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ORIGINAL PAPER

# Light microscopic study on *Eimeria* species infecting Japanese quails reared in Saudi Arabian farms

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**Abstract** Japanese quails *Coturnix coturnix japonica* reared in economic farms were individually investigated for coccidian infections. The results indicated the absence of infections in birds younger than 1 month. An *Eimeria* infection rate of up to 80% was detected in birds 7–9 weeks old with a general infection rate of 29%. The infection rate decreased to 21.42% in birds older than 10 weeks. Morphometric characteristics of freshly shed, unsporulated oocysts were taken. These oocysts appeared pale yellow in color, were oval to subspherical in shape being limited by a bilayered oocyst wall of 1.2 μm. The unsporulated oocysts measured 17.73±12.92×12.79±1.69 μm (mean of 100) and possessed a polar granule, a micropyle and an oocyst residuum. The sporulation took 72 h and resulted in the formation of four elongated sporocysts containing two sporozoites, in addition to a stieda body and a sporocyst residuum. The life cycle of this *Eimeria* species was followed in experimentally infected quails. Three asexual generations (at 60, 78, and 96 h p.i.) were detected in the epithelium of the small intestine before the sexual cycle started at 84 h p.i. The prepatent period was 5 days, while the patent period covered 6–7 days. Besides this well-defined species, another *Eimeria* species occurred, the oocysts of which were excreted in low numbers and were characterized by the absence of a micropyle and an oocyst residuum. These oocysts measured 15.73±2.22×14.18±1.89 μm (mean of 100) and sporulated already within 60 h.

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## Introduction

Quails are reared in the most important modern poultry industry in Saudi Arabia. They are farmed on a very wide scale to be used as a protein source instead of pigeons and turkeys in neighboring countries such as Egypt. Coccidiosis is one of the major parasitic diseases limiting development of this industry (Abdel-Ghaffar et al. 1990; Bashtar et al. 1991, 1992b; Dalloul et al. 2007). Nowadays, quail coccidiosis and its planned control have become a major target in all countries (Duszynski and Gutierrez 1981; Mehlhorn 2006, 2008). Several *Eimeria* species were described from the different species of quails in different countries such as *Eimeria coturnicis* from *Coturnix coturnix coturnix*, *Eimeria uzura* and *Eimeria bateri* from *Coturnix coturnix japonica* in India (Bhatia et al. 1965; Rao and Sharma 1992), and *E. uzura* and *Eimeria tsunodai* from *C. coturnix japonica* in Japan (Tsunoda and Muraki 1971; Tsutsumi 1972; Teixeira and Lopes 2002; Teixeira et al. 2004). Other *Eimeria* species had been described in North America (Fisher and Kelley 1977; Duszynski and Gutierrez 1981; Ruff 1985).

The present investigation deals with the natural prevalence of *Eimeria* infections among farmed quails and offers the description of both exogeneous and endogeneous stages of the most common *Eimeria* species found in Saudi Arabia.

## Materials and methods

In the present study, 200 Japanese quails *C. coturnix japonica* (Saudi strain) were investigated. Quails were

collected from three farms around Riyadh, Saudi Arabia, separated according to their age into three groups; young (1–7 weeks), middle aged (7–10 weeks), and old quails (more than 10 weeks). Fresh fecal samples were collected and investigated for the presence of oocysts at intervals of 12 h applying the usual floatation technique (Long et al. 1976). Heavily infected quails were separated for determination of the infection sites and collection of oocysts. Morphometric data and specific characteristics of unsporulated and sporulated oocysts were recorded.

### Prepatent and patent periods

Six coccidian free, 4-week-old quails were orally inoculated each with $1\times10^5$ sporulated oocysts previously collected and identified from naturally infected birds. Fecal samples were collected and examined daily till the first appearance of Eimerian oocysts. Strict precautions were used to prevent infections with other coccidians. The prepatent as well as the patent period were recorded.

### Study of the endogeneous stages

Thirty-six non-infected (4 weeks old) quails were orally inoculated with $1\times10^4$ sporulated oocysts. Three birds of each group were sacrificed at 12, 24, 36, 48, 60, 72, 78, 84, 96, 108, and 120-h post-infection and three other animals were used for collection of oocysts. Small samples from the duodenum and ileum were removed and became immediately fixed in 3% (*v*/*v*) cold and buffered glutaraldehyde, were processed by the usual technique and finally became embedded in paraffin wax. Sections of 3–5 μm in diameter were prepared and stained with hematoxylin and eosin. Stained sections were examined with a Zeiss Research Photomicroscope. Six non-infected birds were kept under the same conditions as controls. All measurements were given in micrometers (μm) and represent mean values of at least 80–100 measurements.

## Results

Fifty-eight out of 200 quails of different ages were found to be infected shedding Eimerian oocysts in their feces. This represented a mean infection rate of 29%. However, no infection was detected among birds less than 1 month old. The infection rate in young quails (7–9 weeks old) was 80% (48/62), while the infection rate decreased to 21.42% (30/140) in older birds. Microscopic examination revealed that only *Eimeria* oocysts were observed in the feces of the infected quails. These oocysts were transparent, covered by a bilayered oocyst wall and could be differentiated morphologically into two types.

**Fig. 1** Photomicrograph of freshly shed unsporulated oocysts of the two types of oocysts; at the *left side* the ovoidal is shown and at the *right* is the spherical. ×1,950

**Fig. 2** Photomicrograph of the sporulated ovoidal oocyst with four sporocysts (*SPC*) each containing two sporozoites (*SPO*): note the polar granule (*Pg*), the stieda body (*St*), the sporocyst residuum (*SPR*), and the large refractile body (*RBO*) at the posterior end of the sporozoite. ×2,750

**Fig. 3** Photomicrograph of a fully sporulated oocyst of the second type showing two sporozoites, a sporocyst residuum (*SPR*), and a stieda body in each sporocyst. Note the presence of the polar granule (*Pg*), the two layers of the oocyst wall (*OL*, *IL*), and the refractile body of the sporozoite (*RBO*). ×2,800

**Fig. 4** Photomicrograph of a developing early meront (*DSCH*) within a parasitophorous vacuole (*PV*) in the intestinal epithelium. ×2,300

**Fig. 5** Photomicrographs of first generation meronts (*DSCH*) at different developmental stages. ×2,300

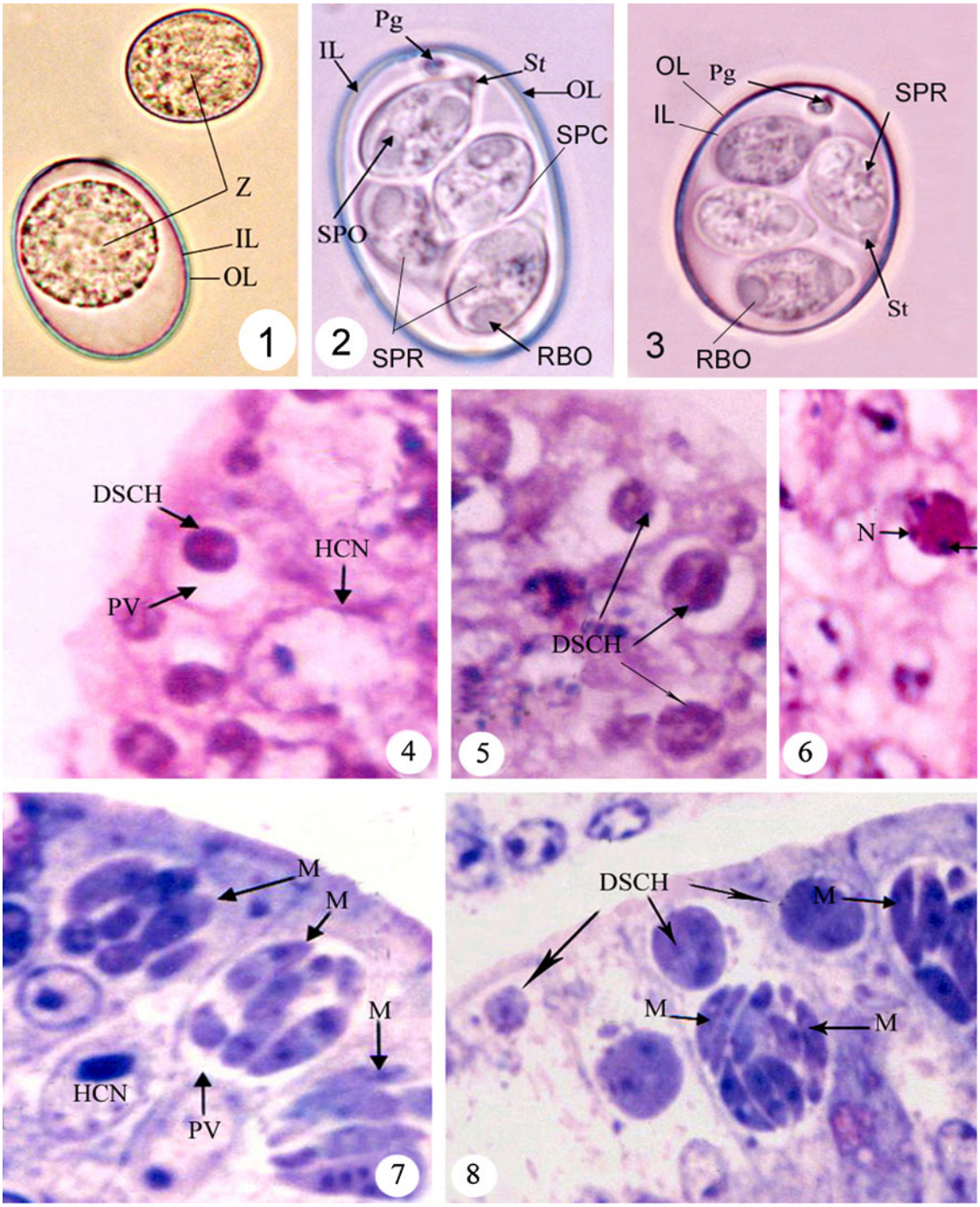
**Fig. 6** Photomicrographs of first generation meronts at different developmental stages. ×2,300

**Fig. 7** Photomicrographs of mature meronts of the first and second generations containing mature merozoites (*M*) and some developing meronts. ×2,350

**Fig. 8** Photomicrographs of mature meronts of the first and second generations containing mature merozoites (*M*) and some developing meronts (*DSCH*). ×2,350

The oocysts of type one were transparent, covered by a smooth double-layered wall and appeared ovoidal to ellipsoidal in shape. The sporont usually occupied the entire cavity of the freshly shed oocysts. These oocysts were abundant, measured 13.90–22.21 μm, (mean 17.73± 2.92 μm) in length and 10.81±14.79 μm in width, with a mean of 12.79±1.69 μm. The shape index (length/width) reached 1.39 (Fig. 1). At the beginning of the sporulation, the zygote was condensed in the middle of the oocyst and the nuclei underwent two successive divisions producing finally four sporoblasts. When the sporulation proceeded each nucleus of the four sporoblasts divided once again producing two daughter nuclei, finally giving rise to two sporozoites, which were situated in four thick-walled ovoidal sporocysts measuring 6.13±0.24× 4.31±0.29 μm. These sporocysts contained a prominent stieda body (Fig. 2). A micropyle, polar granule, and an oocyst residuum were observed in each oocyst. Sporulation of oocysts (97–98%) occurred within 72 h at room temperature (26–28°C). The sporozoites measured 3.6× 0.96 μm and each contained a large posterior refractile body (Fig. 2).

The second type of detected oocysts was scantty, appeared transparent, spherical in shape, and was surrounded by a thick double-layered wall. These oocysts measured 15.73±2.22 μm in length (range 11.71–16.81 μm) and 14.18±1.89 μm in width (range 11.63–15.83 μm) with a shape index of 1.11 (Fig. 1). Their sporulation occurred within 60 h producing four elongated sporocysts, each measuring 7.51±0.98×4.75± 0.25 μm, containing two elongated sporozoites and a




sporocyst residuum. This second oocyst type was characterized by the absence of a micropyle and an oocyst residuum (Fig. 3).

## Site of infection

Macroscopic and microscopic examination of the jejunum, ileum, rectum, and caecum indicated that the developmental stages of both oocyst types were found mainly in the epithelial cells of jejunum, ileum, and only occasionally in the rectum (in heavy infections), but they were never seen in the caecum.

## Prepatent and patent periods

Microscopic examination of freshly shed feces revealed that the prepatent period was 5 days, while the patent period reached 6–7 days.

### Endogeneous stages

Examinations of stained preparations of sections of the ileum indicated the presence of three merogonic cycles terminating at 60, 78, and 96 h p.i. Each developmental stage was seen within a bright parasitophorous vacuole. Uninucleated meronts of the first generation were seen 24 h p.i, in the mucosa and submucosa of the intestinal epithelium (Fig. 4). Multinucleated meronts measuring 7.11±0.71×4.37±0.34 µm were seen 36–44 h p.i., while fully developed and free merozoites (10–15 merozoites/meront) of this generation were noted 60 h p.i. (Figs. 5, 6, and 7). These merozoites were banana-shaped, measuring 2.1–3.5×0.8–1.11 µm with a mean of 2.9×0.85 µm.

Meronts of the second asexual cycle were noted 68 h, p.i.. Multinucleated meronts with more than nine to 16 nuclei were seen 72 h p.i.. Furthermore, mature meronts of 9.12±1.13×12.23±2.11 µm, each containing 25–35 well-formed merozoites of 1.9–3.4×0.7–0.9 µm with a mean of 2.41×0.81 µm were usually observed 78 h, p.i. (Fig. 8). Merozoites of the second generation invaded new host cells to start the third asexual cycle or developed into gamonts initiating the sexual cycle.

Mature meronts of the third generation measured 11.26±0.97×12.11±1.15 µm were observed 96 h p.i. producing nine to 17 elongated merozoites (Fig. 9).

The development of sexual stages in the present study began about 84–96 h p.i., when merozoites of the second and third generations invaded the host cells and differentiated into micro- or macrogamonts (Fig. 10). Early small microgamonts of 9.51±1.01×6.61±1.11 µm were recognized by their large number of dark small nuclei distributed all over the whole surface of the gamont (Fig. 11). Numerous flagellated microgametes were detected 96–108 h (Fig. 12). Young macrogamonts were firstly detected 84 h p.i. These stages were spherical to ovoid in shape, being provided with a central prominent large nucleus and contained mostly a distinct nucleolus. Mature macrogametes of 13.17±1.61×10.11±1.57 µm were situated within a large bright parasitophorous vacuole and contained many homogeneous dark wall-forming bodies being arranged at their periphery. Many reserve food materials such as lipid droplets and amylopectin granules were seen 108 h p.i. inside these stages (Figs. 13 and 14). Young oocysts with nondifferentiated zygotes were detected 108–114 h p.i. (Figs. 15 and 16). Fully formed oocysts of 17.73±2.92×12.79±1.69 µm occurred in the feces of the infected quails after 5 days p.i. (Fig. 16).

It was observed that the infected sites of the villi were usually swollen, inflamed, and contained a bloody mucosa. Infected cells were flattened and swollen and their nuclei were pushed aside. All parasitic stages were situated above the host cell nucleus. Greenish mucoid bloody feces were excreted on the eighth day p.i. Infected quails usually showed signs of inappetance, weight loss, and retarded growth. In some cases a number of birds (1–5%) died at 8–10 day p.i..

## Discussion

Coccidian infections especially with *Eimeria* species are one of most dangerous diseases facing poultry production industry. Many Eimerian species are highly pathogenic to their hosts causing great economic losses. Infections with a single *Eimeria* species are rare (Mehlhorn 2006). For species diagnosis the oocyst is used as the most easily accessible stage in many coccidians (Abdel-Ghaffar et al. 1990, 1991; Bashtar et al. 1992a; Abd Al-Aal 2000; Mehlhorn 2008).

The available information on coccidian species infecting quails in Saudi Arabia is very scarce and only a few species of *Eimeria* were identified (such as *Eimeria tahamensis* by Amoudi 1987) from the Arabian quail (*Coturnix delegorguei arabica*).

Regarding natural infections among farmed quails, the present study recorded two *Eimeria* species on the basis of the morphological and morphometric characters of their oocysts and the sporulation time. Type 1 was ovoid to ellipsoidal and occurred in large numbers, while type 2 was spherical to subspherical and rare. The comparison of the morphometric characters of type 1 oocysts (Table 1) with those of other *Eimeria* species recorded in quails indicated that this is a new species.

### Sporulation time

The sporulation time recorded in the present study was 72 and 60 h for the first and second *Eimeria* species, respectively. These periods are longer than the 27 h reported for *E. bateri* (Norton and Peirce 1971), the 24 h for *E. uzura* (Tsunoda and Muraki 1971), the 18 h for *Eimeria lettyae* (Ruff 1985), and the 48 h for *Eimeria colini* (Fisher and Kelley 1977) and *E. tahamensis* (Amoudi 1987). The sporulation time may decrease in crowds of oocysts or depend on contamination. This may support the opinion that different sporulation times may be related to different experimental factors or laboratory techniques or to the lack of adequate oxygen (Long et al. 1976; Koudela and Vitovec 1998; Abd Al-Aal 2000).

### Site of infection

Stages of the endogeneous development of *Eimeria* species of the first type were found along the epithelium of the jejunum, ileum, and occasionally in the rectum in severe

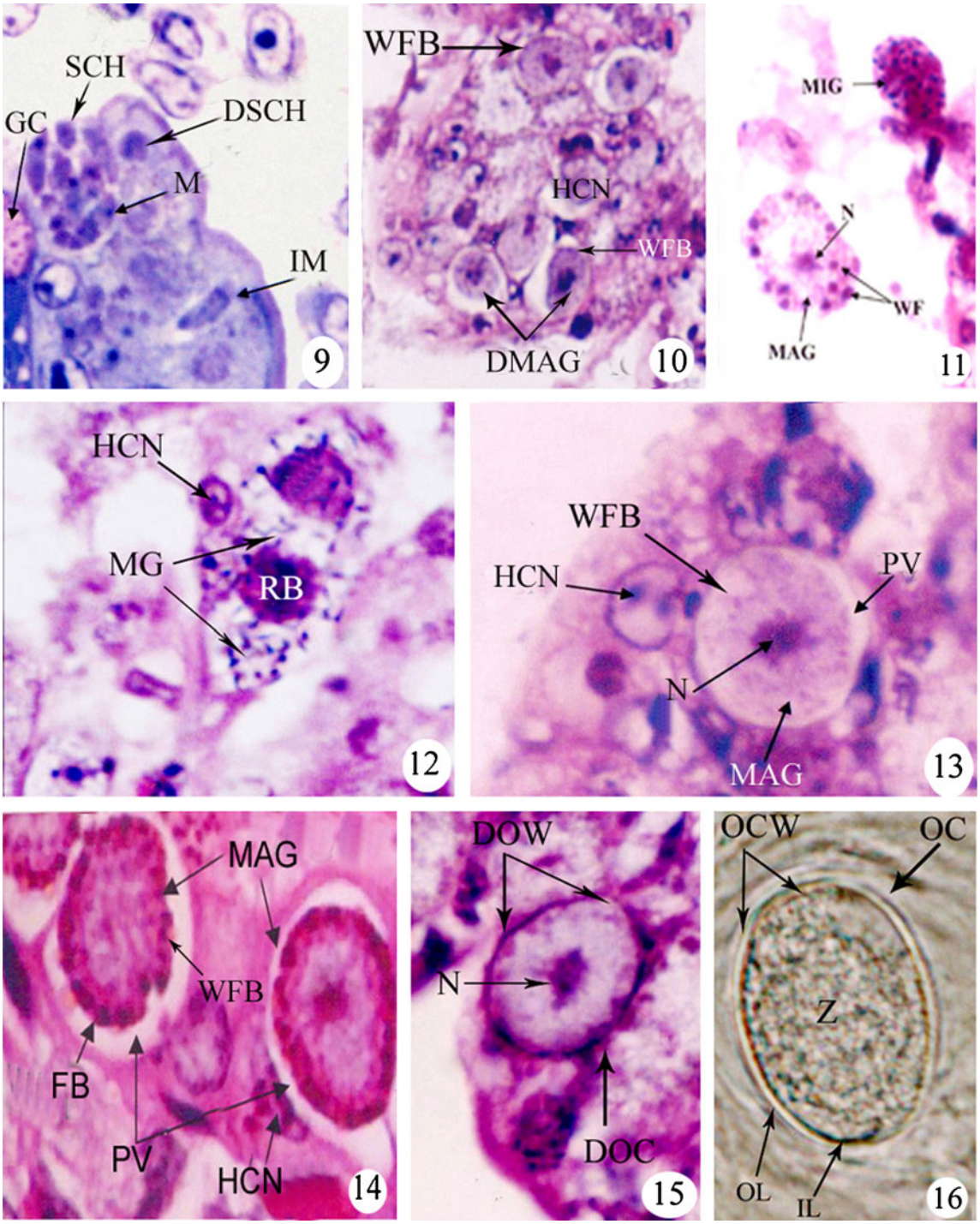



**Fig. 9** Photomicrograph of mature meront (*SCH*) and a developing one (*DSCH*) of the third generation. ×2,350
**Fig. 10** Photomicrograph of developing micro- and macrogamonts. ×1,900
**Fig. 11** Photomicrograph of a macrogamete (*MAG*) with wall-forming bodies (*WF*) and a microgamont (*MIG*) with many nuclei (*N*). ×2,100
**Fig. 12** Photomicrograph of a mature microgamont with many flagellated microgametes (*MG*) and a residual body (*RB*). ×2,300
**Fig. 13** Photomicrographs of mature macrogametes (*MAG*) with wall-forming bodies (*WFB*). ×2,300
**Fig. 14** Photomicrographs of mature macrogametes (*MAG*) with wall-forming bodies (*WFB*). ×2,300
**Fig. 15** Photomicrograph of a fertilized macrogamete within which the fusion of the neighboring wall-forming bodies starts to form the developing oocyst wall (*DOW*). ×2,300
**Fig. 16** Freshly shed oocyst after 11 days p.i.. ×2,650

**Table 1** Comparative data of several Eimerian species from quails (*Corturnix* species)

| Species | Host | Morphology | Measurements | | Prepatent period | Sporulation time | Struc. | References |
|---|---|---|---|---|---|---|---|---|
| | | | L×W | L/W | | | | |
| *E. uzura* | *C. japonica* | Ovoid, elliptical | 24.2×18.7 | 1.30 | 4 days | 24 h/25°C | 1–4 Pg<br>*Mp., | Tsunoda and Muraki 1971<br>*Ruff et al. 1984<br>Teixeira et al. 2004 |
| *E. bateri* | *C. coturnix japnonica* | Ovoid, elliptical, subspherical | 17.6×23.2 | 1.31 | 4 days | 27 hrs/27°C | Pg | Bhatia et al. 1965<br>Norton and Peirce 1971<br>Teixeira et al. 2004 |
| *E. tsunodai* | *C. japonica* | Ovoid | 18.4×14.1 | 1.36 | – | – | Pg | Tsutsumi 1972<br>Teixeira et al. 2004 |
| *E. tahamensis* | *C. delegorguei arabica* (Arabian quail) | Ellipsoidal | 41.2±1.34×28.4±0.81 | – | – | 48 h/25°C | Pg | Amoudi 1987 |
| *E. crusti* | Mountain quail | Ellipsoidal, subspherical | 26.0×21.2 | 1.23 | – | – | Pg | Duszynski and Gutierrez 1981 |
| *E. colini* | Bob white quail | Ellipsoidal | 24.8×20.9 | 1.2 | 84–96 h | 48 h/25°C | Mp | Fisher and Kelley 1977 |
| *E. lettyae* | Bob white quail *C. coturnix* | Oval | 21.1×17.2 | 1.22 | 88–91 h | 18 h/25°C | – | Ruff 1985 |
| Present study type (1) | *C. japonica* | Ellipsoid to ovoid | (17.73±2.92)×(12.78±1.69) | 1.39 | 5 days | 72 h | Mp OR Pg | Present study |
| Present study type (2) | *C. japonica* | Spherical to subspherical | (15.73±2.22)×(14.18±1.89) | 1.11 | – | 60 h | Pg | Present study |

All measurements in microns (μm)

*Mp* micropyle, *Pg* polar granule, *OR* oocyst residuum, *RB* refractile body, *SR* sporocyst residuum, *ST* stieda body, *Ssb* substieda body, – no data available, *L* length, *W* width

infections, but was never seen in the caecum. This observation agrees with the findings in *E. bateri* from quails (Norton and Peirce 1971). Developmental stages of *E. uzura* were found only in the lower part of the duodenum and jejunum and in moderate numbers in the ileum (Ruff et al. 1984). Furthermore developmental stages of *E. lettyae* were reported in the lamina propria, duodenum, ileum, and caecum (Ruff 1985), while stages of *E. tsunodai* were recorded in the caecum (Tsutsumi 1972). The prepatent period was determined as 5 days in the present study which is longer than the 4 days recorded in *E. uzura* and *E. bateri* from quails (Tsunoda and Muraki 1971; Norton and Peirce 1971; Teixeira et al. 2004). The patent period, which was recorded as 6–7 days in the present *Eimeria* species is in agreement with some reports on other *Eimeria* species (Abdel-Ghaffar et al. 1990, 1991; Bashtar et al. 1991, 1992b; Teixeira et al. 2004).

## Endogeneous stages

### 1. Asexual phase

The exact number of asexual generations in species of the genus *Eimeria* is not fixed (Abdel-Ghaffar et al. 1991; Bashtar et al. 1992b; Mehlhorn 2006). In the present investigation three generations of merogony were recorded. Some authors reported two and three or even five asexual generations in other *Eimeria* species infecting different birds (Koura et al. 2001; Dai et al. 2005; Matsler and Chapman 2006). Four generations were reported in *E. bateri* (Norton and Peirce 1971) from the quail *C. coturnix japonica*, five generations in *E. lettyae* from *Collinus virginianus* (Ruff 1985).

In the present investigation, the first asexual generation is completed within 60 h, which is quite different from other *Eimeria* species reported in quails (Table 1) and other birds (Dai et al. 2005; Matsler and Chapman 2006). The second asexual cycle is completed at 78 h p.i., while the time recorded in *E. bateri* was 54 h (Norton and Peirce 1971), 40 h in *E. lattyae* (Ruff 1985) from quails and 72–102 h or longer for *Eimeria* species from other birds (Dai et al. 2005; Matsler and Chapman 2006). The time recorded for the third asexual cycle was 96 h p.i. which is a longer period than that recorded for *Eimeria* species from quails (Table 1).

The number of merozoites yielded from meronts per cycle and measurements of these meronts and their merozoites is quite different and may be a species-specific character in the genus *Eimeria* (Abdel-Ghaffar et al. 1990, 1991; Mehlhorn 2006). In the present study the first generation meronts measured 7.11±0.71×4.37±0.34 µm and produced 10–15 merozoites/meront. In this concern first generation meronts of *E. bateri* measured 9.6×10.9 µm producing eight to 18 merozoites (Norton and Peirce 1971). In *E. uzura* these meronts measured 10.1×11.9 µm producing eight to 18 merozoites (Tsunoda and Muraki 1971), while they measured 7.0×9.4 µm in *E. lettyae* (Ruff 1985). Regarding the duration, measurements, and the number of the produced merozoites in the three asexual generations recorded in the present investigations, different measurements and time elapsed to complete this cycle. Observations reported on both second and third generation meronts in the present investigation were also different from previous results on *Eimeria* species infecting quails. The most interesting character of all these results is the development of merozoites by means of an ectomerogony which is common among the majority of the coccidians (Abdel-Ghaffar et al. 1991; Koura et al. 2001; Mehlhorn 2006, 2008).

### 2. Sexual phase

The sexual phase of the life cycle is of great significance, since it plays an important role in the host specificity. In addition, the start of the sexual phase usually takes place after a specific number of asexual generations (Abdel-Ghaffar et al. 1991; Bashtar et al. 1992b; Mehlhorn 2006). The sexually differentiated merozoites develop after host cell invasion into gamonts and finally produce micro- or macrogametes in separate parasitophorous vacuoles as was reported in all *Eimeria* species studied before (Scholtyseck 1973, Mehlhorn 2006, 2008).

## Microgametogenesis

In the present study mature microgametes were observed 96 h p.i., while in other *Eimeria* species longer periods were recorded: 132 h in *Eimeria tenella* (Nakai et al. 1992) and 96–108 h in *Eimeria stigmosa* (Pecka 1992). Varying sizes of microgamonts and varying numbers of microgametes produced were reported for many species of *Eimeria* (Nakai et al. 1992, Teixeira et al. 2004, Dai et al. 2005, Matsler and Chapman 2006, Mehlhorn 2006, 2008).

## Macrogametogenesis

Macrogamogony involves the transformation of sexually female differentiated merozoites into macrogametes. This process includes changes in shape from elongate to spherical including the enlargement of the nucleus and nucleolus (growth phase). As development proceeded, the peripherally arranged wall-forming bodies and large amounts of food reserve (lipids and amylopectin granules) appeared in the cytoplasm (differentiation phase). These results coincide with those reported for many other *Eimeria* species (Abdel-Ghaffar et al. 1991; Bashtar et al. 1992b; Dai et al. 2005; Mehlhorn 2006).

In the present study, early macrogamonts were observed 84 h p.i., while mature macrogametes were seen 108 h.

After fertilization the wall-forming bodies fused together giving rise to the oocyst wall. Oocysts with a double wall were shedded in the feces of the infected host beginning 5 days p.i.. The appearance of the bilayered oocyst wall is usually accompanied with the disappearance of the wall-forming bodies in the young oocyst. These results are in agreement with previously reported results in many *Eimeria* species (Norton and Peirce 1971; Ruff 1985; Abdel-Ghaffar et al. 1991; Teixeira et al. 2004; Mehlhorn 2006).

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