OBSERVATIONS ON THE LIFE CYCLE OF AN EIMERIA SP. OF CHICKENS IN PENINSULAR MALAYSIA 1

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INTRODUCTION
Coccidia can cause serious disease in many domestic animals and are of great importance in veterinary and medical parasitology. Several studies of Eimeria sp. of chickens have been made in Malaysia (Colley, 1971; Colley & Malling, 1972; Malling & Colley, 1971, 1972; Malling et al. 1972; Rajamanickam, 1971) however, little work has been done on the coccidia of chickens.

Coccidia are closely related to other important parasites in the subphylum Apicomplexa such as the malarial parasites, piroplasms, toxoplasms, and psittacosomes. Therefore, a detailed study of the life cycle of a member of the genus Eimeria provides an introduction to one of the most important groups of parasitic protozoa.

In this paper the life cycle of a species of Eimeria of chickens in Malaysia is studied. The purpose of this study is to learn details of the life cycle of an important parasitic protozoan, including its morphology, physiology, host-parasite intracellular relationships and pathogenicity.

MATERIALS AND METHODS
Oocysts of an unidentified Eimeria sp. from a naturally infected Malayman chicken were obtained from Dr. Mary Fernanado, Department of Parasitology, University of Malaya Medical Faculty, Kuala Lumpur.

Oocysts were observed by direct smear and also by Sheather's sugar flotation method (Levine, 1957).

The oocysts were placed in 2.5% sodium dichromate (K2Cr2O7) in 70 ml glass Petri dishes and allowed to sporulate at room temperature (30°C average). The oocysts were measured with an ocular micrometer and the size of the oocyst was determined by the McMaster counting chamber method (Hammoud & Long, 1973). Sporulated oocysts were centrifuged, washed in water and reconstituted.

The concentrated oocysts were administered to chickens per os using a Lml hypodermic syringe fitted with a flexible plastic tube. Approximately 1,000 oocysts were administered to 8 chickens (group A) and about 100,000 oocysts to 6 chickens (group B). Chickens were 6 weeks old at the time of infection. A daily collection of faeces from the infected chickens was made. Oocysts were detected by using the sugar flotation technique.
Sporulation of oocysts from the fresh feces was observed microscopically at room temperature (average 30°C) and photographs were taken.

A sample of 100 oocysts were observed at intervals of 10 – 12 hours and the number of oocysts at each stage of sporulation was recorded. Every 12 hours the chickens were observed for symptoms and signs of infection. The observation was continued for 24 days. Every 24 hours, for 6 days, an infected chicken from group A was killed and post-mortem studies were made.

Impression smears from the small intestine, mid-intestine, large intestine and cecum were fixed in Bouin's and Giemsa stain (Garraham, 1965). Tissues from adjacent areas was fixed in Bouin's fluid, processed through alcohol series, embedded in paraffin and sectioned on a rotary microtome. Slides were stained with iron hematoxylin and Heidenhain's hematoxylin (Oxleger & Knoll, 1971). Chickens in group B were killed on the 6th and 5th days following infection and the same procedure as above was followed.

Photographs of all stages were taken with a Leitz Ortholux microscope with automatic camera.

RESULTS

1. Exogenous Development

Sporulation. Unsporulated oocysts are ellipsoidal to ovoid with a smooth, uniformly thick wall composed of two layers (Figs. 1, 2). The unsporulated oocyst contains a single cell, the sporont (Fig. 2). In the first stage of sporogony cytoplasm of the sporont becomes condensed and spherical and is separated from the oocyst wall by a space. At this stage a polar granule forms (Fig. 3). Karyokinesis occurs rapidly, resulting in 8 nuclei (Fig. 4). The cytoplasm then contracts and divides (Fig. 5). Cytoplasmic division results in 6 undifferentiated sporoblasts (Fig. 6). Each sporoblast develops into a membrane bounded sporocyst containing 2 sporozoites.

Morphology of sporulated oocysts. Fifty sporulated oocysts measured 16.8 millimeters - 22.6 millimeters by 14.8 millimeters to 20.7 millimeters, range 20.6 millimeters by 17.9 millimeters. An oocyst polar granule is present. Oocyst residuum and micropyle are absent (Fig. 7). The sporocyst size was 9.8 millimeters to 12.8 millimeters by 4.9 millimeters to 6.9 millimeters, with a mean of 11.0 millimeters by 6.2 millimeters. A Stieda body is present. Each sporocyst contains two sporozoites and sporocyst residuum, and two transparent globules, one anterior and one posterior to the nucleus (Fig. 8). Some oocysts were completely sporulated after 34 hours, 95% were sporulated at 106 hours (Table 1).

A small number of oocysts were observed in the feces of chickens in group A 3 days after infection. By the 5th day many oocysts were being produced. After the 6th day fewer oocysts were detected; however, the infection persisted until day 24.
II. Endogenous development

Sporocysts
First generation schizogy. Oocysts and sporocysts break open in the intestine of the chicken. Sporozoites are liberated and enter host cells. Each sporozoite rounds up within the host cell and forms a trophozoite.

Trophozoites were observed 24 hours after inoculation. They are about 2-3 millimeters in diameter in Bouin's fixed tissue section. Each trophozoite has a single large nucleus and granular cytoplasm. Older trophozoites are surrounded by a clear area, the parasitophorous vacuole (Fig. 5).

First generation schizonts are found throughout the intestine; however, localization in the host tissue varies. In the small intestine schizonts are found in large numbers in the epithelial cells of the villi (Fig. 10) and crypts of Lieberkühn, and in lesser numbers in the lamina propria and muscularis mucosa (Fig. 11, 12). In the mid-intestine, large intestine and cecum, 1st generation schizonts are usually restricted to the crypts of Lieberkühn and the lamina propria.

First generation schizonts were seen at various stages of nuclear division. Schizonts with 2-14 nuclei were observed (average 10). Immature schizonts range from 3-5 millimeter in diameter in section. In young 1st generation schizonts the nuclei are not arranged in any particular order but are scattered irregularly throughout the cytoplasm.

After 68 hours mature 1st generation schizonts with meronts were observed. Mature schizonts range from 5-7 millimeter in diameter (average 6.0) (Fig. 13). In impression smears schizonts average about 8 millimeters in diameter (Fig. 14). Meronos average about 1.7 millimeter in length. The 1st generation meronts are banana-shaped, with one end slightly pointed, and the other broad and rounded.
The nucleus is more or less in the centre and occupies almost the entire width of the merosomite. The cytoplasm of the merosomite appears granular.

**Second generation schizonts.** Mature 1st generation merosomites leave the host cells and penetrate new host cells. Merosomites round up, form trophozoites and 2nd generation schizonts by division. Young 2nd generation schizonts were observed 48 hours after infection. The majority were found in the lamina propria of the villi, although some are also seen in epithelial cells of the villi (Fig. 17). Young 2nd generation schizonts seen in section have 2-4 nuclei. Mature 2nd generation schizonts are about 4-5 microns in diameter (average 4.3), with 6-11 nuclei. The nuclei are smaller than in the first generation schizonts.

Second generation merosomites are also banana-shaped with deeply curved nodules and with one end more pointed than the other, they average 1.7 mill microns in length. Host nuclei may be flattened due to the presence of the parasite within the cell (Fig. 16).

**Gametocytes.**

Approximately 72 hours after inoculation the second generation merosomites penetrate epithelial cells or cells in the lamina propria and round up to form gametocytes. At first it is difficult to distinguish microgamogonites from macrogamogonites (Fig. 18). However, in impression smears stained with Giemsa, it was observed that young microgamogonites generally have slightly large nuclei than macrogamogonites at the nucleate stage. Like the schizonts, microgamogonites and macrogamogonites are surrounded by a parasitophorous vacuole during the later stages of their development. Macrogamogonites are more numerous than microgamogonites.

**Macrogametocytes.** Young macrogamogonites are spherical or ellipsoidal. They are characterized by the presence of a large nucleus and numerous, dense wall forming nodules. Initially, wall forming bodies are scattered throughout the cytoplasm of the microgamogonite (Fig. 17). In old microgamogonites the wall forming bodies migrate to the periphery of the cell and coalesce to form the outer wall of the oozyt's. This process presumably occurs after the macrogamogonite is fertilized by a microgamogonite. At this stage the parasites lie within a large parasitophorous vacuole (Fig. 18). The shape of the host nucleus may be completely changed due to the presence of the parasites (Fig. 19).

The mature macrogamogonites are 5-5.5 mill microns in diameter in section, impression smears they average about 8 mill microns in diameter. Macrogamogonites and oozyt's are seen in greatest numbers in the lamina propria (Fig. 20).

**Microgametocytes.** Some second generation merosomites become microgamogonites. They are differentiated from microgamogonites by the presence of multiple nodules, which appear to develop in clumps or rows within the cytoplasm. This gives the impression of figures in the young microgamogonites (Fig. 11). The mature microgamogonite is packed with larger elongate nuclei. Flagella of the microgamogonites are sometimes faintly visible (Fig. 22). Mature microgamogonites are 3-7 mill microns in diameter in section. Mature oozyt's rupture the host cell and are released in to the lumen of the intestine. The entire life cycle is shown diagrammatically in Fig. 23.
not the entire egg granules, leave the host's trophozoites behind and become the thick, less motile cysts found in the liver cells of the host. In this state, the protozoa have 2-4 nuclei each in the first stage of multiplication.

III. Pathogenicity

In group A (infection with 1000 oocysts) all chickens looked healthy and their appetite was good throughout the course of the infection. On the fourth day, slight diarrhea was observed. On the fifth and sixth days there was diarrhea and the feces contained mucus, but chickens still appeared healthy and active with good appetite. No blood was observed in the feces. On the seventh day, there was only slight diarrhea and the chickens appeared to be completely healthy thereafter.

During post-mortem examination no signs of hemorrhage were observed; however, edema and mucus were seen in the lumen of the intestine especially on day 5 and 6.

DISCUSSION

Coccidiosis of domestic chickens is widespread in distribution and is a disease with serious economic consequences. However, not all species of Eimeria occurring in chickens are of equal pathogenicity. Therefore, the first step in diagnosis of coccidiosis is species identification. Both morphological and biological characteristics are used to separate the species of coccidia. There are differences in the morphological characters of both the endogenous and exogenous stages of the life cycle. The structure of the oocysts is commonly used to identify the species (Levine & Evans, 1965).

Several morphological characters are used to differentiate oocysts. Not all species have the same characters and their presence or absence helps to differentiate the species.

Oocysts vary in shape and size. The oocyst wall is made up of one or two, or rarely three layers. There may be a micropyle at one end of the oocyst in some species. An oocyst residuum and polar granule may be present. In addition, a refractile polar granule and an oocyst residuum may be present. The oocyst residuum may be compact or may consist of a few, or many, scattered granules.

The sporocysts also vary in shape and size and may have a nipple-like projection at one end, the stieda body. A sporocyst residuum may also be present as a compact body or consisting of few to many granules (Levine & Evans, 1965).

The sporozites are found within the sporocysts. Sporozites are usually elongated, with one end broader than the other. The nucleus is either central or near one end. There may be one, or two refractile globules in each sporozoite. Levine (1962) estimates that the size of these characters at least 2,454,756 morphologically different oocysts are possible in the genus Eimeria.

In Table 2 the oocyst morphology of all known species of Eimeria from domestic chickens is shown and compared with the Eimeria sp. described in this paper.

From these data it may be seen that the oocyst of the Malaysian Eimeria sp. differs morphologically from the oocysts of all other Eimeria spp. shown in Table 2.
<table>
<thead>
<tr>
<th></th>
<th>E. tenella</th>
<th>E. necatrix</th>
<th>E. brunat</th>
<th>E. acervulina</th>
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<td>common</td>
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<td><strong>Oocyte Morphology</strong></td>
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<td>ovoid, smooth, ovoid</td>
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<td>absent</td>
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<td></td>
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<td>elongated, ovoid</td>
<td>ovoid</td>
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<td>present</td>
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<td>noid-wade</td>
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<td>-----------</td>
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<td>E. maxima</td>
<td>E. solt</td>
<td>E. praecen</td>
<td>E. augeni</td>
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<td>common</td>
<td>rare</td>
<td>prevalence unknown</td>
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<td>ovoid, smooth</td>
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<td>16 by 18</td>
<td>21 by 17</td>
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<tr>
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<td>elongated, ovoid</td>
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<td>elongated, ovoid</td>
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<tr>
<td>19 by 9</td>
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<td>11 by 1</td>
<td>11 by 9</td>
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<td>absent</td>
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<td>present</td>
</tr>
<tr>
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<td>E. benetii</td>
<td>E. ascriculae</td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Prepatent period</td>
<td>7 days</td>
<td>7 days</td>
<td>5 days</td>
<td>4 days</td>
</tr>
<tr>
<td>Patent period</td>
<td></td>
<td></td>
<td>12 days</td>
<td></td>
</tr>
<tr>
<td>Sporulation time</td>
<td>1-2 days</td>
<td>1-2 days</td>
<td>1-3 days</td>
<td>1 days</td>
</tr>
</tbody>
</table>

**Sickening**

<table>
<thead>
<tr>
<th>Generation</th>
<th>E. coli</th>
<th>E. mastii</th>
<th>E. benetii</th>
<th>E. ascriculae</th>
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</thead>
<tbody>
<tr>
<td>1st generation</td>
<td>above the host cell in cecum</td>
<td>small intestine above the host cell</td>
<td>small intestine above the host cell</td>
<td>superficially in anterior small intestine</td>
</tr>
<tr>
<td>2nd generation</td>
<td>above the host cell in cecum</td>
<td>-</td>
<td>-</td>
<td>posterior small intestine, rectum, cecum, below the host cell</td>
</tr>
<tr>
<td>3rd generation</td>
<td>cecum, below the host cell</td>
<td>-</td>
<td>-</td>
<td>small intestine</td>
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</table>

**Carcinogenicity**

- Macroganons: below the cell in cecum
- Microganons: below the host cell in cecum

**Pathogenicity**

<table>
<thead>
<tr>
<th>E. coli</th>
<th>E. mastii</th>
<th>E. benetii</th>
<th>E. ascriculae</th>
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</thead>
<tbody>
<tr>
<td>causes hemorrhagic inflammation, lesions in cecum</td>
<td>chronic type small intestine swollen, filled with clotted and indistinct blood</td>
<td>hemorrhagic inflammation in rectum and colon</td>
<td>causes mostly in the small intestine</td>
</tr>
<tr>
<td></td>
<td>E. maima</td>
<td>E. mitis</td>
<td>E. pruvae</td>
</tr>
<tr>
<td>-----</td>
<td>----------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>4 days</td>
<td>4-6 days</td>
<td>4-5 days</td>
<td>4 days</td>
</tr>
<tr>
<td>Few days</td>
<td>10 days</td>
<td>4 days</td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>1 day</td>
<td>2 days</td>
<td>2 days</td>
</tr>
</tbody>
</table>

**Superficially in anterior small intestine**
- About the host cell in small intestine
- Epithelium cells of the villi, below the host cell
- Mostly in crypts of Lieberkühn, above host nuclei, throughout intestine
- Mostly in lamina propria, below host nuclei, throughout intestine

**Small intestine**
- Branches the host cell in small intestine
- Branches the host cell in small intestine

**Lesion mostly in the small intestine**
- Lesions mostly no distress, non-pathogenic
- Pinched hematomas in small intestine and also cataracts inflammation
- Swelled oedema throughout intestine and oedema, no hematomas
Only two species of Eimeria have oocysts morphologically similar to the Malaysian species; these are *E. maxima* and *E. brunetti*. *E. maxima* differs from the Malaysian species in being smaller and in having a spongy cyst residuum. *E. brunetti* is similar morphologically but according to Pellethead (1965) it has a single oocyst wall. Levine (1967) is not clear on this point.

In addition to oocyst morphology a second group of criteria used in differentiating coccidian species is the location of the endogenous stages in the host. All species of *Eimeria* in chickens are found in the intestine; however, within the intestine different species are found in different regions.

The location of parasites within the host tissue or cell may differ with different species even in the same region of the intestine. Some may be found in the epithelial cells at the tip of the villi, others only in the crypts of Lieberkühn, and others in the submucosa and muscularis mucosae. Within the host cell some species may reside above the host cell nuclei, others beneath them (Levine, 1967). In addition, prepatent and patent periods and sporulation time of oocysts may vary between different species. The endogenous stages of *Eimeria* sp. of domestic chickens are compared with Malaysian *Eimeria* sp. in Table 2.

In Table 3 it may be seen that the Malaysian *Eimeria* sp. differs from *E. brunetti* in several respects. First generation schizonts develop in the crypts of Lieberkühn rather than in the epithelial cells of the villi and second generation schizonts develop in the lamina propria rather than in the tips and sides of the villi. Location of schizonts also helps distinguish it from *E. maxima* and the other species shown in Table 3.

A third method of differentiating coccidian species is pathogenicity. While many species of coccidia are pathogenic many others are not. Pathogenicity depends on a number of factors, for example, the number of host cells destroyed per infecting oocyst and the location of the parasites in the host tissues and within the host cell. The size of the infecting dose or doses, the degree of acquired or natural immunity of the host are also important.

If disease is present, the signs are diarrheal in nature. There may or may not be blood in the feces, depending on the parasite species and the severity of the infection (Levine & Ivins, 1965).

In chickens two main types of pathology are associated with the hemorrhagic and nonhemorrhagic forms of coccidiosis. With *E. maxima* and *E. stercoralis*, severe hemorrhage is associated with the development of large 2nd generation schizonts which occur in crypts in intersporulated tissue. Although some blood loss can be observed in severe infections with other species, it is relatively small compared with that caused by *E. maxima* or *E. stercoralis*. With the other species in the flock, the damage to the intestine is caused by excessive gametocytes and schizonts parasitizing the villi (Hammond & Lloyd, 1975) (Table 3). Perhaps the reason that the Malaysian *Eimeria* sp. is relatively non-pathogenic is because its endogenous stages are spread throughout the intestine. In addition, its schizonts and gametocytes are of moderate size.

Although the time necessary for sporulation of oocysts is given in Table 3, this is not a reliable criterion for differentiating coccidian species. The reason
for this it that sporulation time varies markedly with temperature. If this
cracter is to be used taxonomically temperature of oocysts must be strictly
regular.

The prepatent and patent periods may also vary in length depending
on the age of the host, general condition and previous infection. Therefore,
this information should also be carefully standardized before it is used for
diagnosis of species.

In this study the important stages in the life cycle of an unidentified
Eimeria sp. have been observed: sporulation, oocyst morphology, endogenous
development, and pathogenicity. On the basis of the evidence gathered it
seems likely that this parasite is an Eimeria sp. of chickens that has not been
described previously. However, further, more detailed studies will be necessary
to confirm this.

SUMMARY

The life cycle of an unidentified Eimeria sp. from domestic chickens in
Malaysia was studied. Sporulation of oocysts was completed in 34 hours at
room temperature (30°C). Oocysts are ellipsoidal to oval averaging 21 by 18
millimicrons with a yellowish double wall. There is a polar granule. Sporocysts
are elongated and oval averaging 11 by 5 millimicrons in size with a Stieda
body and containing a sporocyst residuum.

First generation schizonts develop throughout the intestine, mainly in the
crypt of Lieberkühn but also in the muscularis mucosae, lamina propria and
epithelial cells above the host nucleus. About 14 merozoites are produced.

Second generation also develop throughout the intestine, mainly in the
lamina propria, below the host nucleus. About 12 merozoites are produced.

Gametocytes are numerous in the mid-intestine, large intestine and
cecum; and are mainly in the lamina propria.

Pathogenicity was mild. Diarrhoea and m racecd edemas were the only
clinical signs observed and the infection was self-limiting, ending after about
20 days.

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I am also grateful to Miss E. Tawasachotin for providing technical assistance and
to Miss Sui Zabadih for typing the manuscript.

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——, R. Mullin, S.W. 1971a Isopora hylopetia and Eimeria bantengi n.sp. (Protozoa:
Eimeridae) from the lesser gibbon Hylonomys zululius Muller, 1839 in Malaya. SEA J.

——, 1971b Caecodorus parasiticus (Protozoa: Eimeridae) of the lesser mouse deer Tragulus


Fig 1. Fresh unsporulated oocysts of *Eimeria* sp. × 450.

Fig 2. Unsporulated oocysts *Eimeria* sp. containing sporont (sp). × 1,100.
Fig. 3. - Earliest stage in sporulation of oocyst. Note rounded sporont (sp) and polar granule (pg). \( \times 1,000 \).

Fig. 4. - Sporulation process at time of nuclear division. Nuclei are indicated by arrows. \( \times 1,000 \).
Fig. 5.— Sporulation process at time of cytoplasmic division. × 1,100.

Fig. 6.— Oocyst with 4 undifferentiated sporoblasts (sb). × 1,100.
Fig. 7.—Sporulated oocysts showing polar granule (pg), sporocysts (sc) and outer and inner walls of the oocyst (ow), (iw). × 1,100.

Fig. 8.—Sporocyst. Note the Stieda body (s), sporozoites (sz) and sporocyst residuum (r). × 1,100.
Fig. 9.—Section of large intestine in the region of the crypt of Lieberkuhn. Note a young trophozoite (t) lying within a parasitophorous vacuole (pv) in the host cell (h). Bouin’s fixed, Heidenhain’s hematoxylin stained. × 1,100.

Fig. 10.—First generation schizont (s—l) in epithelial cells of the small intestine. Note that the parasites lie above the host nucleus (hn). A parasitophorous vacuole (pv) may be seen surrounding one schizont. Bouin’s fixed, Heidenhain’s hematoxylin stained. × 1,100.
Fig. 11.-- First generation schizonts (s-1) lying in parasitophorous vacuole (pv) in lamina propria of the large intestine. Bouin's fixed, Heidenhain's hematoxylin stained. × 1,100.

Fig. 12.-- First generation schizonts (srows) in crypts of Lieberkuhn (cl) and mucosa mucosa (mm) of midintestine. Bouin's fixed, Heidenhain's hematoxylin stained. × 450.
Fig. 13.—Mature 1st generation schizont in crypt. Note merozoites (m) and parasitophorous vacuole (pv). Bouin’s fixed, Heidenhain’s hematoxylin stained. $\times 1,100$.

Fig. 14.—Impression smear of 1st generation schizont. Bouin’s fixed, Giemsa stained. $\times 1,100$. 

m = merozoite, pv = parasitophorous vacuole, Heidenhain’s hematoxylin.
Fig. 15.—Second generation schizonts (arrows) in lamina propria of large intestine. Bouin’s fixed, Heidenhain’s hematoxylin stained. × 1,100.

Fig. 16.—Second generation schizonts (arrow) in lamina propria of large intestine. Note flattened host nucleus (hm) in infected cell. Bouin’s fixed, Heidenhain’s hematoxylin stained. × 1,100.
Fig. 17. Young macrogamonts (ma) in epithelial cells of large intestine. Note wall forming bodies (wb) and nucleus (n) of macrogamont. Bouin's fixed, Heidenhain's hematoxylin stained. × 1,100.

Fig. 18. Older macrogamont (ma) surrounded by parasitophorous vacuole (pv) in epithelial cell of large intestine. Note coalescence of wall forming bodies to form outer wall (ow). Bouin's fixed, Heidenhain's hematoxylin stained. × 1,100.
Fig. 19.— Impression smear showing macrogamont. Note reaction of host cell nucleus (hn) to parasitic invasion. Bouin’s fixed, Giemsa stained. × 1,100.

Fig. 20.— Macrogamonts (mg) and oocytes (o) in lamina propria of large intestine. Bouin’s fixed, Heidenhain’s hematoxylin stained. × 1,100.
Fig. 21.—Young microgamont (m) in epithelial cell of large intestine, showing aggregations of nuclei. Bouin's fixed, Heidenhain's hematoxylin stained. × 1,100.

Fig. 22.—Mature microgamont containing numerous microgametes, in lamina propria of large intestine. Bouin's fixed, Heidenhain's hematoxylin stained. × 1,100.
Fig. 23.—Life cycle of *Eimeria* sp. from the chicken.

1–3 Exogenous stages
1. Unsporulated oocyst
2. Sporulation
3. Sporulated oocyst

4–20 Endogenous stages
4. Excretion of oocyst and sporocytes
5. Free sporozoites penetrate host cell and forms trophozoite
6. Trophozoite development in intestine,
   a) epithelial cells,  
   b) lamina propria, 
   c) muscularis mucosae, 
   d) crypt of Lieberkühn
7. First generation schizont in host cell
8. Free 1st generation merozoite
9. Trophozoite development, primarily in lamina propria
10. Second generation schizont in host cell
11. Free 2nd generation merozoites
12. Young microgamont
13. Older microgamont
14. Mature microgamont
15. Free microgamete
16. Young macrogamont
17. Older macrogamont with wall forming bodies
18. Older macrogamont at time of fertilization by microgamete
19. Development of oocyst wall
20. Oocyst with sporont