ENDOGENOUS DEVELOPMENT AND PATHOGENICITY OF EIMERIA ANGUSTA IN THE RUFFED GROUSE, BONASA UMBELLUS

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ENDOGENOUS DEVELOPMENT AND PATHOGENICITY OF *EIMERIA ANGUSTA* IN THE RUFFED GROUSE, *BONASA UMBELLUS*

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ABSTRACT: Oocysts of *Eimeria angusta* were recovered from the cecum of a captive ruffed grouse which died of coccidial typhlitis. Five experimental passages were made in grouse over a 2-yr period. Prepatent period was 6.5–7.0 days; sporulation time at 22 C was 54–60 hr. Endogenous stages were not found in grouse killed 1 and 2 days after inoculation (DAI). A few immature schizonts were in cecal epithelium 4 DAI. Developing and mature schizonts, and undifferentiated gamonts were in cecal epithelium 5 DAI. Developing micro- and macrogamonts, but no oocysts, were present 6.5 DAI. Fibrinohemorrhagic typhlitis, associated with large numbers of gamonts and oocysts in the epithelium, was found 9 DAI. Cecal glands were hypertrophic and there was a heavy mixed inflammatory cell reaction. Diarrhea, depression and reduced feed intake occurred 7–10 DAI with most severe signs and greatest oocyst passage 8 or 9 DAI. Most infected birds lost weight 5–8 or 9 DAI, but none died.

INTRODUCTION

Two species of coccidia, *Eimeria angusta* and *E. bonasae* were described from the cecum of the ruffed grouse by Allen (1934), and redescribed by Todd et al. (1970). The taxonomy of the coccidia of grouse is confused (Pellerdy, 1974) and Todd et al. (1970) considered it unlikely that similar oocysts in other species of grouse were *E. angusta* and *E. bonasae*. However, mortality caused by cecal coccidiosis attributed largely to *E. angusta* has been reported in wild sage grouse, *Centrocercus urophasianus* (Simon, 1940; Honess and Post, 1968). Cecal coccidiosis was also implicated in the "grouse disease" that devastated populations of red grouse (*Lagopus lagopus scoticus*) in Great Britain (Committee of Inquiry on Grouse Disease, 1911). As part of the investigation of "grouse disease" in Scotland, Fantham (1909, 1910a, b) described endogenous stages of coccidia in naturally infected grouse, and in grouse chicks, chickens and pigeons infected experimentally (he claimed) with coccidial occysts from grouse.

In this paper we report natural mortality associated with *E. angusta* typhlitis in a captive ruffed grouse, and observations on sporulation time, prepatent period, endogenous stages and pathogenicity in experimentally infected ruffed grouse.

MATERIALS AND METHODS

A 5-mo-old ruffed grouse, hatched in an incubator from an egg collected in the wild and raised in wire-bottomed caging in the aviary of the Department of Zoology until its death, was diagnosed as having cecal coccidiosis in October 1977. Oocysts in cecal scrapings were sporulated in 2.5% potassium dichromate and some were measured under oil immersion on a Reichert Zetopan microscope using a calibrated eyepiece micrometer. The remainder were stored in 2.5% potassium dichromate at 4 C.

Over the subsequent 2 yr, groups of grouse (Table 1) were inoculated orally with a strain of oocysts derived from this original culture, resulting in five serial passages. Clutches of wild-collected eggs were hatched in sanitized incubators in early June. Grouse were reared to 5 days of age in sanitized brooders using honeybee larvae and unmedicated duck starter crumbles. Then, with the exception of grouse in Groups 1 and 5, which were reared in the Department of Zoology aviary, they were transferred to an isolated rearing room where grouse had never been held. Here they were fed duck

TABLE 1. Summary of oocyst passage in groups of ruffed grouse experimentally inoculated with *Eimeria angusta*.

Group	Date	No. of birds	Age	Inoculum	Oocysts passed	Passage level
1	Nov. 1977	2	6 mo	original	no	—
2	June 1978	8	12 days	original	yes	1
3	July 1978	4	7 wk	passage 1	yes	2
4	Aug. 1978	12	10 wk	passage 2	yes	3a
5	May 1979	2	11 mo	passage 2	no	—
6	June 1979	2	16 days	passage 2	yes	3b
7	July 1979	2	6 wk	passage 3b	yes	4a
8	Aug. 1979	5	8 wk	passage 3b	yes	4b
9	Aug. 1979	4	10 wk	passage 4a	yes	5

starter crumbles and were housed in cardboard boxes, with wire floors of appropriate mesh size, and roofed with soft nylon netting.

Experiments were carried out in a separate tight isolation room. Birds were housed in pairs or individually in wire-bottomed boxes with netting tops, or in standard steel rabbit cages. Fecal samples taken prior to inoculation and examined by saturated sodium nitrate flotation contained no coccidial oocysts.

Six- to 12-mo-old aviary-reared birds (Groups 1 and 5) were inoculated in attempts to multiply the culture, and in the case of Group 5, so that substantial numbers of fresh oocysts might be available to carry out experimental inoculation of young grouse chicks.

Group 2 (Table 1) consisted of eight experimental birds, and two uninoculated controls. Pairs of birds were inoculated orally with 50, 500, 5,000 or 10,000 oocysts derived from the original case. The objectives were to test the viability of the inoculum, to generate more oocysts for subsequent work, to determine the prepatent period and to titrate, if possible, a pathogenic dose in young chicks. Feces were collected daily and examined for oocysts by flotation. Birds were killed 13 days after inoculation (DAI).

The four birds in Group 3 (Table 1) received single oral doses of 2,500, 5,000, 7,500 and 10,000 oocysts, in order to further define the prepatent period and produce greater numbers of oocysts. Feces were examined daily 1–5 DAI, and at 12-hr intervals form 5 to 8 DAI.

In order to investigate the endogenous development of *E. angusta*, four birds in Group 4 were each inoculated with 100,000 oocysts and pairs were killed 1 and 2 DAI. A further eight birds, each inoculated with 25,000 oocysts, were killed in pairs, 4, 5, 6.5 and 9 DAI. Im-

mediately after death of the bird, the intestinal tract was removed and intestine from nine sites was opened and fixed in Serra's fixative for 4 hr, washed in 95% alcohol and ultimately stored in 10% formalin. The sites examined were: descending duodenum; ascending duodenum; jejunum midway between end of duodenal loop and Meckel's diverticulum; jejunum at Meckel's diverticulum; small intestine at a level opposite the tips of the ceca; ileocecal junction, including the origin of both ceca; middle of one cecum; tip of same cecum; mid large intestine. Several 6-μm sections were cut from wax-embedded tissue from each piece of gut, and stained with hematoxylin and eosin for microscopic examination.

Multiplication of a fresh oocyst culture was the objective of inoculation of birds in Group 6, which received 2,500 oocysts each. Feces were collected 7–14 DAI.

Grouse in Groups 7, 8, and 9 were each inoculated with 25,000 oocysts, in attempts to define the clinical effects of infection in growing grouse. Two uninfected control birds of comparable age were monitored with each of Groups 8 and 9. Demeanor of birds and characteristics of feces were observed daily. Feed consumption was subjectively assessed, as severe spillage and fouling of feeders precluded weighing feed. Body weight of birds in Groups 8 and 9 was measured. Experiments were terminated 10 or 12 DAI.

Birds were killed by cervical transection. Feces and cecal scrapings were sporulated in 2.5% potassium dichromate in flasks fitted with a compressed air bubbler, as sporulation was often poor in thin layers of potassium dichromate in shallow dishes. Oocysts were examined microscopically at 6-hr intervals to determine sporulation time. Differences in relative body
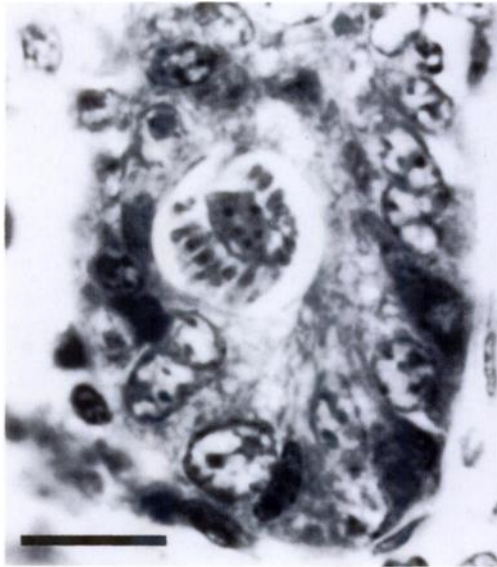
FIGURE 1.   Schizont of *Eimeria angusta* with merozoites developing at the periphery. Cecal gland 5 DAI. H&E. Scale = 20 μm.

weights of infected and control birds were analyzed for statistical significance using Student's *t*-test, at the 5% level.

RESULTS

Oocysts in the original inoculum were irregularly ellipsoidal and had a double oocyst wall, with a micropyle, beneath which a polar body was often visible. Sporocysts had a prominent stieda body, and a diffuse granular sporocyst residuum which obscured the sporozoites, so that usually only two large refractile globules were visible in the sporocyst. Measurements in micrometers of 30 oocysts (mean with range in parentheses) were: oocyst 33.5 (26.0–39.0) long × 17.2 (15.5–18.2) wide; sporocyst 15.0 (13.0–18.2) × 6.2 (5.0–8.0) wide. In these respects they correspond closely with *E. angusta* as redescribed by Todd et al. (1970). Five hundred oocysts examined from the first passage met these criteria suggesting that the culture was pure, though it was not single-oocyst-derived.
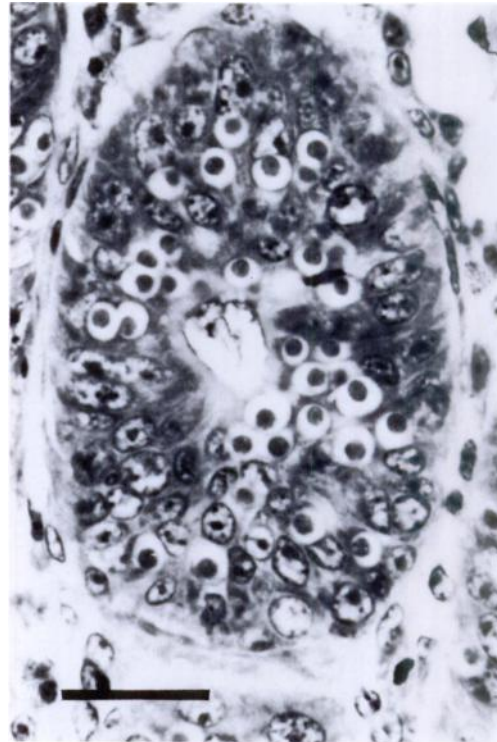

FIGURE 2.   Undifferentiated gamonts of *Eimeria angusta*, often more than one per host cell, in a cecal gland 5 DAI. H&E. Scale = 20 μm.

Attempts to establish patent infections in conventionally reared grouse over 6 mo of age failed (Groups 1 and 5, Table 1), though the same inocula were infective to chicks reared in isolation (Groups 2, 6).

Prepatent period and oocyst passage: Seven DAI the birds in Group 2 which received 10,000 oocysts were passing a few oocysts. Eight DAI, birds inoculated with 5,000 and 500 oocysts had patent infections, and 11 DAI a single oocyst was found in feces of one bird inoculated originally with 50 oocysts. Uninoculated controls did not pass oocysts.

Birds in Group 3 all passed oocysts by 6.5 DAI. Oocyst passage subjectively appeared maximal 9 DAI, but patency persisted until 14 DAI in some birds. In subsequent experiments, infections often
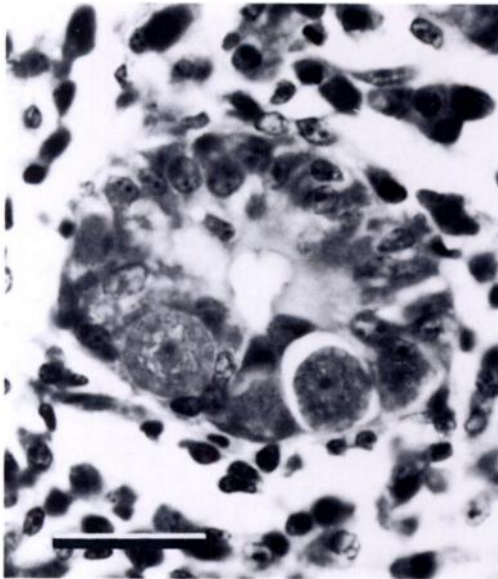
FIGURE 3.   Developing macrogametes of *Eimeria angusta*, about 13 μm in diameter, with refractile wall forming bodies. Cecal gland 6.5 DAI. H&E. Scale = 20 μm.
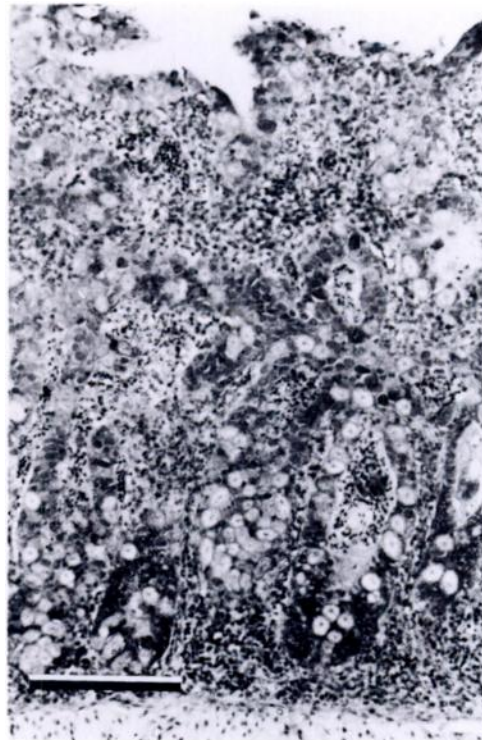

FIGURE 4.   Cecal mucosa 9 DAI with *Eimeria angusta*, with glands lined by epithelium infected with coccidia at various stages of gametogony. The mucosa is thickened, glands contain oocysts, hemorrhage and heterophils, and there is an extensive mixed inflammatory infiltrate in the lamina propria. H&E. Scale = 200 μm.

became patent by 6.5 DAI, and always by 7 DAI. Oocysts became sporulated in 54–60 hr at room temperature (about 22 C).

Endogenous development and associated pathology: Endogenous stages were not found in grouse killed 1 and 2 DAI. Subsequently, stages were seen only in cecal mucosa, but not at the ileocecal junction. Four DAI the earliest protozoan forms found were in the surface epithelium. They were oval or irregularly spherical zoites, about 3 × 5 μm with a deep basophilic cytoplasm containing one to three paler vacuoles, and no obvious nucleus.

Developing schizonts up to about 15 μm in diameter were found 5 DAI in epithelium of glands. Nuclei in early schizonts were present around the periphery of the cytoplasm where merogony occurred. Several mature schizonts with a residual body surrounded by up to 20 narrow falciform merozoites 5–7 μm long, with a

central nucleus, were seen in epithelium of glands (Fig. 1). Zoites about 4–6 μm in diameter with deeply basophilic cytoplasm were present in surface epithelium. Several of the larger of these contained three to four nuclei and were considered to be early schizonts. In several glands many cells each contained up to three zoites 3–4 μm in diameter, with pale basophilic cytoplasm and a single nucleus. These were interpreted as undifferentiated gamonts (Fig. 2).

The grouse killed 6.5 DAI had no oocysts in their feces. Schizonts were seen rarely. Immature gamonts 4–10 μm in diameter, with a prominent nucleus and a

finely vacuolate cytoplasm containing scattered small eosinophilic granules were in epithelial cells in glands and on the surface of the cecum. Larger macrogamonts up to 10 × 15 μm (Fig. 3) had refractile eosinophilic wall-forming bodies around the periphery. Early microgametocytes up to 18 × 22 μm had small nuclei which were distributed on infoldings, and toward the periphery, of the basophilic cytoplasm.

Gross lesions were noted in one of the two grouse killed 9 DAI, 2 days after they began passing oocysts. Cecal content was fluid rather than pasty. There were patchy masses of fibrinous exudate adherent to the mucosa, which had pinpoint hemorrhages and appeared thickened. Microscopically, the cecal mucosa of this bird was much thicker than normal—often exceeding 800 μm, in comparison with a cecal mucosal thickness of 260–320 μm in the four grouse killed 1 and 2 DAI.

The elongate glands were lined by epithelium with a high nucleus/cytoplasm ratio, open vesiculate nuclei, and a moderate number of mitoses. Many surface cells and virtually all cells lining glands in some areas contained one to four gamonts, generally more than 10 μm in diameter, which caused hypertrophy of the host cell and frequently obscured the nucleus and intercellular boundaries (Fig. 4). Immature macrogametes had a nucleus with a prominent nucleolus and a pale basophilic cytoplasm containing small open vacuoles and pale eosinophilic granules, scattered randomly.

Eosinophilic wall-forming bodies were condensed at the margin of larger macrogametes (Fig. 5). Oocysts up to 27 × 15 μm were present in cells in tissue, while free oocysts up to 32 × 15 μm, with a micropyle, were in lumina of glands and in exudate in the cecal lumen. Immature microgametocytes up to 30 × 28 μm had nuclei lined up around the margin, and along infoldings of the cytoplasm, forming blastophore-like arrays in some instances (Fig. 5). Swirling masses of microgametes 3 μm long were present in mature microgametocytes and in the lumina of some glands.

Oocysts, heterophils and masses of bacilli were in gland lumina, and in areas in the lamina propria where glands appeared to have been disrupted or destroyed (Fig. 6). There were erosions of surface epithelium. Heterophils, erythrocytes, oocysts and bacteria were adherent to the surface in some areas. A heavy mononuclear cell infiltrate, including many plasmacytes, was in the lamina propria, with scattered heterophils throughout (Fig. 4). As a result of the proprial infiltrate glands in some areas were abnormally separated from each other.

Casts and fibrinous typhlitis were seen in two of seven grouse killed 10 DAI (Groups 7, 8) but not in birds killed at later times. In the original, naturally infected grouse which died, the cecal lumina contained elongate loose fibrino-hemorrhagic casts, and fluid blood-tinged content. The casts separated readily from the underlying mucosa which was congested and thickened. In section, schizonts, gamonts and developing oocysts were present in nearly all cells in some glands and over the surface in many areas. Extensive hemorrhage and heterophil exudate was in the lumen in association with colonies of bacteria and large numbers of oocysts, which often plugged cecal glands. A heavy mononuclear and heterophil infiltrate was in the propria. Tissues taken at other levels of the intestine were moderately autolysed, but no coccidial stages were seen.

Clinical effects of infection: No experimentally infected birds died of coccidiosis. Diarrhea, characterized by more fluid, lighter colored and occasionally, blood-tinged, cecal droppings was common. It occurred in 19 of 26 infected birds 7–11 DAI, with the most severe signs usually 8 or 9 DAI. At this time birds became quiet
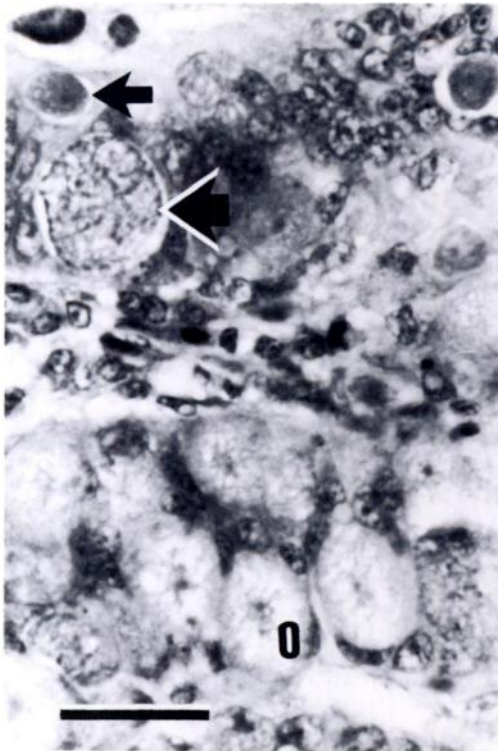
FIGURE 5. Gamonts of *Eimeria angusta* at various stages of development in a cecal gland 9 DAI. Early macrogamonts (small arrow), developing oocysts (O) with condensing wall forming bodies and an immature microgametocyte (large arrow) are present. H&E. Scale = 32 μm.
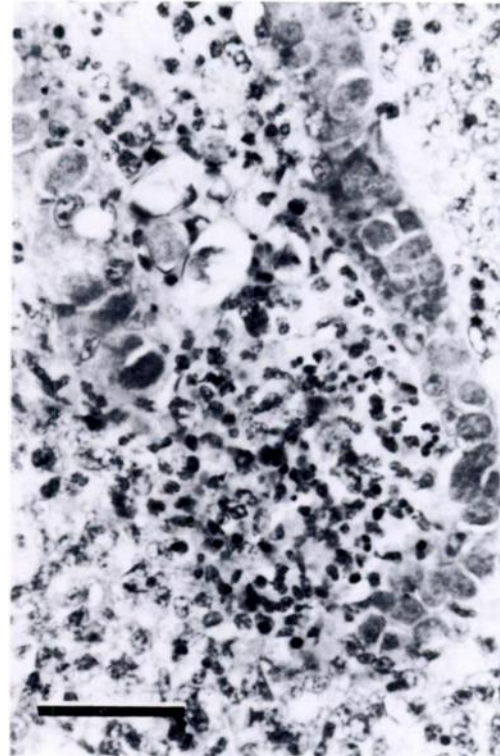

FIGURE 6. Disrupted cecal gland lined by cells containing developing gamonts of *Eimeria angusta*, and containing oocysts and masses of immigrating heterophils 9 DAI. H&E. Scale = 50 μm.

and less excitable, feed consumption appeared to decline and spillage was reduced, and several birds became profoundly depressed, with ruffled feathers. The four control birds in Groups 8 and 9 each gained 15–37 (mean 26) g over the period 0–10 DAI. Three of the nine infected birds lost weight over the same period. Eight of nine had slight but significant weight loss 1–2 DAI, and the same number had a decline in body weight of 8–85 (mean 32) g over the period 5–8 or 5–9 DAI. However, a rapid compensatory gain occurred during the subsequent 2–4 days of observation. Loss of weight was variable in degree and duration among birds (Table 2). The maximum loss was 21% of original body weight.

DISCUSSION

Oocyst production, especially from young birds, following experimental inoculation, was not heavy. Patent infections could not be induced in mature birds, presumably because of immunity resulting from prior exposure or "age resistance." Oocysts stored for 8–10 mo, though apparently viable, also failed to generate heavy infection (e.g., Group 2, where 10,000 oocysts produced only mild signs in 30–40-g birds). Heavy infections occurred only when young birds from the single annual hatch were infected with fresh oocysts. As a result, most experi-

TABLE 2. Body weights of ruffed grouse following inoculation with 25,000 oocysts of *E. angusta*, and of uninoculated controls in the same period. Body weights are expressed as a percent of the body weight on the day of inoculation. Groups 8 and 9 pooled. Inoculated, $n = 9$; Control, $n = 4$ except where noted.

Days after inocu- lation	Body weight mean (standard deviation)		Signifi- cance of differ- ence at 5% level
	Control	Inoculated	
0	100	100	
1	101.0 (2.1)	97.9 (1.6)	+
2	102.2 (0.9)	97.4 (4.3)	+
3	102.1 (2.1)	99.4 (5.4)	−
4	101.6 (1.3)[a]	101.1 (4.4)[c]	−
5	102.1 (1.9)	102.3 (6.2)	−
6	102.7 (1.4)	100.5 (5.8)	−
7	103.9 (2.4)	98.6 (6.1)	−
8	104.0 (2.0)	95.6 (8.3)	+
9	107.2 (3.5)[a]	95.5 (9.4)[c]	−
10	105.9 (2.7)	100.1 (9.2)	−
12	105.2 (2.1)[a]	103.5 (13.4)[b]	−

[a] $n = 2$.
[b] $n = 4$.
[c] $n = 5$.

ments were undertaken using only moderate numbers of oocysts, in birds which had become at least half grown during the period required to multiply the cultures through two passages.

Though no mortality occurred in experimentally infected birds, signs of disease were common and consistent. One bird killed 9 DAI (Group 4) had gross and microscopic lesions similar to those in the naturally infected bird which died, and several others killed 10–12 DAI appeared to have resolving typhlitis. The lesions in ruffed grouse apparently resembled those in sage grouse with coccidiosis, whose ceca were described colorfully as "a shambles" (Honess and Post, 1968).

Pathogenicity is associated with gametogony in the cecum, as visible signs and significant lesions were restricted to the period 6–10 DAI, when late gametogony was proceeding and oocyst production was incipient or maximal. Severe destruction of epithelium was evident 9 DAI, and in the spontaneous mortality it was associated with maturation of large numbers of oocysts. Thickening of the mucosa is related to hypertrophy and hyperplasia of glandular epithelium, and the increase is interstitial inflammatory cells. Loss of protein, and hemorrhage into the cecum may be life-threatening in heavy infections. Mucosal damage may permit invasion by bacteria, which apparently occurred in sage grouse with coccidiosis (Honess and Post, 1968). The transitory weight loss (up to 21% of body weight) which occurred at this time was probably largely related to inappetence. Depression, which was profound in a few birds, might predispose affected chicks to predation in the wild.

Early endogenous stages of *E. angusta* were presumably too sparsely scattered to be encountered in birds killed on 1 and 2 DAI, though they received larger inocula. At least one generation of schizogony occurred in the cecum 4–5 DAI. No stages were found in the cecum prior to that time and schizonts were never numerous. Therefore, it is likely that only a single preceding generation of schizogony occurs, probably elsewhere in more proximal intestine. Schizonts were described in the duodenum and cecum of red grouse, and the duration of schizogony in that host was considered to be 4–5 days (Fantham, 1910a). However, it is probable that more than one species of coccidia was involved, and their relationship to those in ruffed grouse is unclear. All stages in the cecum of ruffed grouse were restricted to the epithelium.

The source of infection for the original spontaneous mortality is obscure. Fecal contamination of eggs or of the feet of field workers may have introduced infection to the aviary, but the birds were reared under conditions generally considered unfavorable to the development of coccidiosis. Experimental inocula of 25,000 oocysts in naive birds of similar size were not fatal, and birds of similar age reared at the same time as the initial case could

not be infected experimentally (Group 1, Table 1).

Coccidiosis in red and sage grouse is considered to be a disease of young chicks. It is conceivable that sufficient oocysts might be generated by early hatched chicks in a locality to increase infection pressure to fatal levels for later hatches, since under favorable conditions a complete cycle might take only 12–14 days. Moist substrate may favor the development of coccidiosis in chicks, as diseased sage grouse chicks were found near "soaks." Development of fly maggots in fluid cecal droppings was considered a possible means of promoting infection of red and sage grouse chicks (Fantham, 1910b; Honess and Post, 1968) since the chicks are insectivorous early in life. The more arboreal habits of ruffed grouse (Johnsgard, 1973) probably mitigate against coccidiosis being a significant problem in that species in nature. However, medication of feed with a coccidiostat seems a worthwhile precaution with captive grouse (Wilson and Wilson, 1978).

ACKNOWLEDGMENTS

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