

Re-evaluation of the life cycle of *Eimeria maxima* Tyzzer, 1929 in chickens (*Gallus domesticus*)

J. P. Dubey and M. C. Jenkins

Research Article

Cite this article: Dubey JP, Jenkins MC (2018). Re-evaluation of the life cycle of *Eimeria maxima* Tyzzer, 1929 in chickens (*Gallus domesticus*). *Parasitology* **145**, 1051–1058. <https://doi.org/10.1017/S0031182017002153>

Received: 6 September 2017
Revised: 18 October 2017
Accepted: 1 November 2017
First published online: 14 December 2017

Key words:

Chickens (*Gallus domesticus*); development; *Eimeria maxima*; life cycle; schizogony.

Author for correspondence:

J. P. Dubey, E-mail: jitender.dubey@ars.usda.gov

United States Department of Agriculture, Agricultural Research Service, Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland 20705-2350, USA

Abstract

A time-course study was conducted to resolve discrepancies in the literature and better define aspects of the *Eimeria maxima* life cycle such as sites of development and both morphology and number of asexual stages. Broiler chickens were inoculated orally with five million *E. maxima* oocysts (APU1), and were necropsied at regular intervals from 12 to 120 h p.i. Small intestine tissue sections and smears were examined for developmental stages. The jejunum contained the highest numbers of developmental stages. At 12 h p.i., sporozoites were observed inside a parasitophorous vacuole (PV) in the epithelial villi and the lamina propria. By 24 h, sporozoites enclosed by a PV were observed in enterocytes of the glands of Lieberkühn. At 48 h p.i., sporozoites, elongated immature and mature schizonts, were all seen in the glands with merozoites budding off from a residual body. By 60 h, second-generation, sausage-shaped schizonts containing up to 12 merozoites were observed around a residual body in the villar tip of invaded enterocytes. At 72 and 96 h, profuse schizogony associated with third- and fourth-generation schizonts was observed throughout the villus. At 120 h, another generation (fifth) of schizonts were seen in villar tips as well as in subepithelium where gamonts and oocysts were also present; a few gamonts were in epithelium. Our finding of maximum parasitization of *E. maxima* in jejunum is important because this region is critical for nutrient absorption and weight gain.

Introduction

Eimeria maxima invades and undergoes asexual and sexual development in the middle intestine altering intestinal nutrient uptake by disrupting gut absorptive capacity. The release of large numbers of merogonic stages causes sloughing of the gut epithelium that in turn sets up an ideal environment for colonization by microbes such as *Clostridium perfringens* leading to necrotic enteritis. *Eimeria maxima* is distinctive among the seven *Eimeria* species infecting chickens because its oocysts and male gamonts are the largest (up to $39 \times 35 \mu\text{m}$) among the avian *Eimeria* species (Levine, 1973). During our efforts to compare two *E. maxima* strains (APU1, APU2, Jenkins *et al.* 2017), it became obvious that there were discrepancies in the literature concerning *E. maxima* schizont development and the number of generations of schizogony. Almost 90 years ago, Tyzzer (1929) described endogenous development of *E. maxima*, as well as naming the species based on the large size of its oocysts. In this seminal study, Tyzzer (1929) found that schizonts were distributed throughout the small intestine, but that the greatest numbers were present in jejunum–ileum. Tyzzer (1929) observed *E. maxima* schizonts at 72, 96 and 120 h, but not at 24 or 48 h p.i. The small size and presence at the surface epithelium of schizonts at 72 and 96 h has generally been confirmed by other authors (Long, 1959; Scholtyseck, 1963). Subsequently, Challey and Johnson (1968) and Millard *et al.* (1972) reported on the first-generation schizonts that were missed previously. However, there is uncertainty concerning the number of schizogonic generations and details of development are lacking because of the small size of schizonts.

The purpose of this study was to identify the primary site of *E. maxima* invasion and development, and resolve discrepancies in the literature concerning intracellular sporozoite morphology and schizogony, particularly the number of schizont stages and site(s) in the intestinal epithelium where each stage of development occurs.

Materials and methods

Eimeria maxima strain and experimental infections of chickens

All studies of the *E. maxima* life cycle described in this study utilized the *E. maxima* APU1 strain, which had been propagated in susceptible chickens every 3–4 months for 10 years after initial isolation (Fetterer and Barfield, 2003; Jenkins *et al.* 2017). The purity of *E. maxima* oocysts was confirmed by microscopy and ITS1-PCR using procedures described elsewhere (Jenkins *et al.* 2006). Broiler chickens (male, Hubbard-Ross 708, Longeneckers Hatchery, Elizabethtown, PA, USA) were each inoculated at 1 week of age orally with 5×10^6 *E. maxima* APU1 oocysts, and intestinal tissues were harvested in two separate studies.

Table 1. Summary of endogenous development of *Eimeria maxima* in small intestine of chickens orally inoculated with five million oocysts

Hours p.i.	Chicken ID	Segment #1 (duodenum)	Segment #2 (jejunum)	Segment #3 (ileum)	Remarks
12	D230	+	+	-	Sporozoites in surface epithelium and lamina propria
24	D216	-	+	+	Sporozoites in glands of Lieberkühn
36	D218	+	+	-	Sporozoites, multinucleated first-generation sporozoite-shaped schizonts
48	D220	-	+	-	Sporozoites, mature first-generation schizonts
60	D222	-	+	-	Mature second-generation schizonts
72	D224	+	+	+	Third- and fourth-generation schizonts. Sporozoites in gland
96	D226	+	+	+	Third-, fourth- or fifth-generation schizonts in villus epithelium, rare in gland and the lamina propria
120	D228	+	+	+	Few fifth-generation schizonts in villus epithelium and lamina propria. Gamonts in lamina propria

+, Positive; -, negative.

Experiment 1

Chickens inoculated with *E. maxima* oocysts were necropsied at 12, 24, 36, 48, 60, 72, 96 or 120 h p.i. The small intestine was divided into three segments: segment #1 consisted of the entire duodenal loop (14–19 cm long), segment #2 consisted of the jejunum from the end of duodenum to Meckel's diverticulum (34–49 cm long) and segment #3 consisting of Meckel's diverticulum to the just anterior of the caeca (23–37 cm long). Each segment was flushed with 10% buffered formalin (BF) from both ends and immersed in 10% BF. After 1–2 h in BF, 12 cross-sections (3–4 mm thick at equal distances) were cut and left in BF for at least 1 day. Formalin fixed tissues were then embedded in paraffin for sectioning (American HistoLabs, Rockville, MD, USA). The remainder of intestinal segments were saved in 10% formalin for further studies. Histological sections were cut at 3–5 μm thick, stained with haematoxylin and eosin and examined for coccidian stages at 1000 \times magnification. In addition, 60 cross-sections were prepared from segment #2 of each of two chickens euthanized at 48 and 60 h after *E. maxima* APU1 inoculation

Experiment 2

The objective of this experiment was to obtain additional details of the life cycle in two chickens inoculated with APU1 strain of *E. maxima*. These chickens were euthanized at 72 or 96 h p.i. and only jejunum (segment #2) was studied. A 5 cm section of intestine was slit open, spread on paper towel and the surface epithelium was very gently scraped with a glass slide, mashed and a 4 \times 2 cm area was smeared on 7.5 \times 2.5 cm glass slides. The smears (100 each at 72 and 96 h) were fixed with methanol, air dried and examined after staining with Giemsa. All smears were examined for coccidian stages at 1000 \times magnification.

Results

Distribution of *E. maxima* stages in intestine

Depending on the duration of infection, stages were seen throughout the small intestine but the jejunum was the most parasitized (Table 1). The entire life cycle was completed by 120 h.

There was no host reaction to the parasite in histological sections.

Sporozoites

Sporozoites excysted *in vitro* and stained with Giemsa were approximately 12 \times 2.5 μm (Fig. 1A). They contained a nucleus

and one or two large refractile bodies. In histological sections of small intestine, sporozoites were detected 12–72 h p.i. and were located singly in parasitophorous vacuoles (PV). At 12 h, sporozoites were in the lamina propria and the epithelium of the villus. At 24 h, sporozoites were intracellular in enterocytes of the glands of Lieberkühn (here after referred as glands). Sporozoites were 6–7 \times 3–3.5 μm and contained prominent RB that were eosinophilic. The nucleus was pushed to side or at the non-conoidal end. Of all sections, only one sporozoite was seen at 72 h.

First-generation schizonts

All development occurred in variable-sized PV in enterocytes of glands at 36–72 h (Fig. 1). Early stages were sporozoite-shaped; round uninucleated stage was not seen. The RB from sporozoites was fragmented into several pieces (Fig. 1C–G). The nucleus was divided into irregular-shaped nuclei that were arranged throughout the schizont, and were often obliterated by RB. Immature schizonts were often irregular in appearance ranging from elongated to pear-shaped or oblong. At 36 h, most organisms seen were sporozoites and only few had multiple nuclei. The details of nuclear division were masked by the RB. Merozoites were observed arising asynchronously from the main mass. Thus, some merozoites were free in the PV, while others were still attached to the main mass (Fig. 1H). Because of the variability of the PV, the schizont size varied containing a maximum of 20 merozoites. Merozoites were 6–7 \times 1 μm and contained a centrally located nucleus (Fig. 1). The lumens of infected glands were dilated and free merozoites were seen in the glandular lumen (Fig. 2A and B). Among many recuts of the jejunum of chicken (D224, Table 1) euthanized 72 h, a single mature schizont was found in gland enterocyte at 72 h; it had 10 merozoites and was located at the base of gland touching the muscular mucosae. The measurements are from tissue sections.

Second-generation schizonts

Mature second-generation schizonts were seen in the jejunum at 60 h. Schizonts were located exclusively in enterocytes at the villar tips (Fig. 2C–I). Immature schizonts were amoeboid to sausage-shaped (Fig. 2D–G), were 10 μm in maximum diameter and contained up to 12 merozoites, sometimes arranged around a residual body (Fig. 2G). The merozoites were 4–5 μm long and contained a centrally located nucleus (Fig. 2G). The measurements are from tissue sections. The merozoites appeared to differ in size (Fig. 2); however, smears were not available to confirm the size differences.

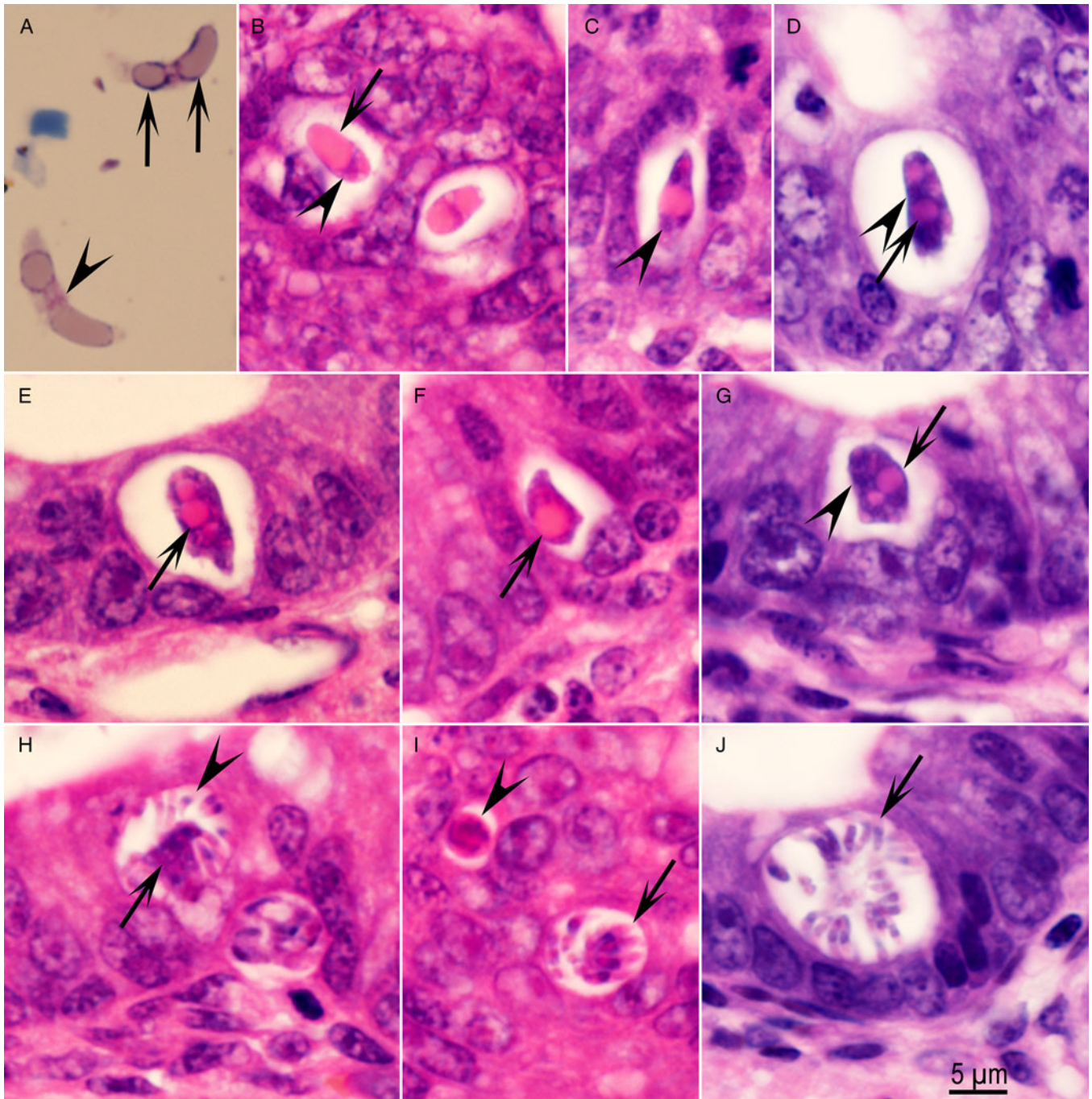


Fig. 1. *Eimeria maxima* sporozoites and the development of first-generation schizonts. (A) Sporozoites released from oocyst *in vitro*. Giemsa stain. (B–I) Stages in histological sections of glands of Lieberkühn of the jejunum of chickens orally inoculated with oocysts, haematoxylin and eosin stain. The luminal part of glands is oriented on the top. In B–G, the refractile body/bodies (RB) are eosinophilic (arrows), the nucleus and nuclei are basophilic and indicated by arrowheads. (A) Two sporozoites. Arrows point to RB. (B) Two sporozoites in separate parasitophorous vacuoles (PV). The nucleus in the sporozoite on the left is located terminally, whereas it is centrally located in the sporozoite on the right. (C) Elongated sporozoite-shaped immature schizont with nuclei around RB. (D–G) Different shaped immature schizonts. (H) Merozoites (arrowhead) arising from the central mass (arrow) of a schizont. (I) Two small schizonts (arrow, arrowhead) with small PV. (J) Mature schizont with radiating merozoites (arrow).

Third-, fourth- and fifth-generation schizonts

The number of generations could not be determined with certainty because of small-sized schizonts and hyperinfection. Therefore, schizogony is described separately at 72, 96 and 120 h.

Seventy two hours

Schizogony was seen in enterocytes, not only at the tips of the villi but also along the villus, up to the opening of glands, but not in

glands. In sections, schizonts were approximately 5 µm in diameter and contained up to 10 merozoites that were approximately 4 µm long, based on measurements in sections (Fig. 3A). Some merozoites were arranged around a central residual body (Fig. 2G).

Only a few schizonts were seen in smears of the jejunum. The immature schizonts were <5 µm in diameter and merozoites were forming even at the three-nucleated stage (Fig. 3B). Two sizes of merozoites were seen – the long merozoites were 7 × 1.5 µm, whereas small ones were 3.5 × 1 µm (Fig. 3C–E).

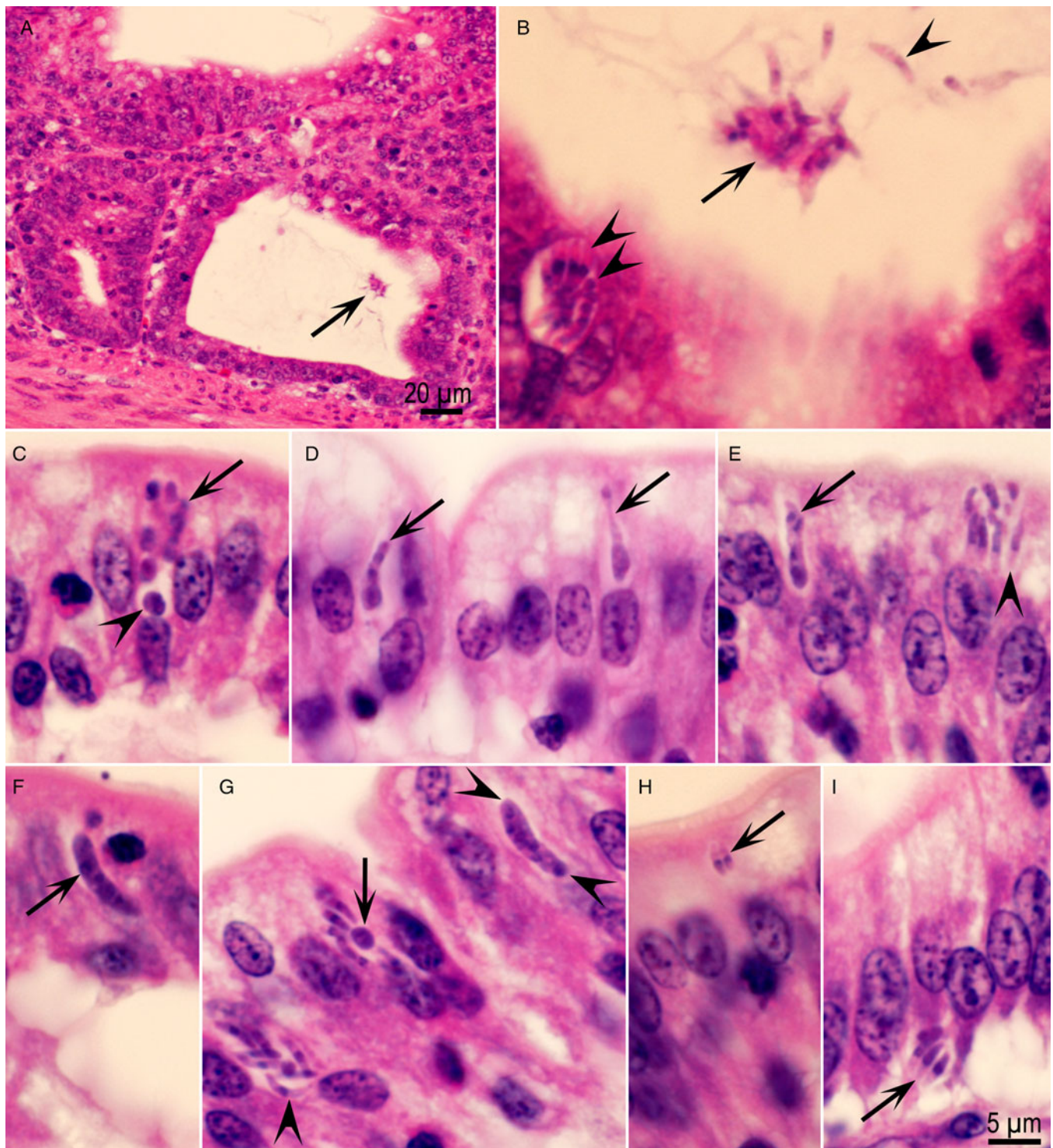


Fig. 2. *Eimeria maxima* schizonts in histologic sections of the jejunum of chickens. Haematoxylin and eosin. (C–I) Second generation of schizonts in the jejunum of a chicken 60 h p.i. Scale bar in B–I is 5 μ m. (A) A dilated gland with a ruptured schizont (arrow), 48 h p.i. (B) Higher magnification of Fig. 2A showing merozoites at the periphery of a central mass (arrow) and free merozoites (arrowhead) away from the main mass. Double arrowheads point to an intracellular schizont. (C) A group of immature schizonts (arrow). Arrowhead points to parasitized parasitophorous vacuole above the nucleus of enterocyte. (D) Two multinucleated amoeboid-shaped immature schizonts (arrows). (E) An elongated immature schizont (arrow) on the left and a mature schizont with merozoites (arrowhead) on the right. (F) A sausage-shaped multinucleated schizont (arrow). (G) Three schizonts in one field. The schizont on far right is immature and sausage-shaped (double arrowheads), the schizont in the middle is mature and has a central residual body (arrow) and the schizont on the far left is mature. Note centrally located nucleus in merozoites (arrowhead). (H) Two merozoites above the nucleus of the enterocyte. (I) Mature schizont (arrow) below the enterocyte nucleus.

Ninety-six hours

Profuse schizogony occurred at 96 h with at least two morphologically distinct types of schizonts (Fig. 4A and B). Most schizonts did not have a residual body and contained long merozoites. Some schizonts had a large residual body and short merozoites (Fig. 4B). Based on histological sections, schizonts were up to 10 μ m in diameter and the

merozoites were 5 μ m (long merozoites) and 2 μ m (short merozoites).

More details of parasite development were clear in smears (Fig. 5). Relatively few schizonts and many merozoites were seen in smears. Immature schizonts were round, oblong, elongated and contained up to 10 nuclei (Fig. 5A–I). Merozoite formation began as early as at the two- or three-nucleated stage (Fig. 5C and E). Two merozoites

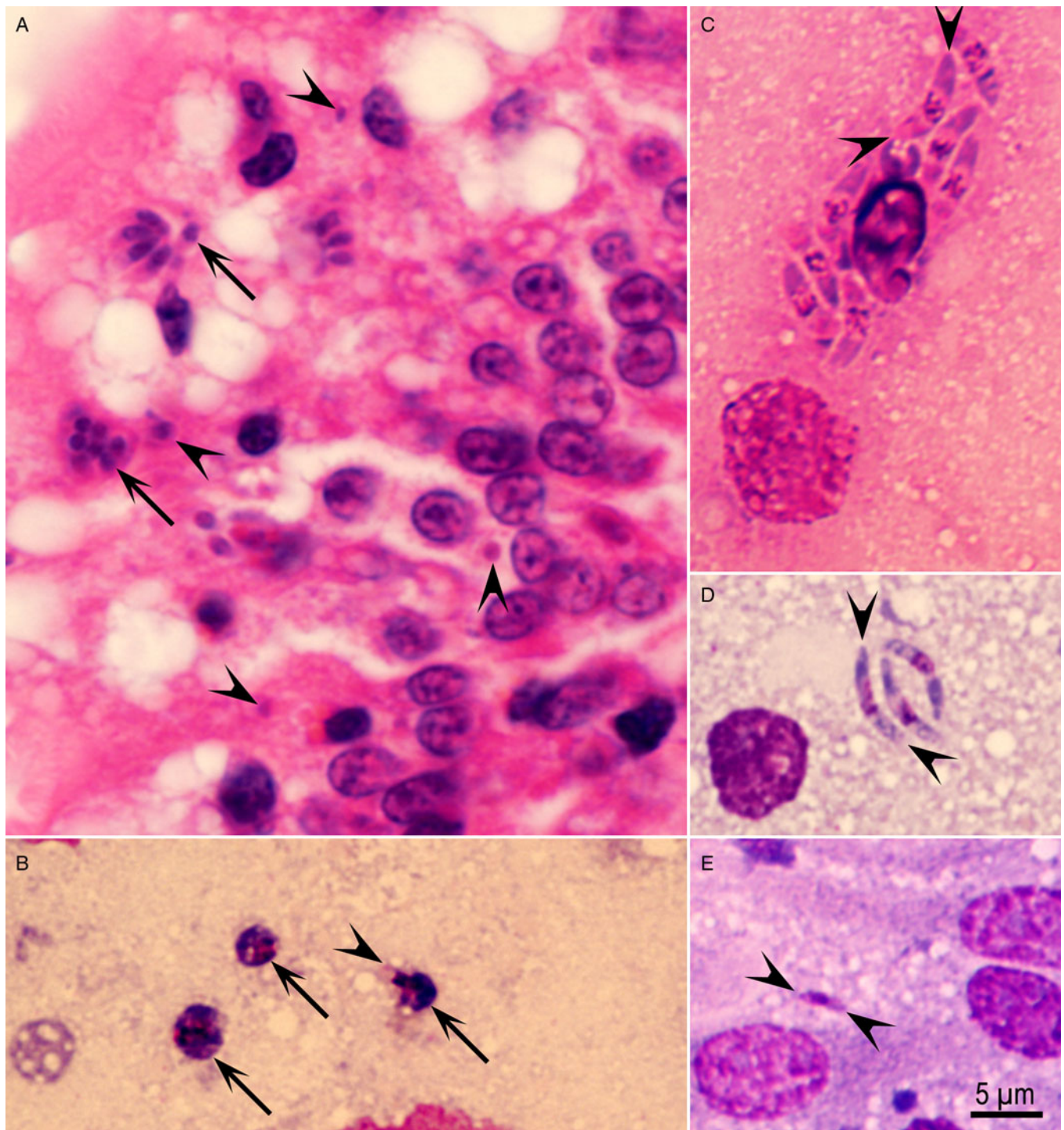


Fig. 3. Schizogony of *Eimeria maxima* in the jejunum of chicken, 72 h p.i. (A) Histological section showing mature schizonts (arrows) and single organisms (arrowheads) at the villar tips. Haematoxylin and eosin stain. (B-D) Smears of the jejunum stained with Giemsa. (B) Three immature schizonts (arrows) with two or three nuclei. Arrowhead points to a merozoite arising at the 2-3 nucleated stage. (C) Mature schizont containing eight banana-shaped 7.5 µm long (arrowheads) merozoites. (D) A group of three slender merozoites (arrowheads). (E) Small (arrowheads) 3 µm long merozoite.

were seen once within a PV (Fig. 5D). Schizonts with small merozoites (3–4 µm long) had a large residual body (Fig. 5J–L); some merozoites were seen in the residual body (Fig. 5K). Different sizes and shapes of merozoites seen are shown in Fig. 5M–P. Clearly, there were two distinct sizes of merozoites; small 3–4 µm vs. long 7.5–8.0 µm (Fig. 5P). These measurements are based on smears.

One hundred and twenty hours

At 120 h, both schizogony and gametogony were seen in histological sections of jejunum. Only a few schizonts were detected

in both the villar epithelium (Fig. 4C) and the lamina propria (Fig. 4D–E). Schizonts were small (5 µm in maximum diameter) and contained up to eight 4 × 1 µm merozoites; they were present both in the epithelium (Fig. 4C) and in the lamina propria (Fig. 4C–E). In histological sections, longitudinally cut merozoites were 4 × 1 µm. These merozoites could be distinguished from the earliest gamonts that were each located in a separate PV.

Male and female gamonts, and fully formed oocysts were seen in the subepithelial tissue, apparently in the lamina propria (Fig. 4D–G).

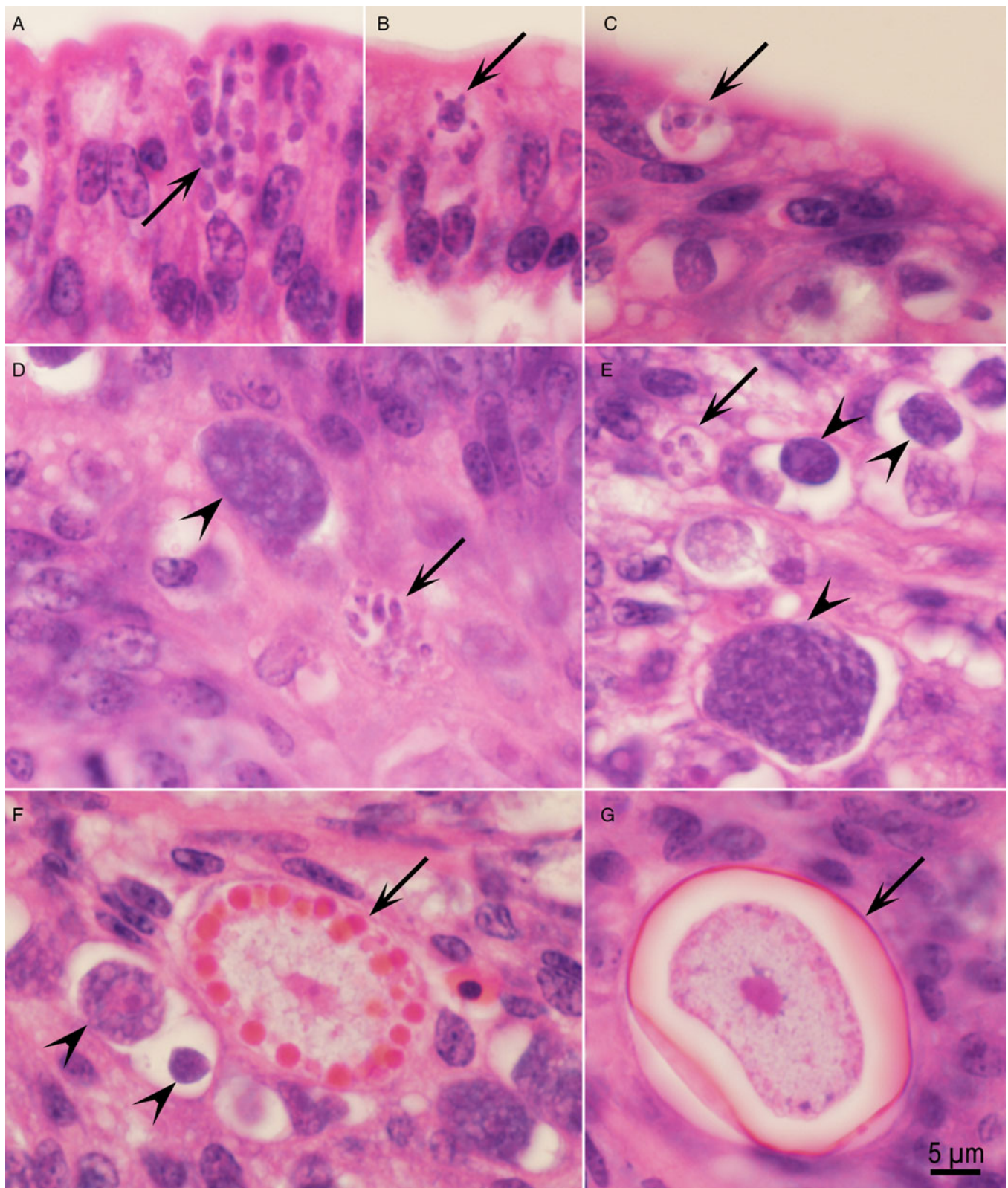


Fig. 4. Asexual and sexual development of *Eimeria maxima* in the jejunum of chickens. Haematoxylin and eosin stain. (A) Numerous schizonts (arrow) above enterocyte nucleus, 96 h p.i. (B) Arrow points to a schizont with small merozoites attached to a large residual body, 96 h p.i. (C) A schizont with four merozoites in the superficial epithelium at the villar tip, 120 h. (D, E) Schizont (arrows) in the lamina propria adjacent to a microgametocyte (arrowheads), 120 h p.i. (F) Mature (arrow) and immature macrogamonts (arrowheads) in the lamina propria, 120 h. (G) Unsporulated oocyst with a central nucleus and oocyst wall (arrow) partly peeled away from the parasitophorous vacuole.

Discussion

The present study provides details of the schizogony of *E. maxima*, such as the number of schizogonous stages and their location in the gut. In the present study, immature first- and second-generation

schizonts were sporozoite-/merozoite-shaped and contained multiple nuclei. In most *Eimeria* species, the asexual development is considered by a divisional process of schizogony where sporozoites round up after invasion of host cells (mostly enterocyte) leading to a uninucleated organism called the trophozoite (Levine, 1973).

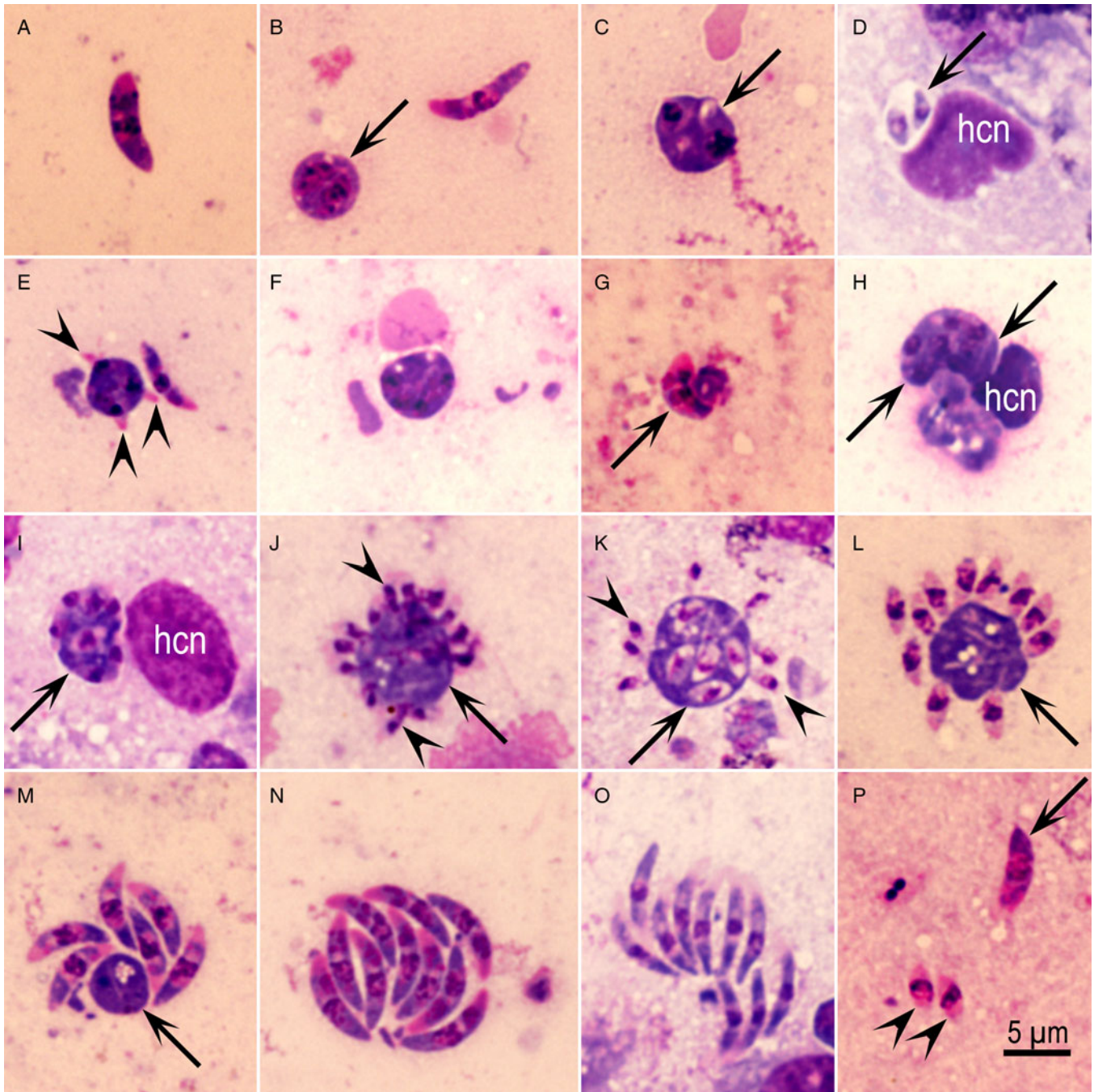


Fig. 5. Asexual *Eimeria maxima* stages in the jejunum of chicken, 96 h p.i. Giemsa stain. (A) Merozoite with two nuclei-like structures. (B) Binucleated small schizont (arrow) and an 8 μm long merozoite. (C) An organism (arrow) with two developing merozoites/nuclei. (D) Two merozoites within one parasitophorous vacuole, apparently binary/endodyogeny division. (E) A trinucleated schizont with developing merozoites (arrowheads). (F) Schizont with four nuclei. (G) Merozoite-shaped schizont with six nuclei. (H) Elongated multinucleated (arrowheads) schizont. (I) Schizont with seven or more nuclei (arrow). (J) Schizont with a large residual body (arrow) and small merozoites (arrowheads) forming at the periphery. (K) Small merozoites (arrowheads) arising from a central undivided mass (arrow) that has fully formed merozoites. (L) Small (3–5 μm) merozoites arranged around a large undivided mass (arrow). (M) Large (7.5 μm) six merozoites around a small residual body (arrow). (N) Group of 10 stout merozoites. (O) Group of 10 slender merozoites. (P) Two small (arrowheads) and a large merozoite (arrow) in one field.

Subsequent development occurs by schizogony, wherein the parasite nucleus divides into four or more nuclei (in some species, such as *Eimeria bovis* into more than 1000 nuclei) before merozoite formation occurs (Levine, 1973). In our study of *E. maxima*, we did not see the trophozoites after sporozoite invasion. Instead, the young schizont remained sporozoite-shaped and the nuclei were distributed throughout the schizont. Additionally, merozoite formation was not synchronous in that some merozoites were fully formed, whereas others were still forming. A similar process was observed for the second-generation schizonts where immature schizonts were sausage-shaped or elongated. Additionally, some

organisms at 96 h appeared to divide into merozoites at the 2–3-nucleated stage, not previously reported for eimerian species.

Based on the present study and review of the literature, there are more than three generations of schizonts in the life cycle of *E. maxima*. First-generation schizonts are distinctive because of their morphology and exclusive location in glands. In general, the first-generation schizonts here appear similar to those reported by Millard *et al.* (1972). However, Millard *et al.* (1972) did not provide details of the development. The differences in the gut region parasitized by *E. maxima* are apparent between our present study (in the jejunum) and that of Millard *et al.* (1972) in the duodenum,

probably due to several factors. For instance, Millard *et al.* (1972) examined only 1 cm of the duodenum, whereas we examined all regions of the small intestine. Millard *et al.* (1972) dosed 11 chickens with two million oocysts of the Houghton strain of *E. maxima* in two breeds of chickens. Two Brown leghorn chickens each were killed at 24, 48, 72 and 96 h p.i. and three Light Sussex chickens were killed at 63 h. Whether the dose, parasite strain or breed of chickens affected results has not been investigated. The size of first-generation schizonts in both studies is similar. Challey and Johnson (1968) in an oral presentation at a regional meeting first alluded to first-generation schizonts in the glands of chickens; details of this paper were never published.

With respect to the location, the first-generation schizonts of *E. maxima* resemble first-generation schizonts of *Eimeria acervulina* that is often found mixed with *E. maxima* in naturally infected chickens. *Eimeria acervulina* first-generation schizonts also occur in gland enterocytes of duodenum and are of the same size as *E. maxima* (Vetterling and Doran, 1966). However, unlike *E. acervulina*, *E. maxima* first-generation schizonts are located in large PV.

The second generation of *E. maxima* was completed between 48 and 60 h; these were absent at 48 h but had matured by 60 h. Unlike the first generation, the second generation occurred at the villar tips. Additionally, some second-generation schizonts were located below the host cell nucleus. Millard *et al.* (1972) also reported small immature schizonts at 63 h p.i. and mature schizonts at 72 h.

The number of generations beyond the first two could not be conclusively identified. The number of parasites seen at 60, 72 and 96 h suggests that schizonts at 72 h were the third generation and those at 96 h were the fourth generation. A possible fifth generation of schizonts was seen at 120 h. Unlike, the previous generations, the fifth generation schizonts were also located in subepithelium along with gamonts. The significance of these schizonts is unknown because by 120 h gamonts had formed.

In the present study, unsporulated oocysts were seen in histological sections at 120 h but not in feces. However, prepatent period for APU1 *E. maxima* is 138 h. Long (1959) mentioned a prepatent period of 121 h for the Houghton strain of *E. maxima*, and in chickens fed single oocyst, the prepatent period was 7 days. These differences may be related to the location of oocysts in the subepithelium and their slow release in to the lumen or the strain of the parasite.

Avian coccidiosis is an intestinal disease caused by protozoa in the genus *Eimeria*. Each year, the disease incurs over \$350 million loss in the USA alone, and over \$1 billion worldwide due to poor

weight gain in infected chickens and the cost of administering anticoccidial drugs and vaccines. The most costly parasite is *E. maxima* because it not only depresses weight gain and feed utilization, but also predisposes chickens to other diseases such as necrotic enteritis, a bacterial disease that causes high mortalities in young chicks. Contrary to previous studies conducted over the last century, our studies revealed that the parasite localizes to an area of the gut involved in nutrient absorption. Although there were no obvious histologic lesions, the extensive replication probably affects absorption. This information will be valuable to poultry and pharmaceutical companies that are considering control measures against avian coccidiosis caused by *E. maxima*.

Acknowledgements. We would like to thank Celia O'Brian, Carolyn Parker and Oliver Kwok for technical assistance. We are indebted to Dr Shiv Kumar Verma for assembling photographs.

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

References

- Challey JR and Johnson CA (1968) Penetration and development of *Eimeria maxima* sporozoites and the static effect of decoquinate. *Poultry Science* **47**, 1660.
- Fetterer RH and Barfield RC (2003) Characterization of a developmentally regulated oocyst protein from *Eimeria tenella*. *Journal of Parasitology* **89**, 553–564.
- Jenkins MC, Miska K and Klopp S (2006) Improved polymerase chain reaction technique for determining the species composition of *Eimeria* in poultry litter. *Avian Diseases* **50**, 632–635.
- Jenkins MC, Dubey JP, Miska K and Fetterer R (2017) Differences in fecundity of *Eimeria maxima* strains exhibiting different levels of pathogenicity in its avian host. *Veterinary Parasitology* **236**, 1–6.
- Levine ND (1973) *Protozoan Parasites of Domestic Animals and of Man*, 2nd edn., Minneapolis, Minnesota, USA: Burgess Publishing Company, 1–406.
- Long PL (1959) A study of *Eimeria maxima* Tyzzer, 1929, a coccidium of the fowl (*Gallus gallus*). *Annals of Tropical Medicine and Parasitology* **53**, 325–333.
- Millard BJ, Bradley JWA and Long PL (1972) The schizogony of *Eimeria maxima* in the chicken. *Zeitschrift für Parasitenkunde* **38**, 77–81.
- Scholtyssek E (1963) Vergleichende untersuchungen über die kernverhältnisse und das wachstum bei coccidiomorphen unter besonderer berücksichtigung von *Eimeria maxima*. *Zeitschrift für Parasitenkunde* **22**, 428–474.
- Tyzzer EE (1929) Coccidiosis in gallinaceous birds. *American Journal of Hygiene* **10**, 269–383.
- Vetterling JM and Doran D (1966) Schizogony and gametogony in the life cycle of the coccidium, *Eimeria acervulina* Tyzzer, 1929. *Journal of Parasitology* **52**, 1150–1157.