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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. [Hamed Mohamed Fayed; Mohamed Abd-Allah Shazly and Sayed Abd El-Monem. Life cycle of *Eimeria rousetti* sp. nov. (Alveolata: Apicomplexa: Eimeridae) infecting the frugivorous bat, *Rousettus aegyptiacus* Geoffroy, 1810 (Mammalia: Chiroptera: Pteropodidae) in Egypt. A light microscopic study. Journal of American Science 2011; 7(12): 292-305]. (ISSN: 1545-1003). http://www.americanscience.org.

**Keyword:** *Eimeria rousetti*, life cycle, Oocysts. Subspherical, merogony and gamogony.

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 (Scholtyscek, 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

1. **Introduction**

Members of family Eimeriidae are alveolate apicomplexan parasites belonging to the eucoccidian suborder Eimerina (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; Bashatar et al., 2003; Taylor et al., 2007; Al-Mathal, 2008; Kim et al., 2010; Lee et al., 2011; Saratis 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; Bashatar 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.
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 Roussettus 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 sporocytes and differentiation of the sporozoites (Figs. 5 & 6).

At the end of sporulation, each sporulated oocyst contained 4 ovoid sporocytes, each with 2 banana-shaped sporozoites and sporocyst residuum (Figs. 5&6). These sporocytes 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, Roussettus aegyptiacus in Egypt by light microscopy.

2. Materials and Methods
Experimental animals used in the present study were the frugivorous bats, Roussettus 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 observed at 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 1x10⁵ 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 deparaffinization, the sections were hydrated in a descending series of ethyl alcohol and 293...
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. 17). The development of each microgamont resulted in the formation of a single microgamete. This type of development did not include any nuclear division. Each microgamete 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 microgametes 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 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. 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.

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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 unilayerd 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 bilayerd oocyst wall in Eimeria bragancaensis infecting the Brazilian bat, Peropteryx macrotris, 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 (Elvasila, 1984) and E. perforans (Scholtyskeck et al., 1971) respectively. In addition, 9 membranes were 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 unilayerd 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 bilayerd oocyst wall in Eimeria bragancaensis infecting the Brazilian bat, Peropteryx macrotris, 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 (Elvasila, 1984) and E. perforans (Scholtyskeck 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. wombatii and E. arundeli (Barker et al., 1979); E. tomoapea (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, 2003a); E. pipistrellus (Alyousif et al., 1999); E. doweri and E. sealanderi (McAllister and Upton, 2009) and finally E. heidti (McAllister et al., 2011). On the other hand, Lainson and Naiff (1998)
asexual merogonic generations (Mehlhorn, 1988). In infection occurs after a species-specific number of role in the host specificity of Coccidia (Scholtyseck significance because it probably plays an important parasite and host cell. allowing the exchange of metabolites between were manipulated by the parasite and functioned later 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 ectomerogogenous 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 endomerogogenous 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 rouseei 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 296
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:**

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

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
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 macrogamont (MAG).

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

Figs.(16): Microgamonts in different developmental stages. A microgamont (MIG) with a number of developing microgametes (MG) and situated in a parasitophorous vacuole (PV). Other microgamonts 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 (WFB I &II) to form the bilayered oocyst wall (OCW).

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