Remarks of *Eimeria labbeana* infection in Egyptian pigeons

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Research Article

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Abstract

This work was conducted to investigate the course of *Eimeria labbeana* infection in Egyptian pigeons (*Columbia Livia*). Thirty squabs were experimentally infected orally with 2.5 x10^4 sporulated *Eimeria labbeana* oocysts. Daily scarification of three squabs was done from day one post-infection (PI) until day 8 PI for following of the endogenous stages in tissue samples and 6 squabs were kept to follow the patent period and daily oocyst shedding. Paraffin-embedded intestinal samples were sectioned and stained for differentiation of parasitic stages. The infected squabs showed greenish watery diarrhea, weakness, rough feathers, and decrease food intake at day 5 PI. The pre-patent and patent periods were 6 and 14 days PI respectively. The oocyst shedding started at day 6 PI and reached the peak at day 8 PI. Histopathological examination revealed the presence of three schizont stages, macro-and micro-gametes, and oocysts in the duodenal and jejunal parts of the small intestine. In conclusion, it is the first study on Egypt on *Eimeria labbeana* in Egyptian pigeons and its biology is like recorded before.

Introduction

Domestic pigeons (Columba livia) belong to the family Columbidae and the order Columbiformes. Domestic pigeons are reared for meat production, gaming purposes, and recently as laboratory animals in research (Radfar et al. 2011; Sood et al. 2017). In Upper Egypt, the domestic pigeons are mainly reared for meat production and racing hobbies by small reared farms and some households, as a source of protein need of the local population (Elseify et al. 2018).

Pigeons suffer from many parasitic diseases, including coccidiosis, which is caused by protozoan parasites of the genus *Eimeria* (*Coccidia: Eimeriidae*). Pigeon's coccidiosis may occasionally be observed in young squabs under intensive rearing conditions, at the age of four to sixteen weeks. While the elder pigeons serve as asymptomatic carriers and remain apparently healthy (Soulsby 1982; Tenter et al. 2002; Ali et al. 2015). Clinical signs of Pigeon's coccidiosis include rough feathers, anorexia, greenish watery diarrhea with mucus, dehydration, loss of body weight, and mortality (McDougald 2003; Abdul Latif et al. 2016; Dong et al. 2018). There are four species of *Eimeria* species in pigeons: *E. columbae, E. columbarum, E. labbeana*, and *E. labbeana-like* (Yang et al. 2016). *E. labbeana* is the most prevalent species in many geographical regions of the world. In Egypt, Gadelhaq and Abdelaty (2019) record the presence of four *Eimeria* species, *Eimeria labbeana, E. columbarum E.columbae* and *E.labbeana*-like, in pigeons in Minia province.

Pigeon's coccidiosis is usually subclinical; however, outbreaks of coccidiosis may occur, which may, in turn, cause high mortality among nestlings and young birds (Yabsley 2008). As published information concerning *Eimeria* infections in pigeons in Egypt is scarce. Therefore, this work aimed to follow and investigate the course of infection of *Eimeria labbeana* in experimentally infected pigeons from field isolates oocysts collected from naturally infected domesticated pigeons in Minia province.

Materials And Methods

All experiments were conducted according to the ethical standards and protocols approved by the Committee of Animal Experimentation of Faculty of Veterinary Medicine, Beni-Suef University, Egypt (BSUV- 33/2019).

Source of *Eimeria labbeana* oocysts

The primary source of *E. labbeana* oocysts was from naturally infected pigeons, then the collection of oocysts was done then sporulate it. For obtaining a continuous source of oocysts; propagation of obtained sporulated oocysts was done in the laboratory. Five pigeons squabs (30th day of age) were experimentally infected orally by$12x10^3$ purified field isolated *Eimeria* species sporulated oocysts (a mixed species of *E. labbeana 95.00%, E. columbarum and E. columbae* are 5.00% oocysts) that collected from naturally infected pigeons at Minia province and previously identified by Gadelhaq and Abdelaty (2019). The fecal samples were collected at the 8th post-infection then the pigeons were sacrificed and intestinal contents were collected to purify the oocysts. The latter was collected and concentrated in saturated sodium chloride solution, washed, sporulated, and counted by using MC Master Technique (Aboelhadid et al. 2019) and kept at 4 ^oC temperature to study the biology.

Experimental design

Thirty apparently healthy squabs (4–6 weeks old) were brought from the local poultry market and examined for coccidiosis for 7 days to confirm coccidia free. Birds were reared on metal cages for one week before infection with *ad libitum* access to food and water. Birds were fed an anticoccidial-free diet (a 20% protein diet). Thirty squabs were experimentally infected with 2.5 $x10^4$ sporulated eimerian oocysts. Daily scarification of 3 squabs was done from day one post-infection (PI) until the day 8 post-infection (PI). The rest of the birds (six birds) were kept to investigate the pre-patent and patent periods. The clinical signs of coccidiosis and daily fecal oocyst count were recorded. Tissue samples were taken from all parts of the intestine (duodenum, jejunum, ileum, colon, cecum, and rectum) to detect the eimerian endogenous stages by histopathological examination. The tissues were processed according to a previously described method (Bancroft and Gamble 2008). Small pieces of each part of intestine tissue were collected in 10% buffered formalin for histopathology. The fixed tissues were washed in running tap water overnight, dehydrated, and infiltrated by paraffin wax. Serial paraffin sections (5 µm thicknesses) were obtained, and the sections were deparaffinized in three, consecutive washings in xylol for 5 min, and rehydrated with five, successive washings with alcohol in descending order of 100, 95, 80, 70, and 50% in deionized water. The histological sections were then subjected to conventional Hematoxylin and Eosin (H and E) staining procedures.

Results And Discussion

E. labbeana sporulated oocysts are ovoid or subspherical in shape, without oocystic or sporocystic residual body, and no micropyle with average size (15-18.9 urn X 14-17.5 µm) (Fig. 1). The clinical signs appeared at day 5 PI of squabs by field isolated *E. labbeana* sporulated oocysts. The results revealed that

the infected squabs suffered from rough feathers, greenish watery diarrhea, arched back, and decrease food intake that caused weakness of squabs at the 6th day PI. These findings are similar to the findings of Stewart (1957), who record these clinical signs.

The pre-patent period started from infection till shedding of oocysts in feces at day 6 PI. The patent period started from the shedding of oocysts in feces till the disappearance of oocysts in feces on day 14 PI. This result coincided with some previous studies of Nieschulz (1925), Stewart, 1957 and Krautwald-Junghanns et al. (2009). However, Bondois (1936) recorded the pre-patent period as seven days, Morini (1950) recorded it at 7 to 8 day PI, and Aleksandra and Pilarczyk (2014) reported it .at day 8 PI. This discrepancy is common due to geographical differences, and species of the pigeons used in the study. In our results, the mean patent period extended to 14 days PI. This agrees with the results of Stewart (1957) that explained that the patent period of *E. labbeana*, in acute stages lasted less than two weeks.

Unsporulated oocysts firstly appeared at day 6 PI with a very low number. These results match those of previous studies (Stewart 1957; Srivastava 1966; Varghese 1977). The oocysts increased gradually till it reached to the peak of its shedding at day 8 PI, then it started to decline from day 9 PI till reached its minimal count at day 14 PI (Table 1). Sporogony occurs outside the host and its duration lasts 1–4 days. This record is similar to the finding of Saikia et al. (2017), who recorded that the highest concentration of oocysts shedding were observed in feces after 8 days of oral infection.

Table 1
Daily oocyst counts per gram feces (OPG) of *Eiemeria labbeana*. in Egyptian pigeons (local isolates)

Days post-infection	Oocyst counts per gram feces (OPG) Mean + SD
6 dpi	2541.67 ± 226.84
7 dpi	200458.30 ± 2444.20
8 dpi	405583.30 ± 954.70
9 dpi	128250 ± 2459.04
10 dpi	34908.33 ± 212.62
11 dpi	32541.67 ± 162.66
12 dpi	20000 ± 100
13 dpi	12100 ± 200
14 dpi	1008.33 ± 162.66

Moreover, the different parasitic stages were detected in the sectioned stained tissues. Pigeons *Eimeria* showed firstly parasitic vacuole, then the multinucleated cell, schizonts, macro and microgametocytes ending by the formation of oocysts in the intestinal epithelium. Trophozoites in the parasite vacuoles and dividing nuclei were reported at day 1, the parasite vacuole size was 12.12 ± 1.08um× 9.5 ± 0.82 um

(Fig. 2A). The first-generation schizont appeared on day 2 PI. It contained several merozoites up to 32 with the average length of merozoite was 5.25 ± 0.74um (Fig. 2A, B, C). On day 3 PI the second-generation schizont appeared and third-generation schizonts at day 4 and 5 PI (Fig. 3A, B, C). The macro-and micro-gametocytes appeared at day 5 PI, its ovoid in shape and its size was 12.09 ± 0.20 um × 8.5 ± 0.12 um in diameter for macrogametocyte and 8.80 ± 0.82um for microgametocytes. These results were similar to those of Nieschulz 1925; Stewart 1957; Srivastava 1966 and Varghese 1977, who reported that the development of *E. labbeana* in the pigeons up to the appearance of the first oocysts in the feces takes exactly six days. Also, the measurements and duration of each stage of different endogenous stages were nearly similar to the measurements of Varghese (1975). In schizonts, the number of merozoites was variable from 20 in first-generation, 10–18 in second-generation schizonts, and 4–8 in third-generation schizonts (Fig. 3). These findings agree with the previous studies that reported data variation in the number of merozoites(6–30) in first-generation schizonts (Varghese 1977 and Mennemeier 1985). The recorded macro-and micro-gametocytes were similar as described by (Varghese 1975, 1976). At day 7 PI, macro-and micro-gametes, as well as oocysts, were detected (Fig. 4C). Parasitic stages were observed mainly in the duodenum and jejunum. Besides, no parasitic stages were detected in the ileum and large intestine. This contrasts with findings from previous studies of Varghese 1977 and Mennemeier 19855, who reported the presence of the parasitic stages in the entire length of the small intestine, mainly located in the midsection. However, our results were similar to Stewart 1957, who reported that *E. labbeana* life cycle stages occurred in the duodenum and jejunum.

Conclusion

We reported that the infection course of *E. labbeana* isolated from Egyptian pigeons is similar to *E. labbeana* described by Varghese (1975). The mean pre-patent period was 6 days, and the mean patent period extended to 14 days PI. The peak of oocyst shedding reached on day 8 PI. There were 3 generations of schizonts before oocysts shedding.

Declarations

Acknowledgment The authors appreciated advices of Prof. Abdelazeem S Abdelbaki, professor of parasitology, Faculty of science, Beni-Suef University in conducting the study.

Authorscontribution All authors have read and agreed to the published data of the manuscript.

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Availability of data and material All the data was analyzed during this study and included in this manuscript

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article

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Figures

Figure 1

E. labbeana sporulated oocysts are ovoid or subspherical in shape, without oocystic or sporocystic residual body, and no micropyle with average size (15-18.9 urn X 14-17.5 µm) (Fig 1).

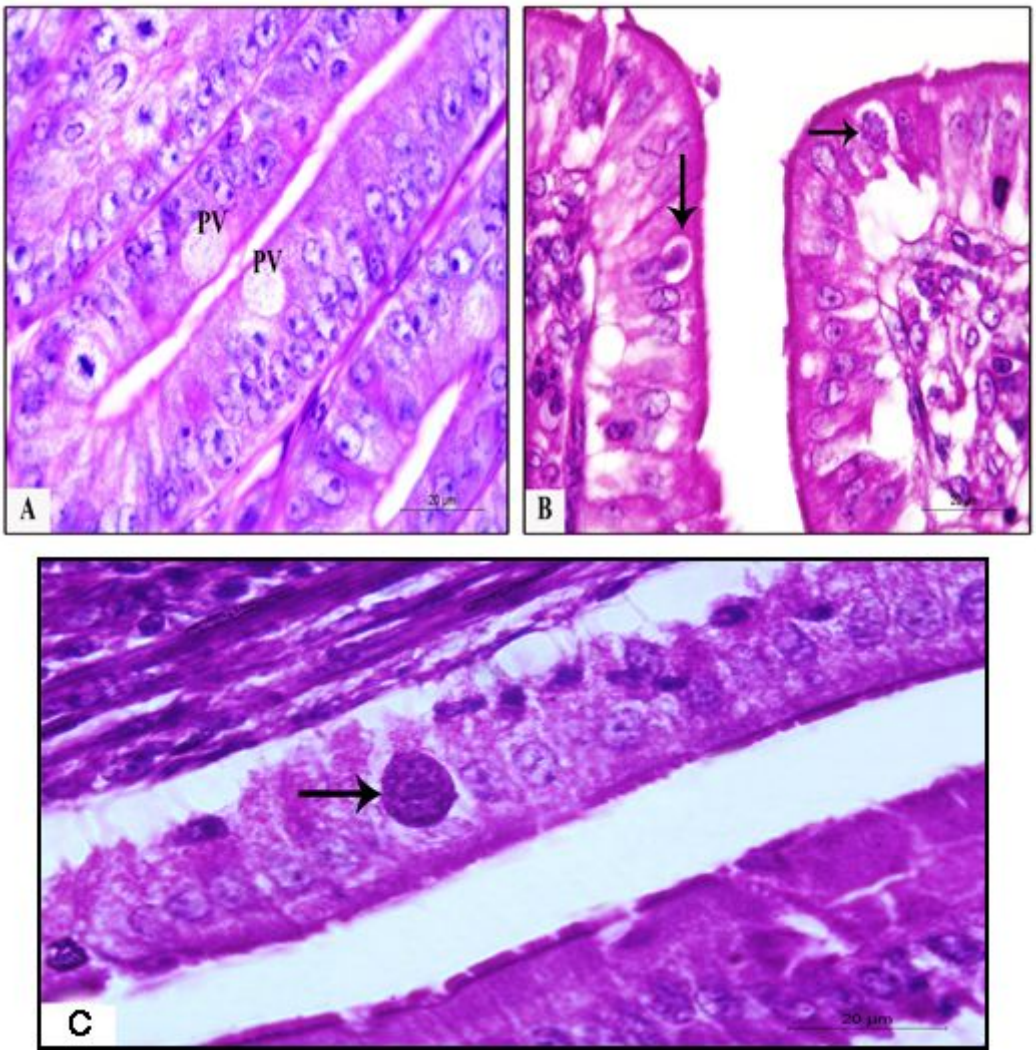

Figure 2

Trophozoites in the parasite vacuoles and dividing nuclei were reported at day 1, the parasite vacuole size was 12.12 ± 1.08um× 9.5± 0.82 um (Fig 2A). The first-generation schizont appeared on day 2 PI. It contained several merozoites up to 32 with the average length of merozoite was 5.25± 0.74um (Fig 2A, B, C).

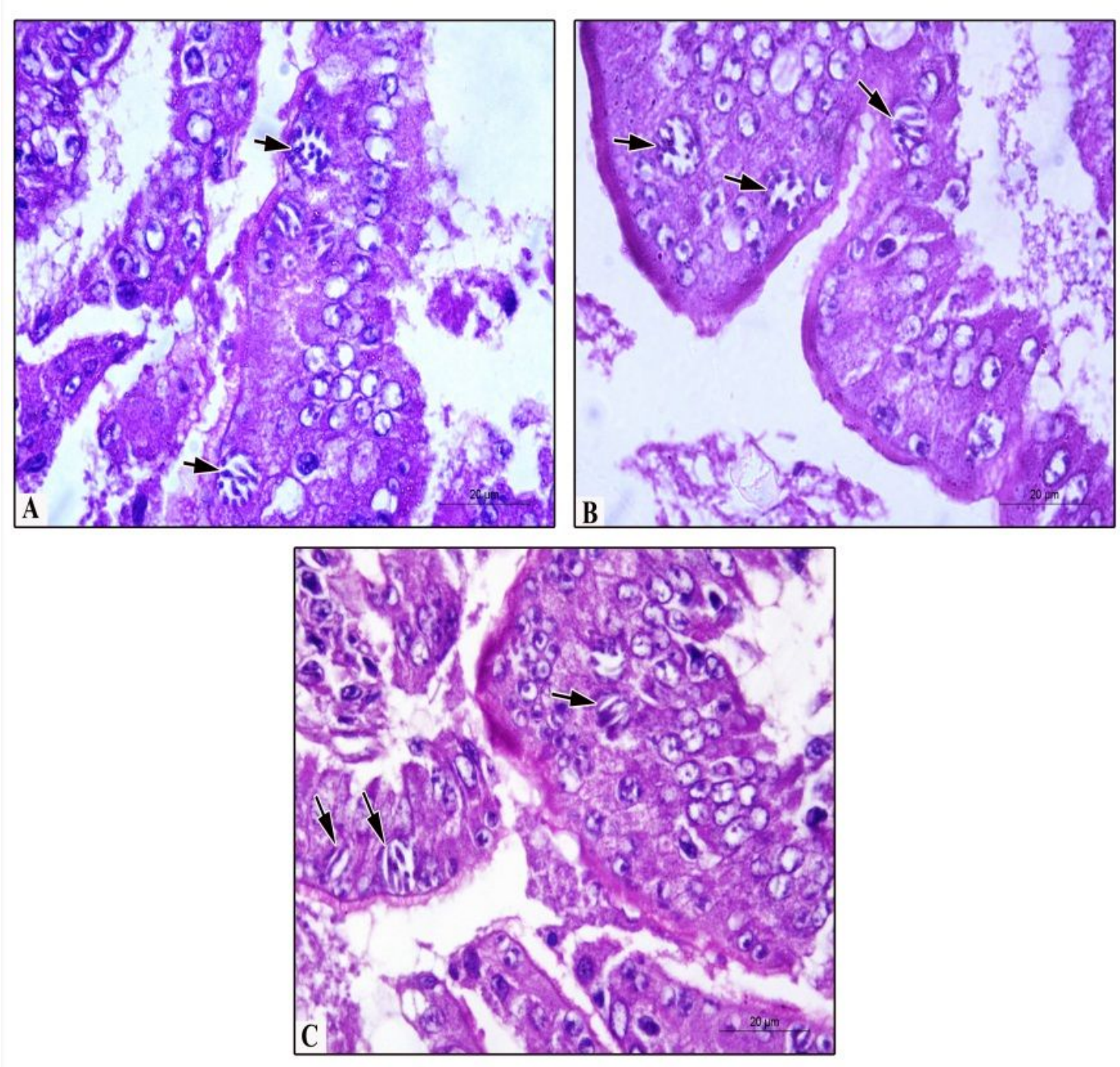

Figure 3

On day 3 PI the second-generation schizont appeared and third-generation schizonts at day 4 and 5 PI (Fig 3 A, B, C).

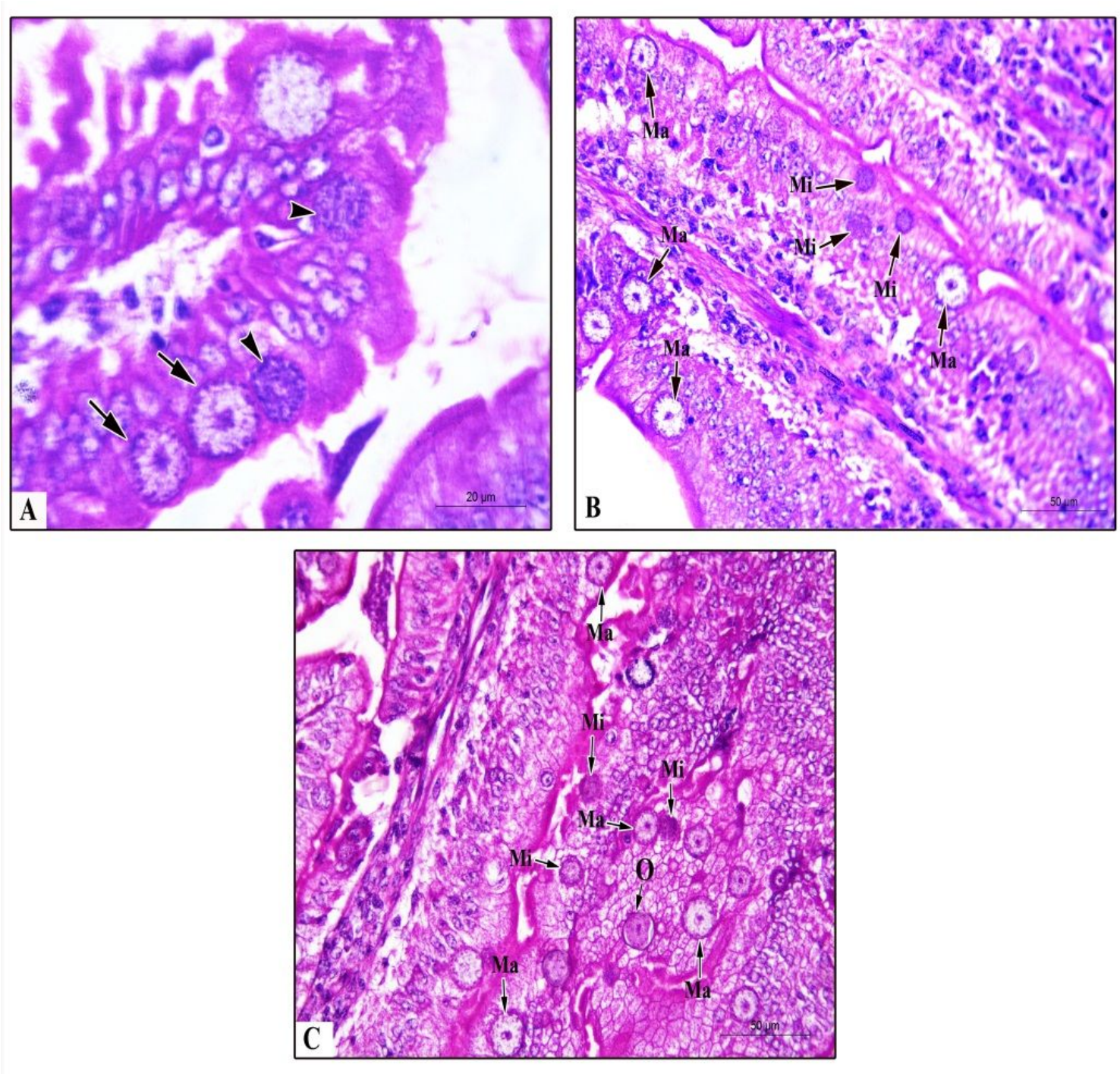

Figure 4

The recorded macro-and micro-gametocytes were similar as described by (Varghese 1975, 1976). At day 7 PI, macro-and micro-gametes, as well as oocysts, were detected (Fig. 4 C).

Supplementary Files

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