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The Life Cycle and Pathogenicity of *Eimeria fulva* Farr, 1953 in Domestic Goslings

Yabin DAI[1, 2], Mei LIU[2], Nongyue HE[1], Jianping TAO[3] and Xingyou LIU[4]

[1]State Key Laboratory of Bioelectronics, Southeast University, Nanjing, Jiangsu; [2]Poultry Institute, Chinese Academy of Agricultural Sciences, Yangzhou, Jiangsu; [3]College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu; [4]Henan Institute of Science and Technology, Xinxiang, Henan, China

Summary. The life cycle and pathogenicity of *Eimeria fulva* were studied. Eighteen, 10-day-old, artificially-reared coccidia-free goslings (*Anser cygnoides* var. *domestica*) were inoculated orally with 7.0×10^5 -1.5×10^6 sporulated oocysts of *Eimeria fulva* and killed at intervals from 24 to 288 h post-inoculation (PI). Parts of the visceral organs including intestines, kidney, liver, gallbladder, and spleen from inoculated goslings were fixed, sectioned, and observed microscopically. The results revealed that at least 2 generations of meronts occurred in the life cycle of *E. fulva*. The first generation completed maturation at about 72 h PI, and the second at about 144 h PI. Each meront contained about 15 merozoites. Development of gamonts began at about 144 h PI. The prepatent period was 7.5 days and patency continued for 2.5 days. Sporulation of oocysts occurred in 60-84 h at 25°C. Developmental stages were present in the epithelial cells of the villi and crypts, and in the lamina propria of the whole intestine and cloaca, resulting in local desquamation and necrosis of the epithelium (including crypt epithelium), edema, hemorrhages, and infiltration of inflammatory cells. Histological lesions were pronounced in the jejunum and the ileum. The infected goslings mainly showed diarrhoea, slight bloody feces, but no death. The results suggest that *E. fulva* may be mildly pathogenic for goslings.

Key words: coccidium, *Eimeria fulva*, gosling, life cycle, pathogenicity.

INTRODUCTION

Coccidiosis is an important protozoan disease of domestic animals. The disease is manifested by severe symptoms such as diarrhea, dehydration, and deaths in young animals (Levine 1985). Although coccidia are among the most common parasites of geese worldwide, coccidiosis in geese is not generally known as a severe problem (Soulsby 1982, Gajadhar *et al*. 1983). Nevertheless, there are numerous case reports where extensive morbidity and mortality have occurred in both domestic and wild flocks in some areas, especially in Europe, where domestic geese are of great economic importance (Gajadhar *et al*. 1983). In China, goose coccidiosis has been paid little attention for a long time because of its little economic significance, and relevant reports are few. With changes in agricultural practices in recent years, however, geese are now raised on a larger scale. As a result, coccidiosis has occurred in many flocks in many areas, resulting in considerable losses (Dai *et al*. 2004).

Address for correspondence: Yabin Dai, Poultry Institute, Chinese Academy of Agricultural Sciences, 46 Sangyuan Road, Yangzhou, Jiangsu 225003, China; Fax: 86(514)7209132; E-mail: ybdai@126.com

Since the first coccidium *E. truncatum* (then named *Coccidium truncatum*) was observed by Railliet and Lucet in 1890 in the domestic goose (*Anser anser domesticus*) in France, 17 species of the family Eimeriidae, belonging to 3 genera have been described and named from a variety of goose hosts in many areas of the world, primarily identified on the basis of oocyst morphology though the validity of some remains in doubt (Railliet and Lucet 1890, Gajadhar *et al*. 1983). Most species, except *E. truncata*, which is known to occur in kidney, are found in the intestines of geese (Gajadhar *et al*. 1982, 1983). Experimental studies, particularly those with single species infections, have rarely been reported, and the life cycles and pathogenicity of many species are yet to be resolved.

Eimeria fulva Farr, 1953 was first reported from the eastern Canada goose (*Branta canadensis canadensis*) and experimentally transmitted to the domestic goose (Farr 1953). Later, it was found in the lesser snow goose (*A. caerulescens caerulescens*), Aleutian Canada geese (*B. canadensis leucopareia*), and the domestic geese (*A. a. domesticus* in Europe and *A. cygnoides* var. *domestica* in China) (Hanson *et al*. 1957, Greiner *et al*. 1981, Arslan *et al*. 2002, Xie *et al*. 1988, Tao *et al*. 2003, Dai *et al*. 2004). The domestic goose was believed to be the experimental host of *E. fulva*, however, it was found widely in domestic geese with relatively high prevalence (Arslan *et al*. 2002, Tao *et al*. 2003, Dai *et al*. 2004). It appears that the host specificity of *E. fulva* (as well as *E. hermani*) is not so strict as researchers expect (Hanson *et al*. 1957, Gajadhar *et al*. 1983), and that the domestic goose may be the natural host of *E. fulva* (Dai *et al*. 2004). Gajadhar *et al*. (1983) reviewed the life cycle and pathogenicity of *E. fulva*; however, detailed information on them is inadequate until the present study.

MATERIALS AND METHODS

Oocysts. The pure cultures of *E. fulva* were produced in our previous experiment (Dai *et al*. 2004). Briefly, 160 sporulated oocysts of *E. fulva* were picked out under the microscope from a sample obtained in an experimental infection in which this species predominated. Eighty oocysts each were inoculated into 2, coccidia-free, 10-day-old goslings. The feces from both birds were collected during the period of oocyst release (9-10 days post-inoculation [PI]). Oocysts in feces were harvested by screening, sedimentation, flotation in saturated salt solution, and washings. They were then placed in 2% potassium dichromate and incubated at 25°C for 4 days to permit oocysts to sporulate. Sporulated oocysts were examined repeatedly under light microscopy to ensure its purity. Two other coccidia-free goslings were each inoculated with 5000 sporulated oocysts obtained from the previously inoculated birds to produce adequate numbers of oocysts for the present study. Sporulated oocysts were stored at 4°C for about 2 months prior to use.

Experimental birds. Domestic geese (*A. cygnoides* var. *domestica*) were incubator hatched and obtained when 1-day-old. All birds were reared individually in wire cages in isolation rooms and provided with a non-medicated ration and water *ad libutum*. Fecal samples from those birds were collected daily and examined for the presence of oocysts by fecal flotation technique before inoculations were performed. Only coccidia-free birds were used in this study. At 10 days of age, 18 goslings were orally inoculated with 7.0×10^5-1.5×10^6 sporulated oocysts of *E. fulva* and killed with an overdose of pentobarbitone at 24 to 288 h PI (Table 1). Considering the parasites in tissues of infected birds might be few and difficult to find at the early stage of infection, those goslings killed early were inoculated with the higher doses (1.2×10^6-1.5×10^6 oocysts). During the experiments, the clinical signs and gross lesions of infected goslings were observed and recorded in detail.

Collection and preparation of tissues. Immediately after the goslings were killed, intestine, kidney, liver, gallbladder, and spleen were collected and fixed in 10% phosphate buffered formalin (pH7.2). For the intestine, beginning at the pylorus, 3 samples from the duodenum, 4-5 at 15 cm intervals from the jejunum, 2 from the ileum, 3 from the caecum, 2 from the rectum, and 1 from the cloaca were taken. Tissues were embedded in paraffin wax and later sectioned at 4 ìm, then stained with hematoxylin and eosin (H. E). Digital photographs were taken by Cell Image System (CIS-1000, Shanghai Tanon Science & Technology Co., Ltd., Shanghai, China).

Examination and sporulation of oocysts. To determine the prepatent and patent periods, feces from some infected goslings were collected twice daily and examined for the presence of oocysts by flotation using saturated salt solution. When oocysts were present, they were harvested by preparing a slurry of feces and water that was passed through a 400 µm nylon sieve and washed with water twice after centrifugation. Subsequently, oocysts were placed in 2% potassium dichromate in covered Petri dishes at 25°C, and observed 4 times daily until most had completed sporulation.

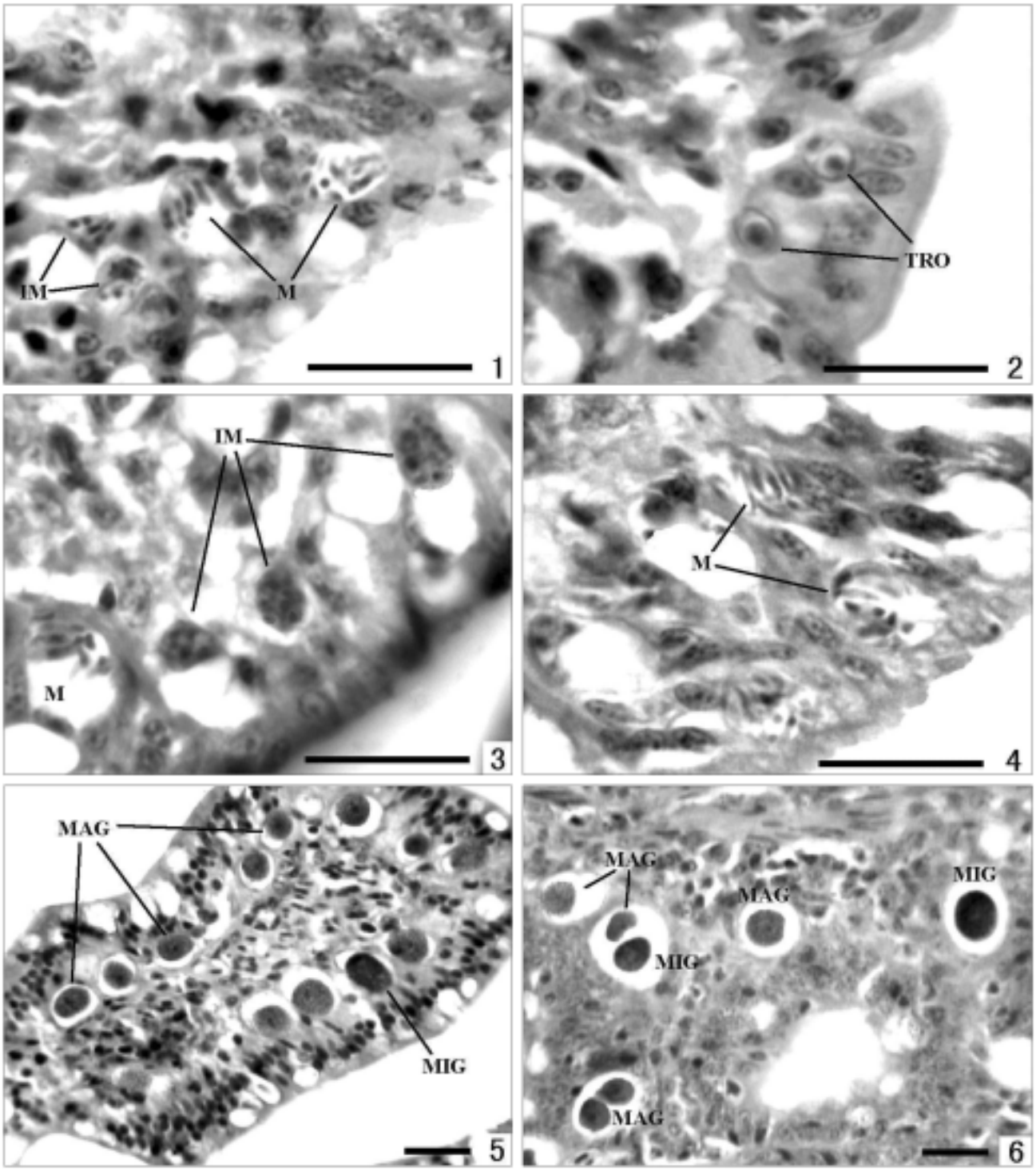
RESULTS

Clinical signs

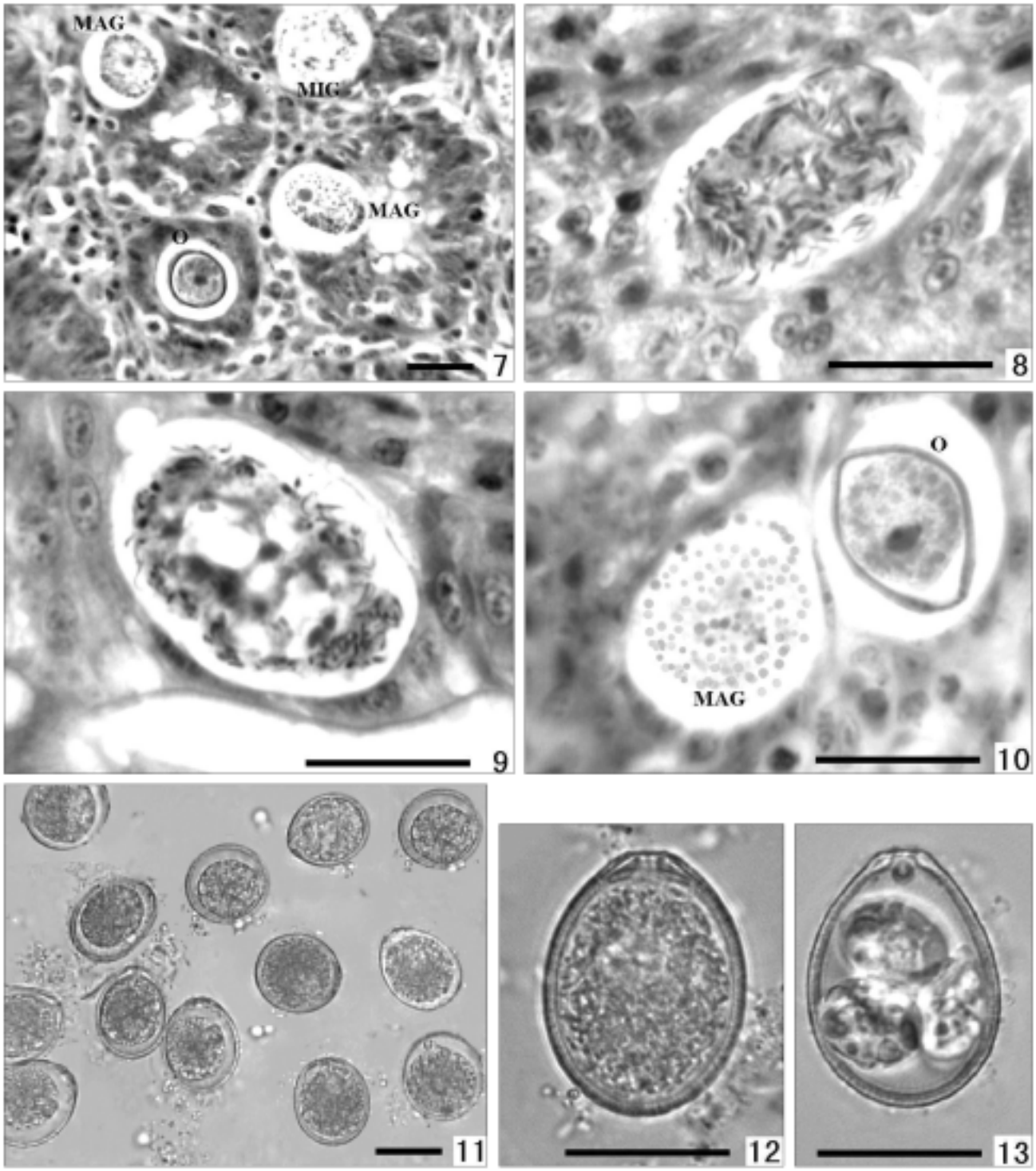
All unkilled birds began to show diarrhea at day 4 PI, and the diarrhea continued through day 9 PI. During this period, the feces generally contained a small amount of necrotic debris, mucus, and blood, and the infected birds remained normal in appetite and vigor. Microscopically, a large number of lipid-like drops were found in the feces. Beginning at day 10 PI, the feces gradually returned to normal.

Life cycle

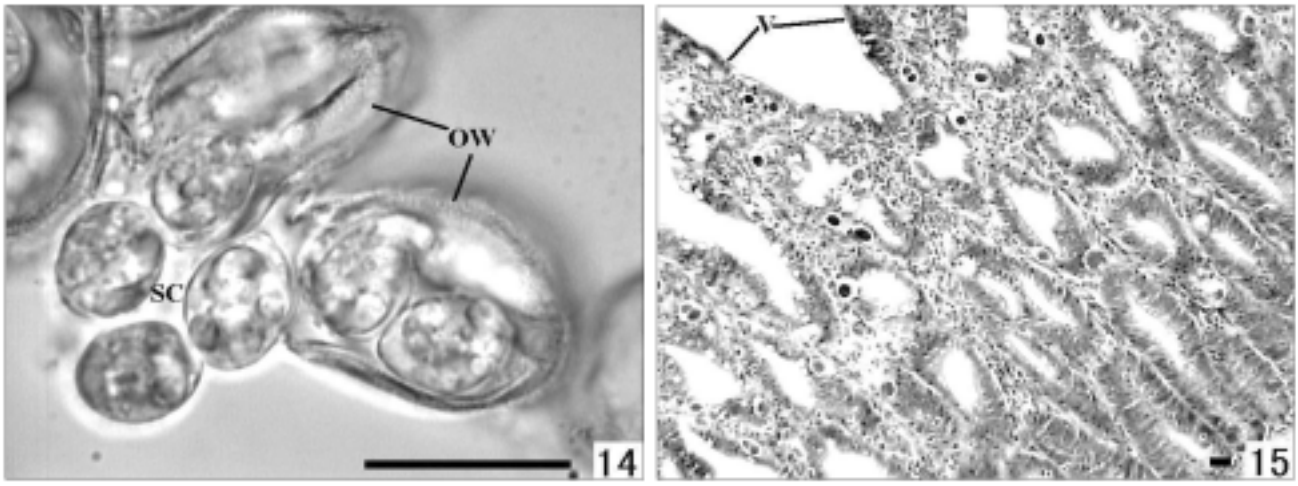
The various stages in the life cycle of *E. fulva* found in infected goslings were showed in Table 1 and fully detailed as follows.



Figs 1-6. *Eimeria fulva*. **1** - immature and mature meronts in the epithelial cells of a villus of the jejunum at 72 h post-inoculation (PI); **2** - trophozoites in the epithelial cells of a villus of the ileum at 72 h PI; **3** - immature and mature meronts in the epithelial cells of a villus of the jejunum at 96 h PI; **4** - mature meronts in the epithelial cells of a villus and in the lamina propria of the ileum at 120 h PI; **5** - young macrogamonts and microgamonts in the epithelial cells of a villus of the ileum at 168 h PI; **6** - young macrogamonts and microgamonts in the crypts and the lamina propria of the jejunum at 192 h PI. IM - immature meront, M - mature meront, MAG - macrogamont, MIG - microgamont, TRO - trophozoite. Scale bars 20 µm.



Figs 7-13. *Eimeria fulva.* **7** - mature macrogamonts, microgamont, and oocyst in the crypts and the lamina propria of the rectum at 216 h post-inoculation (PI); **8** - mature microgamont at 216 h PI with numerous fully developed microgametes; **9** - mature microgamont at 216 h PI with microgametes migrated to its periphery; **10** - mature macrogamont and oocyst in the lamina propria of the caecum at 216 PI; **11** - fresh oocysts isolated from the feces at 204 h PI; **12** - an amplified unsporulated oocyst; **13** - sporulated oocyst. MAG - macrogamont, MIG - microgamont. O - oocyst. Scale bars 20 µm.



Figs 14-15. 14 - *Eimeria fulva* crushed sporulated oocysts. Pitted oocyst walls and sporocytes are showed; **15** - gamonts at various stages in the epithelial cells of the villi and crypts, and in the lamina propria of the ileum at 168 h post-inoculation. Note that no endogenous stages occur in the crypts in the deeper region of mucosa. OW - oocyst wall, SC - sporocyte, V - villus. Scale bars 20 µm.

Meronts and merozoites. Meronts mainly occurred in epithelial cells of the villi and lamina propria of the jejunum, ileum, basis ceci, and rectum, occasionally in the lower duodenum. At 24 h PI, some sporozoites were found in the epithelial cells of villi of the jejunum and ileum. Thereafter, the meronts of different developmental stages were observed at 48-144 h PI (Table 1). Trophozoites, and meronts containing 2-4 nuclei, were found in the epithelial cells of villi and lamina propria at 48 h PI, apparently arising from the sporozoites. Immature and mature meronts, as well as trophozoites, were present at 72 h PI (Figs 1, 2), and both immature and mature meronts at 96 and 120 h (Figs 3, 4). At 144 h PI, only few mature meronts were present; most were trophozoites.

Immature meronts contained 2 to 20 or more nuclei, depending on their developmental degree (Figs 1, 3). Mature meronts were approximately 10 µm in diameter and contained variable numbers of fully developed merozoites (Figs 1, 3, 4). In histological sections, no obvious differences were found among meronts of various generations. Mature meronts contained 8-28 merozoites ($\overline{X}$ =14.92 ± 5.41 SD, n=50).

Fully developed merozoites were slightly curved, with one end rounded and the other end bluntly pointed (Fig. 4). They were approximately 5.0-6.0 µm long and 1.0-1.5 µm wide. Their spherical nuclei were at the wide end of the merozoites. There appeared to be a small amount of undifferentiated residuum left after formation of merozoites in some meronts. Many merozoites were noted in the process of rounding up and becoming trophozoites; apparently, these parasites had just penetrated epithelial cells of villi and crypts (Fig. 2). Trophozoites were located at the base of cytoplasm of host cells and below the nuclei. They contained central nuclei and were surrounded by a clear area.

Gamonts and gametes. Gamonts occurred in the epithelial cells of villi and crypts and in the lamina propria of whole intestines, and cloaca. Development of gamonts apparently began at about 144 PI, but macrogamont and microgamont were undistinguishable morphologically at that time, since both of them developed from a trophozoite, and were in their very early stages. At 168 h PI, young macrogamonts and microgamonts were clearly visible, and gamonts of various stages were present until 240 h PI (Table 1). The macrogamonts were more numerous than the microgamonts.

During the development of macrogamonts, the sizes increased gradually and the color of the cytoplasm paled. Meanwhile, eosinophilic, plastic granules (wall-forming bodies) in their cytoplasm increased in numbers as development progressed. In young macrogamonts, these granules were evenly scattered throughout the cytoplasm (Figs 5, 6); in mature macrogamonts that may have been fertilized those larger, deeply stained granules were in close contact with each other at the periphery of the parasites; others remained dispersed in cytoplasm (Fig. 7). Each of macrogamonts contained a nucleus with a sharply delineated nucleolus in its center.

Table 1. Developmental stages of *E. fulva* found in experimental goslings.

Hours post-inoculation	Gosling No.	Oocysts inoculated ($\times 10^5$)	Developmental stages found
24	B_1 0374	15.0	Some sporozoites in the epithelial cells of villi of the jejunum and ileum.
48	B_1 0376 B_1 0325	12.0 15.0	Trophozoites and meronts containing 2-4 nuclei in the epithelial cells of villi of the jejunum and ileum.
72	B_1 0377	8.0	Meronts at various stages and trophozoites present. Mature meronts and trophozoites predominated. Meronts in the same sites as those at 48 h post-inoculation (PI). Trophozoites in the epithelial cells of villi and lamina propria from middle duodenum to rectum (including basis ceci).
96	B_1 0378 B_1 0324	8.0 7.0	All endogenous stages as at 72 h PI. They were in the same sites as trophozoites at 72 h PI.
120	B_1 0346 B_1 0330	7.0	All endogenous stages as at 96 h PI.
144	B_1 0365	10.0	Few mature meronts in the same sites as the endogenous stages at 96 h PI, and large numbers of trophozoites in the whole intestine and cloaca.
168	B_1 0366	7.0	Trophozoites and young gamonts in the same sites as the trophozoites at 144 h PI.
192	B_1 0364 B_1 0329	10.0 8.0	Large numbers of gamonts at various stages and oocysts in the same sites as the endogenous stages at 168 h PI. A few oocysts shed in the feces.
216	B_1 0379	7.0	All endogenous stages as at 192 h PI. Great numbers of oocysts shed in the feces.
228	B_1 0371 B_1 0324	7.0 8.0	Few mature gamonts and oocysts present. Small numbers of oocysts shed in the feces.
240	B_1. 394	7.0	Very few endogenous stages as at 228 h PI. Few oocysts shed in the feces.
264	B_1. 391	7.0	No endogenous stages present, and no oocysts were detected in the feces.
288	B_1 0328	8.0	Same as at 264 h PI.

Different developmental stages of microgamonts were seen. They contained nuclei stained intensively and uniformly dark blue with hematoxylin. The number of nuclei varied with the developmental degree of the microgamonts. Very young microgamonts were small and contained only a few nuclei. Young microgamonts were in the shape of the host cell, and contained many nuclei (Figs 5, 6). Mature microgamonts contained large numbers of microgametes (Figs 7-9); in some, the microgametes migrated to the periphery of the parasite, with some apparent residual material remaining in the center (Fig. 9). The fully developed microgametes were slender and crescentic or comma-shaped; they measured 4.5-5.5 μm long and about 0.5 μm wide (Figs 8, 9).

Oocysts. Oocysts were found in the same sites as gamonts. The process of fertilization was not observed

in the present study. Young oocysts were found in sections as early as 192 h PI. They possessed a well developed wall and finely granular cytoplasm evenly distributed over the whole oocyst; each contained a nucleus with a central nucleolus (Figs 7, 10). The oocysts differed from the mature macrogamonts in that the eosinophilic, plastic granules at the periphery of the latter had coalesced to form the oocyst wall.

Shedding of oocysts in the feces began at 192 h PI, and continued until 240 h PI (Table 1). The number of oocysts in the feces reached its peak at 204 and 216 h PI, and sharply decreased over the next 24 h. All fresh oocysts (Figs 11, 12) had a bumpy surface and a prominent micropyle; they were filled with the granular cytoplasm (sporont). In 2.5% potassium dichromate solution, oocysts underwent sporogony and were fully sporulated at 25°C after 60-84 h. Sporulated oocysts (Figs 13, 14) were characteristic of *E. fulva*. They were broadly ovoid and slightly flattened at the narrower pole. One hundred oocysts measured 29.79 ± 0.99 (28.0-31.5) × 24.21 ± 1.15 (20.2-25.2) μm with a shape index (length: width ratio) of 1.23 ± 0.08 (1.15-1.38). The bilayered wall was 2.0-2.5 μm thick. The outer layer was brownish yellow, pitted, and transversely striated; the inner layer was smooth and colorless, and expanded around the micropyle. The micropyle was 2.0-3.0 μm wide, and lacked a cap. There was a large refractile polar granule near the micropyle. No oocyst residuum was present. One hundred and twenty sporocysts (Fig. 14) measured 14.22 ± 0.73 (12.5-15.0) × 10.08 ± 0.25 (10.0-11.0) μm with a shape index of 1.41 ± 0.09 (1.25-1.5). Each sporocyst had a prominent Stieda body, and contained a large, coarsely granular residuum and two folded sporozoites.

Pathological changes

Gross autopsy findings. At necropsy, no obvious gross lesions were observed in organs of the goslings at 24 and 48 h. At 72 to 216 h PI, infected birds had watery intestinal contents containing parasites of different stages, and a small amount of blood, and necrotic debris; the intestinal walls were slightly thickened, with some small hemorrhagic points in the mucosa.

Histopathological changes. Examination of histological sections revealed that the meronts mainly developed in epithelial cells of the upper two-thirds of villi, occasionally in those of the bases of villi. Before 72 h PI, meronts were found only in the jejunum and ileum (Table 1), and they were relatively few in number and small in size, no other obvious lesions were present except for the degeneration and necrosis of the infected cells. With further development of the parasite, its numbers increased and the affected region progressively extended upwards and downwards. Until gamogony, the developmental stages were present in the whole intestine (including caecum) and cloaca, but the majority were noted in the jejunum and ileum (Table 1). Gamonts occurred principally in the epithelial cells of villi and in the lamina propria, as well as in the upper regions of crypts (Figs 5-7, 15), except in the duodenum where they were present within epithelial cells only from the middle to tips of the villi. The histological lesions began to manifest progressively at 72 h PI, and were pronounced in the jejunum and ileum during the gamogony and oocyst shedding. Desquamation and necrosis of epithelium of intestine and crypts, infiltration of inflammatory cells, and slight hemorrhage and mucosal edema were usually associated with aggregates of endogenous stages, whereas the tissue structures of intestine generally remained normal. Beginning at 264 h PI, no endogenous stages were present, and only local chronic enteritis was present in some affected areas.

No endogenous stages or histological lesions were found in other tissues.

DISCUSSION

In all coccidia described from geese, four species, *E. fulva*, *E. magnalabia*, *E. striata* and *E. stigmosa*, have sculptured oocyst walls which are pitted and appear striated. *E. fulva* markedly differs from other 3 species in its much larger oocysts and longer prepatent period (Farr 1953, Gajadhar *et al*. 1983, Dai *et al*. 2004). In our previous study (Dai *et al*. 2004), four, 12-day-old coccidia-free goslings were inoculated with oocysts of *E. anseris*, *E. fulva*, *E. hermani*, *E. nocens*, *E. stigmosa* and *Tyzzeria parvula*, and their feces were collected and examined for the presence of oocysts every 12 h after inoculation. When oocysts were present, they were isolated and then identified. At 204 and 216 h PI, the oocyst shedding of *E. fulva* reached its peak, and that of *E. stigmosa* (its prepatent and patent period were 108 h and 60 h, respectively) had ceased. During this period, *E. fulva* oocysts predominated and no *E. stigmosa* oocysts, which might confuse with *E. fulva* oocysts, were present in the fecal samples. It was easy to pick out *E. fulva* oocysts from these samples under light microscope; misidentification was unlikely. Furthermore, the purity of oocysts was repeatedly checked in the

process of oocyst preparation. So we ensure the inoculum used in this study was of a single species.

Like other *Eimeria* spp., *E. fulva* underwent 3 developmental stages, merogony, gamogony, and sporogony, in its life cycle. The observations in the present study showed that first generation meronts matured at about 72 h PI, and the meronts at various stages were present up to 144 h. Gamonts began to develop at about 144 h PI. Accordingly, it was estimated that at least 2 generations of meronts developed in the life cycle of *E. fulva*. There appeared to be no obvious differences among meronts of various generations. Gajadhar *et al.* (1983) described that both meronts and gamonts of *E. fulva* were present in the epithelial cells of tips and sides of villi and in the lamina propria, as well as in the necks and fundi of the intestinal glands at 169 h PI, and that endogenous stages of *E. fulva* require 9 days to complete the life cycle. In the present study, only few meronts were found at 144 h PI, and by 168 h PI no meronts were present. At 192 h PI, oocysts could be detected in the feces, so the prepatent period was about 7.5 days.

Several goose coccidia, *E. hermani*, *E. kotlani*, *E. nocens*, *E. stigmosa* and *Tyzzeria parvula* have been reported to develop intranuclearly (Antukhayev 1976, Ponizovskii and Shibalova 1978, Skeene and Fernando 1978, Shibalova and Morozova 1979, Gajadhar *et al*. 1986). In this study, both meronts and gamonts of *E. fulva* were found to develop beneath the nuclei of host cells. The endogenous development of *E. fulva* appeared to be relatively brief. The prepapent period was about 7.5 days, and all oocysts were released within about 60 h. The short patent period could be explained by the closely synchronous development of endogenous stages.

Severe illness and death of 1- to 2-week-old goslings have resulted from mixed infections with multi-species of coccidia (Randall and Norton 1973, Arslan *et al*. 2002, Dai *et al*. 2004). Severe infections of *E. fulva* in both domestic and Canada geese resulted in a thickening and congestion of the intestinal wall and an accumulation of greenish mucus in the intestinal lumen (Farr 1953, Gajadhar *et al*. 1983). Arslan *et al*. (2002) described the macroscopic and microscopic lesions in intestines and kidneys of goslings that died of severe diarrhea. These birds might be infected with multi-species of coccidia including the renal coccidium *E. truncata*. They had edematous and hyperemic intestinal mucosae, and the intestinal content was watery and brownish red. Hyperemic vessels, epithelial desquamates, and endogenous stages (meronts, gamonts) of coccidia were seen in lamina propria and epithelial cells of the intestinal villi. The kidneys revealed tubular nephrosis and tubular necrosis, and endogenous developing forms of *E. truncata* were also seen in the epithelium of the renal tubules. The results of this study showed that *E. fulva* could invade the whole intestine and cloaca, and cause intestinal lesions similar to those mentioned above. Marked histological changes were present during the gamogony and oocyst shedding, and most pronounced in the jejunum and ileum. However, *E. fulva* appeared to be mildly pathogenic for goslings based on the obtained data. The inoculating doses of 7.0×10^5-1.0×10^6 oocysts could cause diarrhea, slightly bloody feces, but no death. Histological examination showed that of *E. fulva* mainly developed in the superficial regions of intestinal mucosa, and caused less severe lesions than those in the deeper regions.

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