meronts (Figs. 12&13). The host cells were greatly hypertrophied due to the development of the parasite. The host cell nucleus was elongated and flattened at the border facing the parasitophorous vacuole. More than one host cell nucleus were observed at the vacuole edge (Figs. 8-13).

b- Gamogony:

As in other *Eimeria* species, the asexual phase of the life cycle (merogony) was followed by the sexual one (gamogony). In the present investigation, gamogony was detected at 60-96 hrs. p.i. The

recorded trilayerd oocyst wall, composed of an inner thin, colourless one and two outer layers which are thicker, yellowish-brown, prominently striated and in close apposition, in *Eimeria molossi* infecting the bat, *Molossus ater* and unilayered oocyst wall, composed of a single thin colourless layer, in *Eimeria peltocephali* infecting the fresh-water turtle, *Peltocephalus dumerilianus* in Brazil. In spite of, Lainson and Naiff (2000) reported bilayered oocyst wall in *Eimeria bragancaensis* infecting the Brazilian bat, *Peropteryx macrotis*, however, they recorded that the outer layer of the oocyst wall is frequently lost. Meanwhile, McAllister and Upton (1989) and Upton *et al.* (1992a) recorded that oocysts of *Eimeria* species infecting some reptiles were surrounded by a single-layered wall. Moreover, 4 and 5 membranes were observed in the oocyst wall of *E. maxima* (Elwasila, 1984) and *E. perforans* (Scholtyseck *et al*., 1971) respectively. In addition, 9 membranes were reported in the oocyst wall of *Isospora canaria* (Speer and Duszynski, 1975).

The absence of the micropyle, recorded in the present study, was in agreement with some available eimerian species infecting the bats (Wheat, 1975; Duszynski and Barkley, 1985; Duszynski *et al.*, 1988, 1999 a&b; Lainson and Naiff, 1998, 2000; Alyousif, 1999 a&b; Alyousif *et al.,* 1999; Duszynski, 2002; McAllister and Upton, 2009; McAllister *et al.* 2011); the rats (Abdel-Ghaffar *et al.,* 1991; Duszynski *et al.,* 1992; Shazly *et al.,* 1997; Ahmed *et al.,* 1999; Slapeta *et al.,* 2001) and reptiles (Upton *et al.,* 1992 a&b; Abdel-Gawad *et al.,* 1995; Fayed, 1997, 2003; Slapeta *et al.,* 2003; Snow *et al.,* 2011). On the other hand, the presence of the micropyle was reported in other eimerian species (Barker *et al.,* 1979; Scott and Duszynski, 1997; Fayed, 1997 in the second type of oocysts; Bashtar *et al.,* 2003; Alyousif *et al.,* 2010). The presence of the polar granule in the eimerian oocysts in the present study showed similarity with other eimerian oocysts infecting the bats (Wheat, 1975; Duszynski and Barkley, 1985; Duszynski *et al.*, 1988, 1999 a&b; Scott and Duszynski, 1997; Lainson and Naiff, 1998, 2000; Alyousif, 1999 a&b; Alyousif *et al.,* 1999; McAllister and Upton, 2009; McAllister *et al.* 2011). On the other hand, the absence of the polar granule was also recorded in some other eimerian oocysts (Sakran *et al.,* 1994; Fayed, 1997, 2003; Shazly *et al.,* 1997; Snow *et al.,* 2011).

The present investigation showed the presence of an oocyst residuum in the sporulated oocysts which was in agreement with some *Eimeria* species (Barker *et al.,* 1979; Duszynski and Barkley, 1985; Duszynski *et al.*, 1988, 1999 a&b; Abdel-Ghaffar *et al.,* 1991; Fayed, 1997, 2003; Shazly *et al.,* 1997; Alyousif, 1999 a&b; Alyousif *et al.,* 1999; Bashtar *et al.,* 2003; Hofstatter and Guaraldo, 2011; Couch *et al.,* 2011). lower percentage of natural infection (3% and 3.57% respectively) with *Eimeria* species and explained their hypothesis due to the high flying nocturnal activities and different geographical distributions of these flying mammals. Similarly, Scott and Duszynski (1997) recorded 5% and 11% natural rates of infection for two new *Eimeria* spp. infecting the bats *Myotis* spp in New Mexico, California and Bolivia, USA. At the same time, Duszynski and Barkley (1985); Seville and Gruver (2004) and McAllister *et al.* (2011) reported 12%; 6.6% and 10% natural rates of infection respectively in bats, *Tomopeas ravus*; *Myotis* sp. and *Perimyotis subflvus* infecting with new *Eimeria* spp. On the other hand, the rate of natural infection of bats with *Eimeria* spp. may exceed 25% as recorded by Alyousif *et al.* (1999) [25%]; the present study [32%]; Duszynski *et al.* (1999b) [50%]; McAllister and Upton (2009) [63.6%].

2. Exogenous stages:

The oocysts are the most easily accessible stages of any coccidium and in some cases many *Eimeria* species are known only from the morphological and characteristic features of their oocysts (Gottschalk, 1974; Cerná, 1976; Abdel-Ghaffar *et al.,* 1991; Bashtar *et al.,* 1992b, 2003; Fayed, 1997, 2003; Shazly *et al.,* 1997; Duszynski *et al.* 1999 a&b; Slapeta *et al.,* 2001,2003; Duszynski, 2002; Adriano *et al.,* 2003; Couch *et al.,* 2003; Al-Ghamdy *et al.,* 2005; McAllister and Upton, 2009; McAllister *et al.* 2011; Hofstatter and Guaraldo, 2011). The presence or absence of a polar cap or micropyle; shape of the sporocysts; presence or absence of residual bodies, polar granules, Stieda and substieda bodies represent very important criteria for detection of eimerian species (Marinkelle, 1968; Wheat, 1975; Cerná and Rysavý, 1976; Barker *et al.,* 1979; Abdel-Ghaffar *et al.,* 1986a; Mehlhorn, 1988, 2001; Fayed, 1997, 2003; Shazly *et al.,* 1997; Lainson and Naiff, 1998, 2000; Alyousif, 1999 a&b; Zhao *et al.,* 2001; Bashtar *et al.,* 2003; Seville and Gruver, 2004; McAllister and Upton, 2009; Fritzler *et al.,* 2011; McAllister *et al.* 2011; Hofstatter and Guaraldo, 2011).

In the present study, only one type of oocysts was recorded. These oocysts were surrounded by a bilayered oocyst wall. Similar results were obtained in other eimerian species of bats *as E. macyi* (Wheat, 1975); *E. wombati* and *E. arundeli* (Barker *et al.,* 1979); *E. tomopea* (Duszynski and Barkley, 1985); *E. tadarida* (Duszynski *et al.*, 1988); 2 new *Eimeria* spp. from the bats *Myotis* spp. (Scott and Duszynski, 1997); *E. chiropteri* (Alyousif, 1999a); *E. kuhliensis* (Alyousif, 1999b); *E. pipistrellus* (Alyousif *et al.,* 1999); *E. dowleri* and *E. sealanderi* (McAllister and Upton, 2009) and finally *E. heidti* (McAllister *et al.,* 2011). On the other hand, Lainson and Naiff (1998)

al., 1986b, 1990; Koura *et al.,* 2001; Slapeta *et al.,* 2001; Fayed, 2003). On the other hand, three asexual generations (Clarkson, 1959; Fernando, 1974; McDonal and Rose, 1987; Shazly *et al.,* 1997; Ahmed *et al.,* 1999) and four asexual generations (Ernst and Chobotar, 1978; Pakandl *et al.,* 1996) were reported in some other *Eimeria* species. Moreover, five asexual generations were also observed in other *Eimeria* species (Cheissin, 1940; Ruff *et al.,* 1980; Pakandl and Coudert, 1999; Shazly *et al.,* 2005).

It is recorded in the present parasite that its uninucleated meronts were spherical or subspherical and this result runs in agreement with that reported for other *Eimeria* species (Abdel-Ghaffar *et al.,* 1986b, 1990; El-Toukhy, 1994; El-Toukhy *et al.,* 1997; Fayed, 1997, 2003; Shazly *et al.,* 1997, 2005; Ahmed *et al.,* 1999). The shape and position of the multinucleated meronts of the present parasite were similar to other *Eimeria* species but different in the measurements (Cheissin, 1940; Pellérdy, 1974; Abdel-Ghaffar *et al.,* 1986b, 1990, 1991; Ahmed *et al.,* 1992, 1999; Lainson and Naiff, 1998, 2000; Fayed, 1997, 2003; Shazly *et al.,* 1997, 2005). The yielded number of merozoites per each mature meront may be a species-specific character of *Eimeria* species (Levine and Ivens, 1990; Ahmed *et al.,* 1992, 1999). In the present study, mature meronts with 6-15 fully- differentiated merozoites were observed within a clear parasitophorous vacuole. Similar results were recorded by Abdel-Ghaffar *et al.* (1986b, 1990, 1991); Ahmed *et al.* (1992, 1999); Fayed (1997, 2003); El-Toukhy *et al.* (1997) and Shazly *et al.* (1997, 2005). These merozoites reported herein were developed through an ectomerogonous manner. Ectomerogony is the most common recorded mechanism of merozoite formation among Coccidia (Scholtyseck, 1973; Ahmed *et al.,* 1992, 1999; Mehlhorn, 2001; Slapeta *et al.,* 2001). However, Hammond (1973) reported an endomerogonous type of merozoite formation among some other eimerian species. Furthermore, Entzeroth *et al.* (1998) described the structure and function of the parasitophorous vacuole in *Eimeria* species and claimed that the parasitophorous vacuole membrane was manipulated by the parasite and functioned later in the developmental cycle as a molecular sieve, allowing the exchange of metabolites between parasite and host cell.

b- Gamogony:

The sexual phase of the life cycle is of great significance because it probably plays an important role in the host specificity of Coccidia (Scholtyseck *et al.,* 1971, 1977). The sexual phase in any eimerian infection occurs after a species-specific number of asexual merogonic generations (Mehlhorn, 1988). In However, such oocyst residuum is lacking in sporulated oocysts of other *Eimeria* spp. (Wheat, 1975; Scott and Duszynski, 1997; El-Toukhy *et al.,* 1997; Lainson and Naiff, 1998, 2000; Slapeta *et al.,* 2001; Adriano *et al.,* 2003; McAllister and Upton, 2009; Alyousif *et al.,* 2010; Fritzler *et al.,* 2011; McAllister *et al.* 2011; Snow *et al.,* 2011; Hofstatter and Kawazoe, 2011). Furthermore, the measurements and characteristic features of eimerian sporocysts can be used as criteria to differentiate between *Eimeria* species (Mehlhorn, 1988, 2001; Daszak and Ball, 2001; Lainson, 2002; Duszynski, 2002; Adriano *et al.,* 2003; Bashtar *et al.,* 2003; Alyousif *et al.,* 2010; Fritzler *et al.,* 2011; Hofstatter and Kawazoe, 2011). In the present study, sporocysts of *Eimeria rousetti* measured 11.34 x 6.62 µm, contained Stieda and substieda bodies at the pointed end and a sporocyst residuum. These results are in agreement with that reported in some other *Eimeria* species (Barker *et al.,* 1979; Duszynski and Barkley, 1985; Duszynski *et al.*, 1988, 1999 a&b; Fayed, 1997, 2003; Scott and Duszynski, 1997; Shazly *et al.,* 1997; Lainson and Naiff, 1998, 2000; Alyousif, 1999 a&b; Alyousif *et al.,* 1999, 2010; Slapeta *et al.,* 2001; Adriano *et al.,* 2003; Bashtar *et al.,* 2003; McAllister and Upton, 2009; Fritzler *et al.,* 2011; McAllister *et al.* 2011; Hofstatter and Guaraldo, 2011; Couch *et al.,* 2011; Hofstatter and Kawazoe, 2011).

3. Endogenous stages:

Most studies carried out on *Eimeria* parasites mainly recorded the exogenous stages only, but many authors gave a great attention to the endogenous stages as well (Ernst and Chobotar, 1978; Abdel-Ghaffar *et al.,* 1986b, 1990; Ahmed *et al.,* 1992, 1999; El-Toukhy *et al.,* 1997; Fayed *et al.,* 1996 a&b; Fayed, 1997, 2003; Shazly *et al.,* 1997, 2005; Lainson and Naiff, 1998, 2000; Paperna and Lainson, 1999; Shazly, 2002; Al-Ghamdy *et al.,* 2005).

a- Merogony:

In the present study, merogony took place in the *lamina propria* and the epithelial cells of the middle third of the small intestine of the experimentally infected bats at 25-60 hrs. p.i.. Similar observations were reported for some other *Eimeria* species (Cheissin, 1940; Pellérdy, 1974; Abdel-Ghaffar *et al.,* 1986b, 1990, 1991; Ahmed *et al.,* 1992, 1999; Pakandl *et al.,* 1996; Fayed, 1997, 2003; Shazly *et al.,* 2005). Also, merogony occurred only in one generation during the present study and this result runs in agreement with that reported for *E. obtusi* (Fayed, 1997). However, the exact number of asexual merogonic generations among *Eimeria* is not fixed (Clarkson, 1958, 1959; Mehlhorn, 1988). Two asexual merogonic generations were recorded for some *Eimeria* species (Rutherford, 1943; Clarkson, 1958; Pellérdy, 1974; Long, 1982; Abdel-Ghaffar *et*

oocysts were formed in the intestinal epithelial cells and fusion of the wall-forming bodies (types I & II) to produce the bilayered oocyst wall could be observed at the periphery.

Species diagnosis:

***Eimeria rousetti* sp. nov.**

Type-host: Frugivorous bats *Rousettus aegyptiacus.*

Type-locality: Giza, Beni-Suef and Fayoum provinces in Egypt.

Type-specimens (hapantotypes): Oocysts in 10% formalin, slides and phototypes are deposited in the parasitological collection of Zoology Department, Faculty of Science, Cairo University, Egypt.

Site of infection: The *lamina propria* and the epithelial cells of the middle third of the small intestine of the experimentally – inoculated bats.

Prevalence of natural infection: 32%.

Sporulation time: 85-90 hrs at 28±3°C.

Prepatent period: 4 days.

Patent period: 12-14 days.

Number of asexual merogonic generations: One generation

Etymology: The specific name of the present parasite is derived from the name of the host genus *Rousettus.*

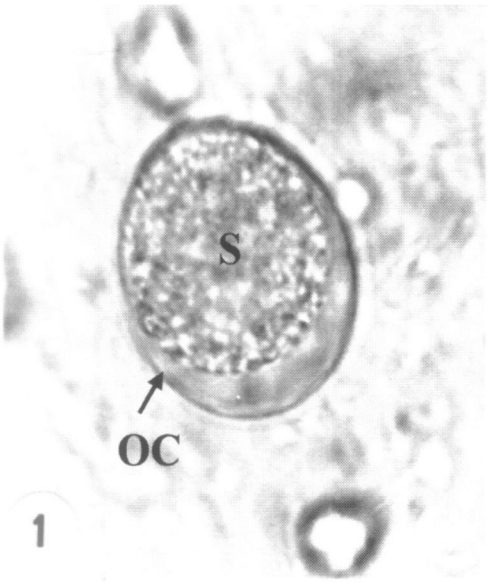
Abbreviations used in figures:

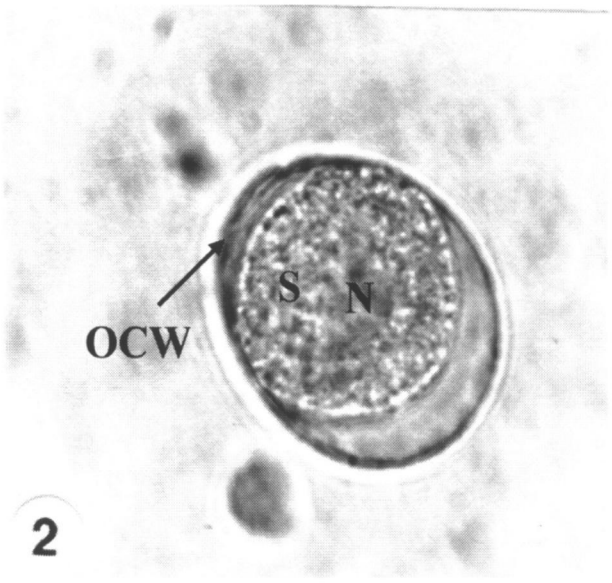
DMS	Developing merozoites
HCN	Host cell nucleus
IL	Inner layer of oocyst wall
M	Meront
MAG	Macrogamont / Macrogamete
MG	Microgamete
MIG	Microgamont
MS	Merozoite /s
N	Nucleus
OC	Oocyst
OCR	Oocyst residuum
OCW	Oocyst wall
OL	Outer layer of oocyst wall
PG	Polar granule
PV	Parasitophorous vacuole
RB	Residual body
S	Sporont
SbT	Substieda body
SP	Sporoblast
SPC	Sporocyst
SPCR	Sporocyst residuum
SPR	Sporozoite
ST	Stieda body
WFB	Wall-forming bodies
ZY	Zygote

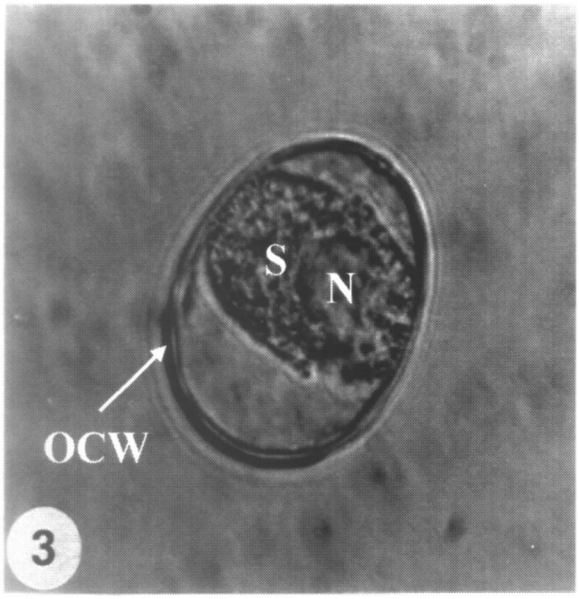
the present study, gamogony was detected at 60-96 hrs. p.i. The produced merozoites invaded new epithelial cells of the middle third of the small intestine of the experimentally inoculated bats and were differentiated into spherical gamonts. Similar results were recorded in gamonts of most *Eimeria* species (Ernst and Chobotar, 1978; Abdel-Ghaffar *et al.* 1986b, 1990; Bashtar, 1991; Ahmed *et al.* 1992, 1999; Fayed *et al.,* 1996 a&b; Al-Hoot, 1997; El-Toukhy *et al.,*1997; Fayed, 1997, 2003; Shazly, 2002; Al-Ghamdy *et al.,* 2005).

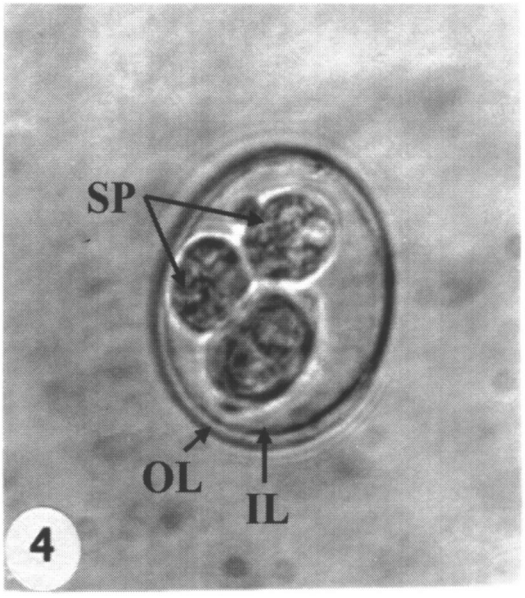
Microgamonts in the present study were characterized by the presence of a large number of small nuclei either randomly spread in the cytoplasm or peripherally arranged, producing a large number of microgametes. Similar observations were also reported in many other *Eimeria* species (Abdel-Ghaffar *et al.* 1986b, 1990; Bashtar, 1991; Ahmed *et al.* 1992, 1999; Fayed *et al.,* 1996a; Al-Hoot, 1997; El-Toukhy *et al.,*1997; Fayed, 1997, 2003; Shazly, 2002; Al-Ghamdy *et al.,* 2005). Using light microscopy in the present study made no way to count and/or to describe the characteristics of the microgametes.

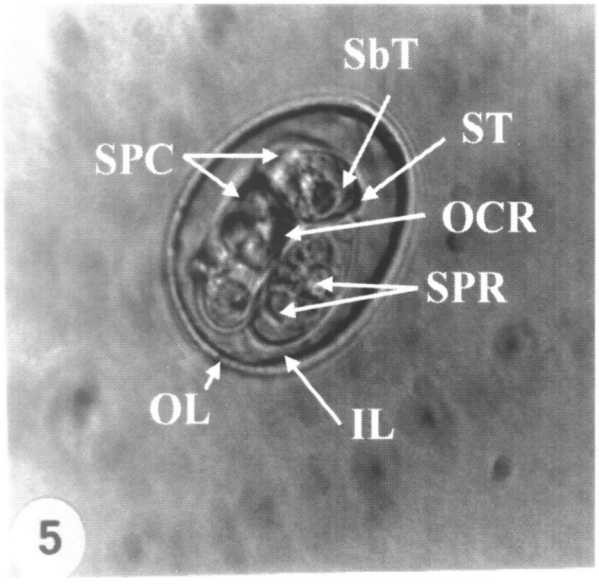
Meanwhile, macrogamonts in the present study were characterized by their large size, accumulation of many food materials in their cytoplasm and their distinct nucleus. After growth, the two types of wall-forming bodies (types I & II) developed and arranged in their peripheral cytoplasm forming macrogametes. The same results were obtained in most *Eimeria* species (Scholtyseck, 1979; Abdel-Ghaffar *et al.* 1986b, 1990; Mehlhorn, 1988; Ahmed *et al.* 1992, 1999; Bashtar *et al.,* 1992a; Fayed *et al.,* 1996b; Al-Hoot, 1997; El-Toukhy *et al.,*1997; Fayed, 1997, 2003; Al-Ghamdy *et al.,* 2005). Both types of wall-forming bodies were named after their fate or function rather than the order of their appearance. Thus, the WFB I, which appeared after the WFB II, giving rise to the outer layer of the future oocyst wall and the WFB II form the inner layer (Scholtyseck *et al.,* 1966; Mehlhorn, 1972, 2001; Speer *et al.,* 1973; Bashtar *et al.,* 1992a; Fayed *et al.,* 1996b; Al-Hoot, 1997; Al-Ghamdy *et al.,* 2005). Although the events of fertilization are still not completely clarified in many recorded species of *Eimeria*, detailed studies regarding the changes associating fertilization and oocyst wall formation of *Eimeria* species were recorded (Scholtyseck, 1973; Scholtyseck *et al.,* 1971; Mehlhorn, 1972, 2001; Abdel-Ghaffar *et al.* 1986b, 1990; Bashtar *et al.,* 1992a; Fayed *et al.,* 1996b; Al-Ghamdy *et al.,* 2005). The present study showed that after fertilization, zygotes or young

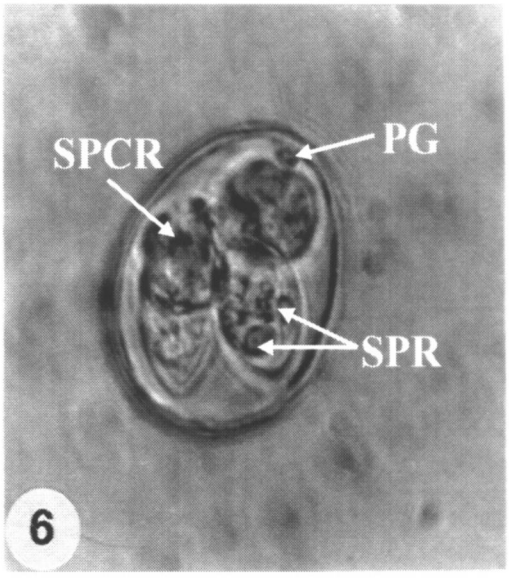

S
OC
1

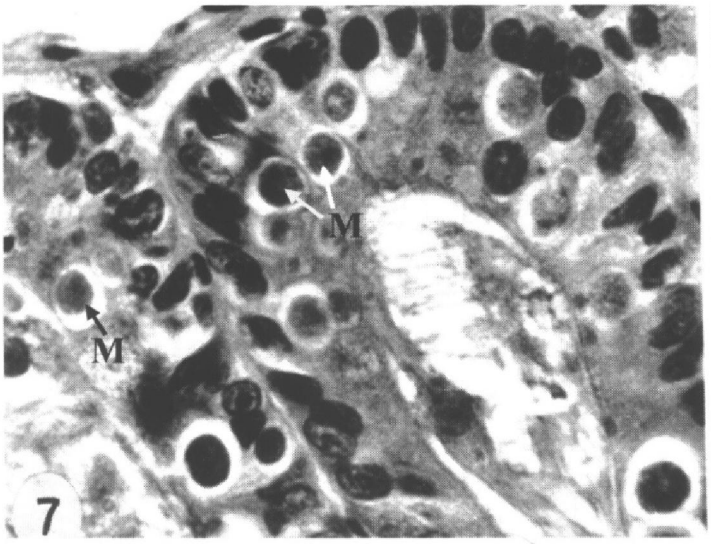

OCW
S
N
2

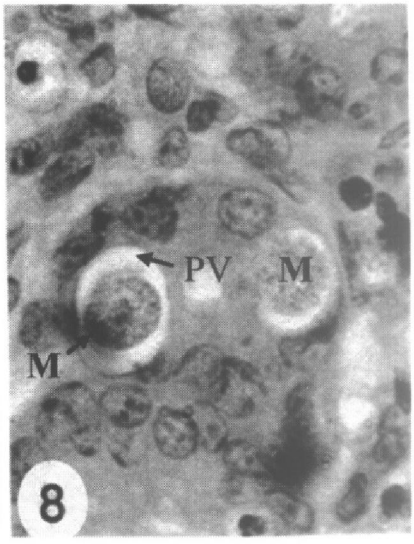

S
N
OCW
3

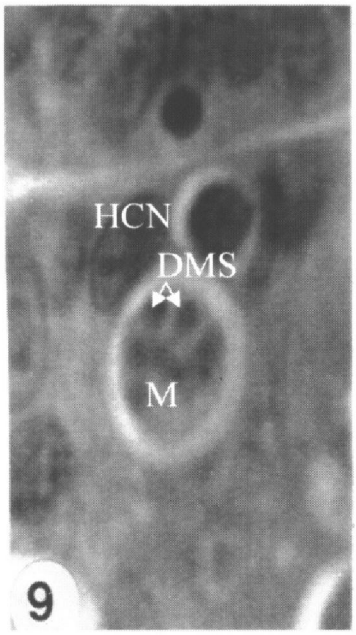

SP
OL
IL
4

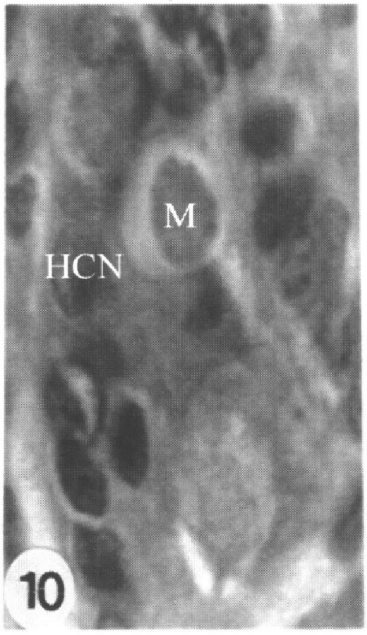

SbT
ST
SPC
OCR
SPR
OL
IL
5

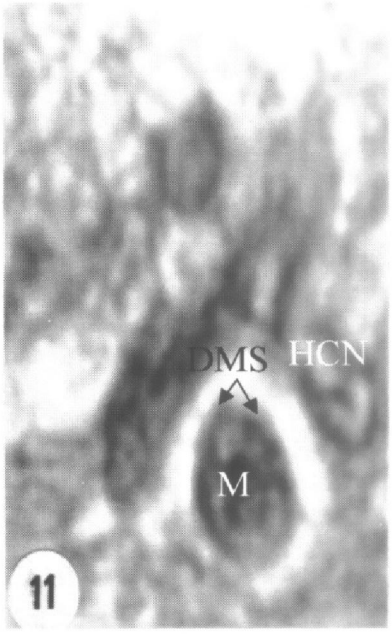

SPCR
PG
SPR
6

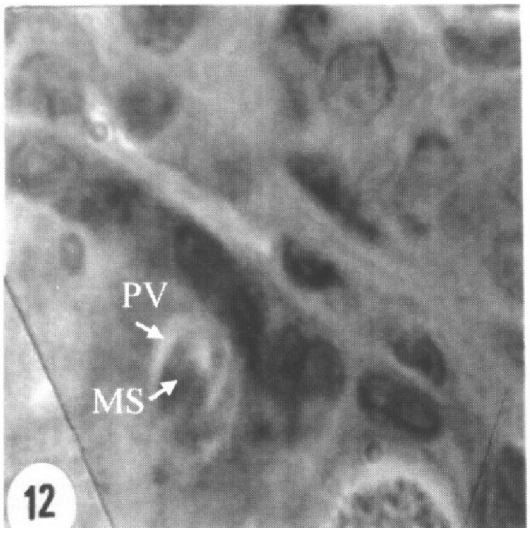

M
M
7

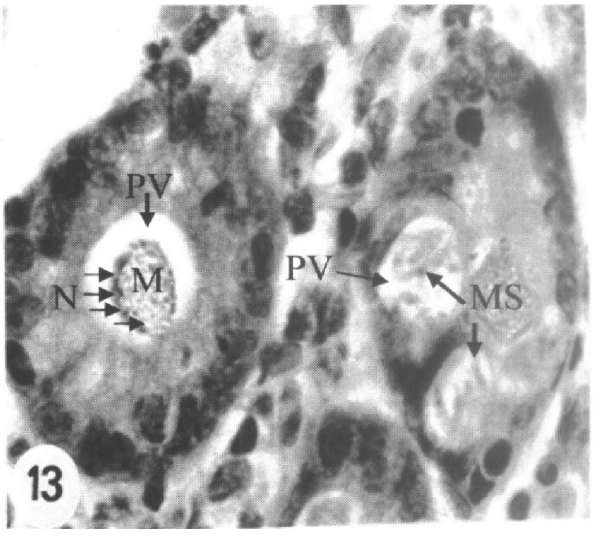

PV
M
M
8

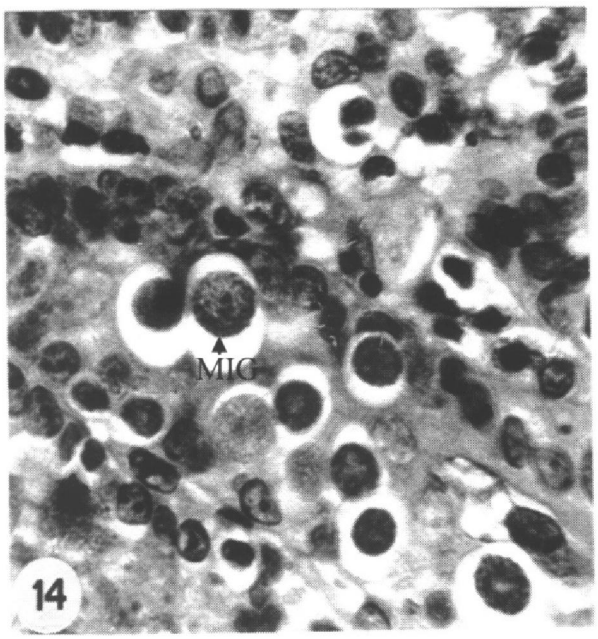

HCN
DMS
M
9

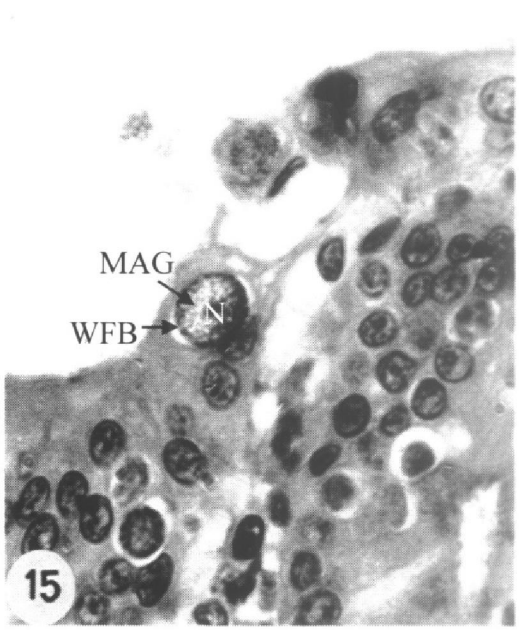

M
HCN
10

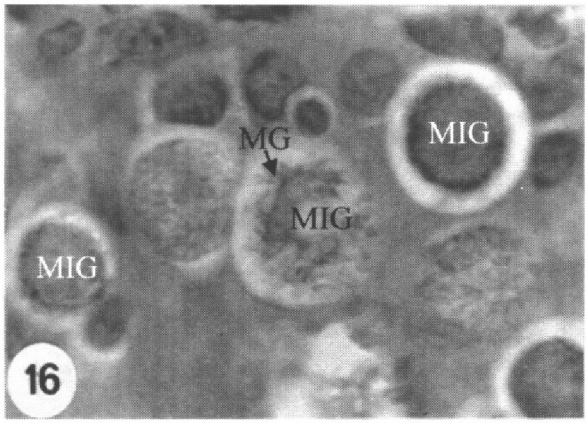

DMS
HCN
M
11

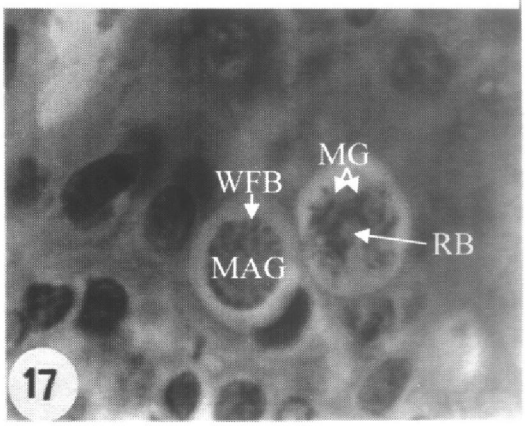

PV
MS
12

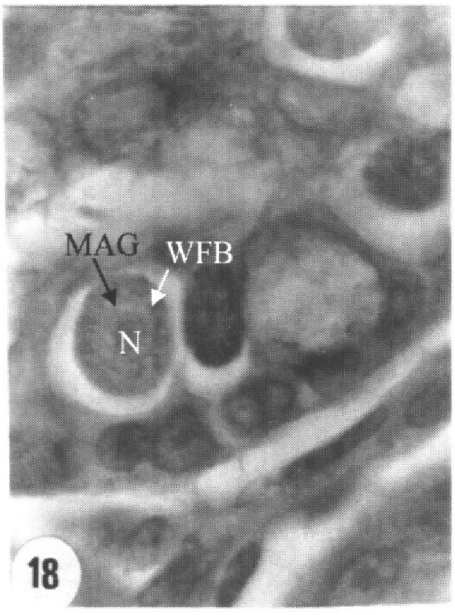

PV
N
M
PV
MS
13

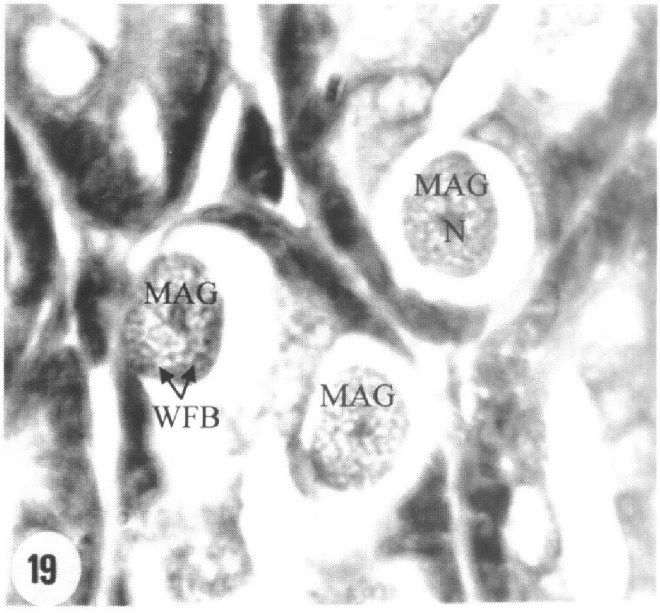

MIG
14

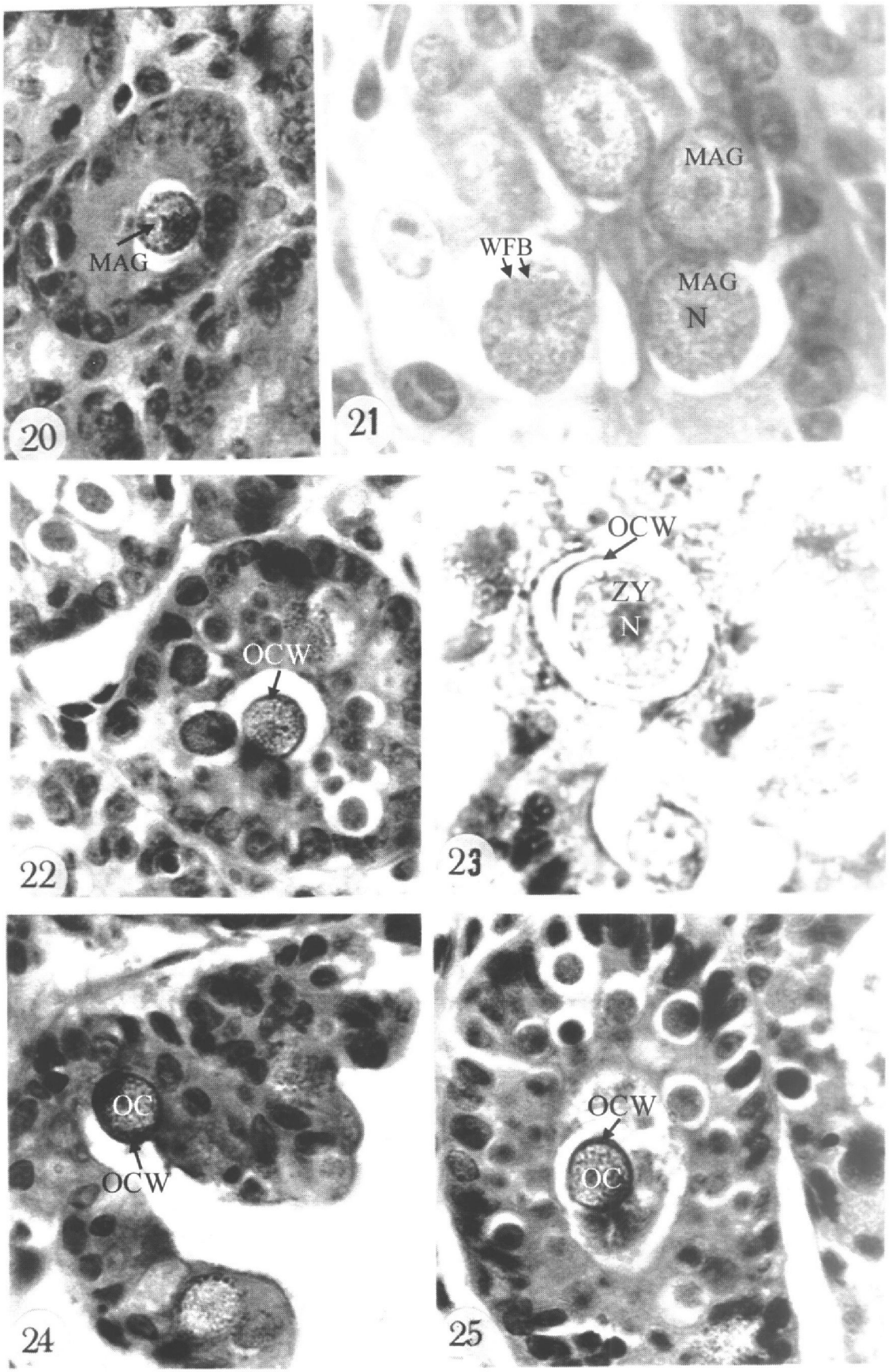

MAG
N
WFB
15


MG
MIG
MIG
MIG
16


WFB
MG
RB
MAG
17


MAG
WFB
N
18


MAG
N
MAG
WFB
MAG
19


MAG
20
MAG
WFB
MAG
N
21
OCW
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OCW
ZY
N
23
OC
OCW
24
OCW
OC
25

Figure Explanations: All photos (X: 1600)

Figs. (1-6): Exogenous stages of *Eimeria rousetti* sp. nov. infecting the Egyptian frugivorous bats *Rousettus aegyptiacus*.

Fig.(1): Fresh non-sporulated oocyst (OC), the sporont (S) occupies the entire volume of the oocyst; no micropyle or polar granules were observed.

Fig.(2): Non-sporulated oocyst; the sporont (S) began to condensate towards one pole of the oocyst. Note the presence of distinct nucleus (N) and oocyst wall (OCW).

Fig.(3): Non-sporulated oocyst (OC), evagination of the sporont (S) appeared. Note the presence of distinct nucleus (N) and oocyst wall (OCW).

Fig.(4): Oocyst (OC) with three sporoblasts (Sp). Note the bilayered oocyst wall (OCW) formed of an outer layer (OL) and an inner one (IL).

Fig.(5 Sporulated oocyst (OC) with 4 sporocysts (SPC), each with two sporozoites (SPR). Note the presence of oocyst residuum (OCR);the stieda (ST) and substieda (Sbt) bodies**.**

Fig.(6): Sporulated oocyst (OC) with 4 fully sporulated sporocysts (SPC), each with two sporozoites (SPR). Note the presence of polar granule (PG) and sporosyst residuum (SPCR).

Figs.(7 - 13): Light photomicrographs showing the merogonic stages of *Eimeria rousetti* sp.nov. experimentally infecting the Egyptian frugivorous bats *Rousettus aegyptiacus*. They occurred in lamina propria and the epithelial cells of the middle third of the small intestine at 25 – 60 hrs P.I. All specimens are stained with haematoxylin and eosin (X 1800).

Fig.(7): Early uninuclear meront (M) were seen 25 – 30 hrs p.i. inside a clear parasitophorous vacuole (PV).

Figs.(8): Developing meronts (M) within a clear parasitophorous vacuole (PV).

Figs.(9): Multinucleated meront (M) showing the beginning of the formation of the developing merozoites (DMS).

Figs.(10): Multinucleated meront (M).

Figs.(11): Multinucleated meront (M) showing invagination of the developing merozoites (DMS).

Figs.(12): Mature meront with fully formed merozoites (MS) in the (L.S.) within PV; each merozoite is banana-shaped.

Figs.(13): Meronts in different merogonic stages; one is multinucleated (M) with peripherally arranged nuclei (N) and the other meronts are mature with fully formed merozoites (MS). Each meront appeared within a parasitophorous vacuole (PV).

Figs.(14 - 21): Light photomicrographs showing the different gamogonic stages of *Eimeria rousetti* sp.nov. in the epithelial cells of the middle third of the small intestine of the experimentally infected bats. Seen from 60 – 96 hrs p.i.

Figs.(14): Young gamonts were observed, each in spherical shape with central nucleus (N) and located in a big PV. One of these gamonts is the young microgamont (MIG).

Figs.(15): Developing macrogamont (MAG) with peripherally arranged wall-forming bodies (WFB).

Figs.(16): Microgamonts in different develop-mental stages. A microgamont (MIG) with a number of developing microgametes (MG) and situated in a parasitophorous vacuole (PV). Other micrgamonts are immature and with peripherally arranged nuclei.

Figs.(17): A microgamont (MIG) with developing microgametes (MG) and residual body (RB). A macrogamont is also showed with a number of wall-forming bodies (WFB).

Figs.(18): A macrogamete (MAG) with peripherally arranged wall-forming bodies (WFB) and a central nucleus (N).

Figs.(19|& 21): Macrogametes (MAG) with wall-forming bodies (WFB) and a central nucleus (N).

Figs.(20): A macrogamete (MAG) with peripherally arranged wall-forming bodies (WFB).

Figs. (22-25): Newly-formed zygotes (ZY) or young oocysts (OC) were observed in the epithelial cells of the middle third of the small intestine of the experimentally infected bats. They are spherical or oval in shape and situated with a clear parasitophorous vacuole (PV). Note that the fusion of the wall-forming bodies of types I &II (WFBI &II) to form the bilayered oocyst wall (OCW).

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