# LIFE CYCLE AND BIOLOGY OF EIMERIA LETTYAE SP. N. (PROTOZOA: EIMERIIDAE) FROM THE NORTHERN BOBWHITE, COLINUS VIRGINIANUS (L.)

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# LIFE CYCLE AND BIOLOGY OF *EIMERIA LETTYAE* SP. N. (PROTOZOA: EIMERIIDAE) FROM THE NORTHERN BOBWHITE, *COLINUS VIRGINIANUS* (L.)

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ABSTRACT: A new species of coccidium, *Eimeria lettyae* sp. n. (Protozoa: Eimeriidae) was recovered from feces of the northern bobwhite, *Colinus virginianus* (L.), from Pennsylvania and Florida. Oocysts measured 21.1 μm (16.4 to 25.8) by 17.2 μm (14.1 to 21.2); index (L/W ratio) = 1.22. Oocysts lacked a micropyle, residuum, and polar granules. Sporozoites penetrated the upper ½ of the villi, then moved to the lamina propria at the base of the villi. There were five asexual generations, all of which developed above the nucleus of the host cell. Meronts measured 9.4 × 7.0 μm, 18.6 × 11.2 μm, 11.8 × 10.1 μm, 7.1 × 6.2 μm, and 20.2 × 12.8 μm, respectively. These matured at 32, 40, 48, 56, and 72 hr postinoculation (PI) and contained 12, 50+, 24 to 36, 12 to 24, and 50+ merozoites, respectively. Infection was most intense in the duodenum although some gamonts were found in the ileum and ceca. The prepatent period was 88 to 91 hr PI. Sporulation time was 18 hr at 25 C. The peak of oocyst production was broad and extended from 4 days PI through 14 days PI. Oocysts were passed for at least 67 to 76 days PI. *Eimeria lettyae* sp. n. did not infect chickens (*Gallus domesticus*), domestic turkeys (*Meleagris gallopavo*), ring-necked pheasants (*Phasianus cochicus*), chukar partridge (*Alectoris graeca*), or Japanese quail (*Coturnix coturnix*). Immunizing bobwhite with *E. lettyae* sp. n. did not protect against challenge with *E. dispersa*. Immunizing bobwhites 25 times with $10^2$ or $10^3$ sporulated oocysts of *E. lettyae* did not entirely eliminate oocyst production following challenge with the same species.

## INTRODUCTION

The identity of coccidial species infecting the northern bobwhite is confused. Early workers reported several chicken species from the bobwhite including *Eimeria tenella* and *Eimeria acervulina* (Venard, 1933). The lack of reproducible cross-transmission studies, however, led Levine (1953) to conclude that *Eimeria dispersa* was the only valid species described from the bobwhite. Although Waggoner (1967) and Prostowo and Edgar (1970) described "new species" they were unnamed. A new species, *Eimeria colini*, was named by Fisher and Kelley (1977); however, only the oocyst and sporocysts were described. Subsequently, researchers continued to incorrectly use the chicken species in checklists (Kellogg and Calpin, 1971) or have reported on *Eimeria* sp. (Kocan et al., 1979).

This paper describes a new species, *Eimeria lettyae* sp. n., from the northern bobwhite including the life cycle, prepatent and patent periods, oocyst production, sporulation time, cross-transmission, and cross-immunity.

## MATERIALS AND METHODS

### Coccidia

Fecal samples were obtained from personnel of a game farm in Cooperstown, Pennsylvania who were attributing mortality and loose droppings in northern bobwhites to coccidiosis. Laboratory-raised, coccidia-free bobwhites fed these samples passed oocysts that seemed to be a single species that was relatively uniform in size and shape. Nine successful single oocyst isolations were made from these oocysts and four of these were chosen for further study. Oocysts for life cycle studies were harvested from pooled cultures. Oocysts were stored at 4 C in 2.5% potassium dichromate. A second source of oocysts was intestinal contents of wild northern

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bobwhite collected from Tall Timbers Research Station, Leon County, Florida by Dr. W. R. Davidson (Univ. of Georgia). Oocysts produced by bobwhites fed these droppings produced three distinct types of oocysts based on size and shape. *Eimeria lettyae* sp. n. was isolated from this mixed culture and used for measurement, cross-transmission and cross-immunity studies. The remaining two types of oocysts have not been isolated or identified at this time. The *E. dispersa* used was originally supplied by Dr. S. A. Edgar (Auburn Univ., Alabama).

Inoculation dosages were based on the number of sporulated oocysts. Bobwhites were administered oocysts orally by gastric intubation. All experiments used cultures of oocysts less than 4 mo old except for experiments on production of oocysts where cultures were less than 1 mo old.

### Bobwhites

Northern bobwhites were hatched from eggs incubated in the laboratory and were raised in wire-floored cages and given unmedicated turkey starter and water ad libitum. Uninoculated bobwhites were checked for oocysts periodically by examining intestinal scrapings and concentrated (sugar floated) feces. These birds were never infected. All bobwhites used were 2½ to 3 wk old at the time of inoculation with oocysts.

### Experimental design

*Development:* Experiment I. Thirty-three bobwhites were each inoculated with $1 \times 10^5$ sporulated oocysts/bird. Three bobwhites selected at random were killed at 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144 hr postinoculation (PI). Three uninoculated bobwhites were also killed at 144 hr PI. Tissues were collected from the duodenum, jejunum, ileum, cecal neck, mid-cecal pouch, cecal tip, and large intestine and fixed in 10% buffered formalin.

Experiment II. Twenty-two bobwhites were each inoculated with $1 \times 10^6$ sporulated oocysts. Nine uninoculated birds were caged with the inoculated birds (sentinel birds); six uninoculated controls were caged separately. All bobwhites were transferred daily to clean, coccidia-free, cages. Two inoculated bobwhites were killed at 24, 32, 40, 48, 55, 64, 72, 84, 96, 192, and 288 hr PI. Three uninoculated sentinel bobwhites were killed at 96, 192, and 288 hr PI (nine total). Uninoculated controls were killed at 72 and 288 hr PI, three at each time. Tissues were collected from the regions described above and fixed.

All tissues from the above two experiments were embedded in paraffin, cut to 10 $\mu$m thickness, stained with hematoxylin and eosin (H and E), and examined with bright field illumination. Tissues for photomicrographs were stained with PAS.

Experiments III and IV. Ten bobwhites were each inoculated with $2 \times 10^6$ sporulated oocysts. Two were killed at 1, 3, 6, 12, and 24 hr PI. Tissues were collected from the duodenum, fixed, embedded, and sectioned. In another experiment (Experiment IV), duodenal tissue was collected at 24, 32, 40, and 48 hr PI. Duplicate sets of slides were prepared. One set was stained with H and E, the other was stained by the monoclonal antibody–immunofluorescence technique (IFA staining) described by Augustine and Danforth (1984) and examined with fluorescence microscopy.

*Measurements:* Experiments I and II. Measurements of endogenous stages were made on histological sections using an ocular micrometer. Whenever possible, at least 25 parasites were measured for each stage. Care was taken to insure that tangential or diagonal sections of meronts were not measured. Measurements were confirmed in scrapings from fresh intestine. Oocysts were taken from feces and measured when completely sporulated. One hundred oocysts were measured from the pooled cultures from Pennsylvania and Florida. Fifty oocysts were measured from each of the cultures derived from four additional single oocyst isolations to obtain some indication of size variation.

*Prepatent period:* Experiment V. Five bobwhites were each given $1 \times 10^3$ sporulated oocysts and caged separately. Feces were collected each hour beginning at 87 hr PI. Feces were examined microscopically for oocysts after sugar flotation.

*Oocyst production:* Experiment VI. Five bobwhites were each inoculated with $1 \times 10^3$ sporulated oocysts. Feces over a 24-hr period were collected daily from individual birds on days 3 to 21 PI. After day 21 PI, feces were collected over a 24-hr period twice a week. The last collection was made on day 76 PI. The number of oocysts in each fecal sample was counted using sugar flotation of oocysts in a McMaster chamber and expressed as oocysts produced/bobwhite and oocysts produced/oocyst given.

Experiment VII. Seven groups of three bobwhites each were inoculated with sporulated oocysts, one group with each dosage. Individual birds were given $1 \times 10^2$, $5 \times 10^2$, $1 \times 10^3$, $5 \times 10^3$, $1 \times 10^4$, $5 \times 10^5$, or $1 \times 10^6$ sporulated oocysts. Feces from each group were collected days 4 through 10 PI which corresponded to the days of maximum oocyst production found

in the first oocyst production experiment. Oocysts were counted as above.

*Sporulation time:* Experiment VIII. Five bobwhites were each given 1 × $10^3$ sporulated oocysts. On day 4 PI the dropping pans were cleaned. Feces were collected 1 hr later and the oocysts quickly concentrated using sugar flotation. The oocysts were then allowed to sporulate at 25 C. Beginning at 14 hr, two bobwhites were each inoculated with 1,000 oocysts. This was continued at 2-hr intervals for 8 hr using two new bobwhites each time. Five days later, feces were collected from each of these bobwhites and examined microscopically after sugar flotation. The bobwhites were then killed by cervical dislocation and intestinal scrapings examined for coccidia.

*Cross-transmission:* Experiment IX. Sporulated oocysts (1 × $10^6$) from the Pennsylvania and Florida strains of *E. lettyae* sp. n. were orally administered to each of three chickens, three domestic turkeys, three ring-necked pheasants, three chukar partridge, three Japanese quail, and three bobwhites. Six days PI, feces were collected and both feces and intestinal scrapings were examined for coccidia.

*Cross-immunity:* Experiment X. Cross-immunity induced by the Pennsylvania and Florida isolates was studied by immunizing 10 bobwhites for each isolate with three oral inoculations of 1 × $10^4$ oocysts each. These inoculations were given 4 days apart beginning at 49 days of age. Ten additional bobwhites were not immunized. Three weeks after the last dose, the feces were examined for 3 consecutive days to insure that oocyst production from the immunizing dose had ceased or was very low (<1 × $10^3$ oocysts/bobwhite/day). One half of each group (five bobwhites) was then challenged with 1 × $10^3$ oocysts/bobwhite for each strain. Five unimmunized controls were also inoculated with each strain. Feces were collected 5–9 days post-challenge and oocysts counted. In a similar experiment (Experiment XI), two groups of three bobwhites each were immunized with oocysts of *E. lettyae* sp. n. following the same regimen. One group was challenged with 1 × $10^3$ oocysts of *E. lettyae* sp. n./bobwhite and one group with the same dose of oocysts of *E. dispersa*. Unimmunized controls were also inoculated and feces collected 5–9 days post-challenge.

## RESULTS

### Development

Parasites were not visible in histological sections until 32 hr PI. Sporozoites and trophozoites were, however, readily visible as early as 1 hr PI using the IFA staining method. At 1 hr PI, sporozoites were in epithelial cells lining the villi, but most numerous in the upper ½ of the villi. These sporozoites were located above the host cell nucleus (Fig. 1). By 3–6 hr PI, many of the sporozoites had moved adjacent to or below the host cell nucleus, often locating adjacent to the basement membrane of the host cell. Most were still in the upper ½ of the villi. By 12 hr PI most of the parasites had moved into the lamina propria and begun rounding up. Most were located in the bottom ½ of the villi but even those in the crypt region were generally in the lamina propria rather than crypt cells. Parasites were numerous in IFA-stained sections (500–1,000/section) with fluorescent light even though none was visible with light microscopy of stained sections or with phase contrast of the IFA-stained sections. Only a few parasites (sporozoites and trophozoites) were seen at 24 hr PI, almost entirely in the crypt region. Only a few sporozoites and trophozoites (10–20/section) were seen at 32 and 40 hr PI using IFA staining. At 48 hr PI, there was a large increase in the number of parasites (over 500/section). It was not possible to determine if these were sporozoites or merozoites in the IFA-stained sections. In addition, some immature and mature meronts were found that were positive for IFA staining. All generations of meronts and the gametocytes developed above the nucleus of the host epithelial cell.

*1st generation meront:* Duodenum; 9.4 (8.2–10.6) × 7.0 (5.8–8.2) μm. Immature, 24–36 hr PI; mature, 32–40 hr PI (Fig. 2); 12 merozoites (6.6 × 1.5 μm). Refractile body rare, not visible in maturing meront; meronts located in epithelium on sides of villi and towards tips. Both the meront and the merozoites fluoresced with IFA staining.

*2nd generation meront:* Mostly duodenum, a few in the jejunum at 40 hr PI;

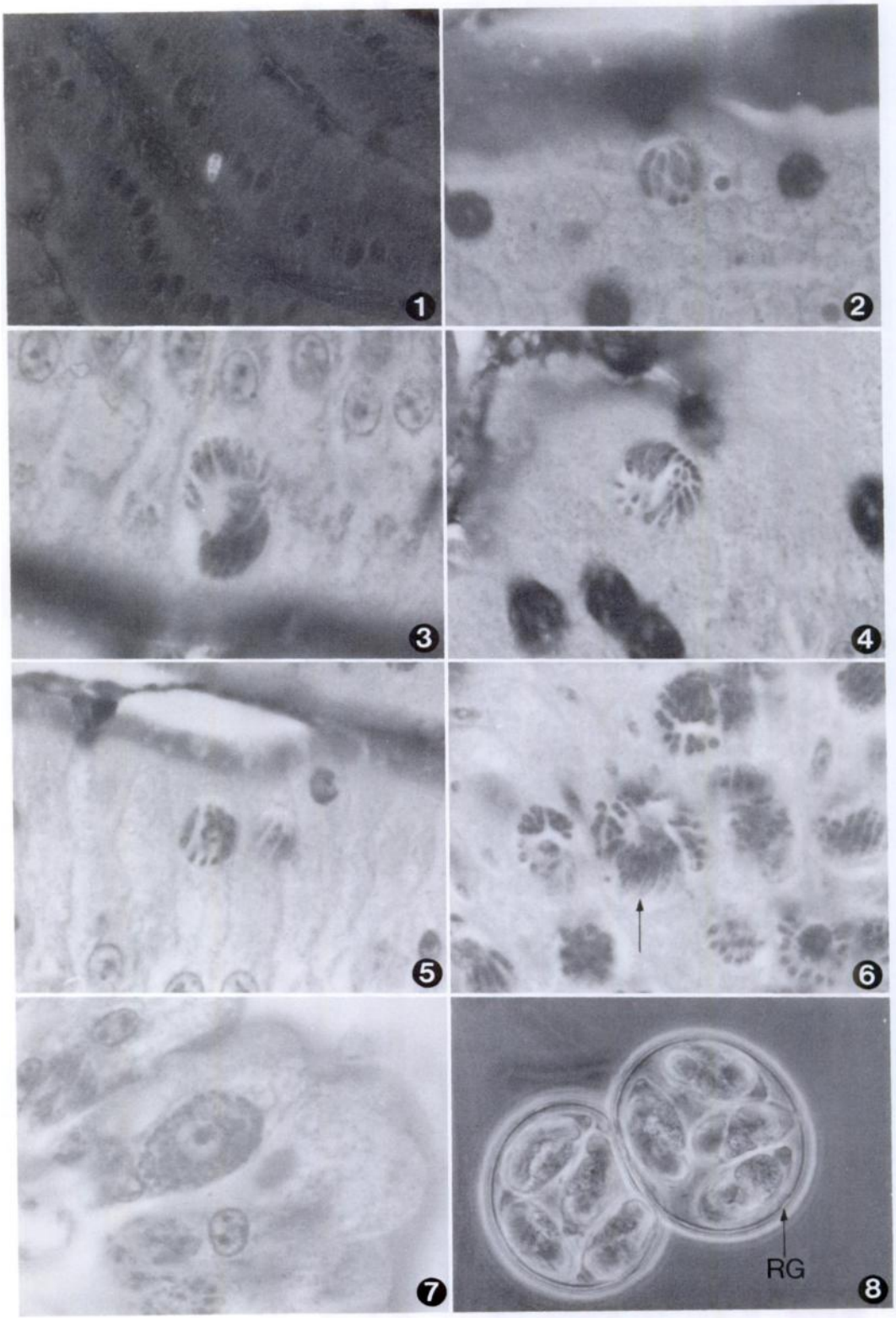

TABLE 1. Dimensions (in micrometers) of oocysts of *Eimeria lettyae* sp. n. from northern bobwhites.

| Source | Isolate (measurement)* | Dimensions | | Index |
|---|---|---|---|---|
| | | Average | Range | |
| Pennsylvania | Mixed | 21.1 × 17.2 | 16.4–25.8 × 14.1–21.2 | 1.22 |
| Pennsylvania | Single oocyst (22.9 × 17.0) | 23.5 × 19.1 | 21.2–25.8 × 17.6–21.2 | 1.23 |
| Pennsylvania | Single oocyst (21.2 × 17.6) | 22.6 × 17.8 | 19.4–25.8 × 15.9–20.0 | 1.27 |
| Pennsylvania | Single oocyst (18.8 × 16.4) | 21.2 × 17.4 | 16.4–25.8 × 14.1–20.0 | 1.21 |
| Pennsylvania | Single oocyst (17.0 × 16.4) | 21.2 × 17.0 | 16.4–25.8 × 14.1–21.7 | 1.25 |
| Florida | Mixed | 23.1 × 19.1 | 17.6–28.2 × 14.7–21.2 | 1.21 |

* One hundred oocysts were measured from each of the Pennsylvania and Florida cultures, 50 from each of the single oocyst isolations.

18.6 (15.0–23.5) × 11.2 (9.4–11.8) μm. Immature, 32–40 hr PI; mature, 40–64 hr PI (Fig. 3); peak at 40–48 hr PI; 50+ merozoites (4.7 × 0.8 μm). Mostly scattered, occasionally in groups of two or three, residual body present. At or close to tips of villi.

*3rd generation meront:* Mostly duodenum, a few in the jejunum; 11.8 (10.6–12.9) × 10.1 (9.4–11.8) μm. Immature, 36–40 hr PI; mature, 40–64 hr PI (Fig. 4); peak at 48–56 hr PI; 24–36 merozoites (6.6 × 1.1 μm). In clusters of four to 12 meronts, generally about ½ way up the villi at 40–48 hr PI, later at tip.

*4th generation meront:* Duodenum and a few in the jejunum; 7.1 (5.9–8.2) × 6.2 (4.7–7.0) μm. Immature, 40–48 hr PI; mature, 56–84 hr PI and beyond (Fig. 5); peak at 56–60 hr PI; 12–24 merozoites (6.6 × 1.1 μm). In clusters from ⅓ above the base of the villi to the tip.

*5th generation meront:* Duodenum, jejunum, occasionally in the ileum; 20.2 (17.6–23.5) × 12.8 (11.8–14.1) μm. Immature, 60–64 hr PI; mature, 72–108 hr PI and beyond (Fig. 6); peak at 72 hr PI, scarce later; 50+ merozoites (7.2 × 1.5 μm). Numerous, sometimes several per cell, residual body often present.

*Gamonts:* Most numerous in the duodenum and jejunum and moderate in the ileum although some found in the cecal neck, cecal pouch, and large intestine; macrogametocytes 16.7 (16.0–18.2) × 12.8 (11.3–14.0) μm; microgametocytes 16.0 (15.2–17.4) × 12.7 (12.2–13.2) μm. Immature, 72 hr PI on (Fig. 7), very numerous, sometimes as many as five/epithelial cell; mature 84 hr PI on; peak 108 hr on.

*Oocysts:* Oocysts were seen in tissue sections at 96 hr PI but not at 84 hr PI. Sporulated oocysts from the feces of bobwhites given the Pennsylvania strain averaged 21.1 × 17.2 μm (Table 1). The strain from Florida was slightly larger (23.1 × 19.1 μm) and in the same range. Isolating single large or small oocysts did not yield strains of consistent oocyst size (Table 1).

Sporulated oocysts each contained four sporocysts that averaged 10.7 × 4.8 μm. Stieda and substiedal bodies present, sporocyst residuum small. Each sporozoite

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FIGURES 1–8. Stages of *Eimeria lettyae* sp. n. from northern bobwhite. Figure 1, IFA–methylene blue stain, fluorescent microscopy, ×500; Figures 2–7, PAS stain, light microscopy, ×1,300; Figure 8, phase contrast, ×1,750. 1. 3 hr postinoculation (PI), sporozoite above host cell nucleus. 2. 40 hr PI, 1st generation meront. 3. 48 hr PI, 2nd generation meront. 4. 56 hr PI, 3rd generation meront. 5. 56 hr PI, 4th generation meront. 6. 72 hr PI, 5th generation meront (arrow), note numerous other meronts cut at various planes. 7. 72 hr PI, maturing macrogamete. 8. Sporulated oocysts, note refractile granule (RG) in oocyst wall.

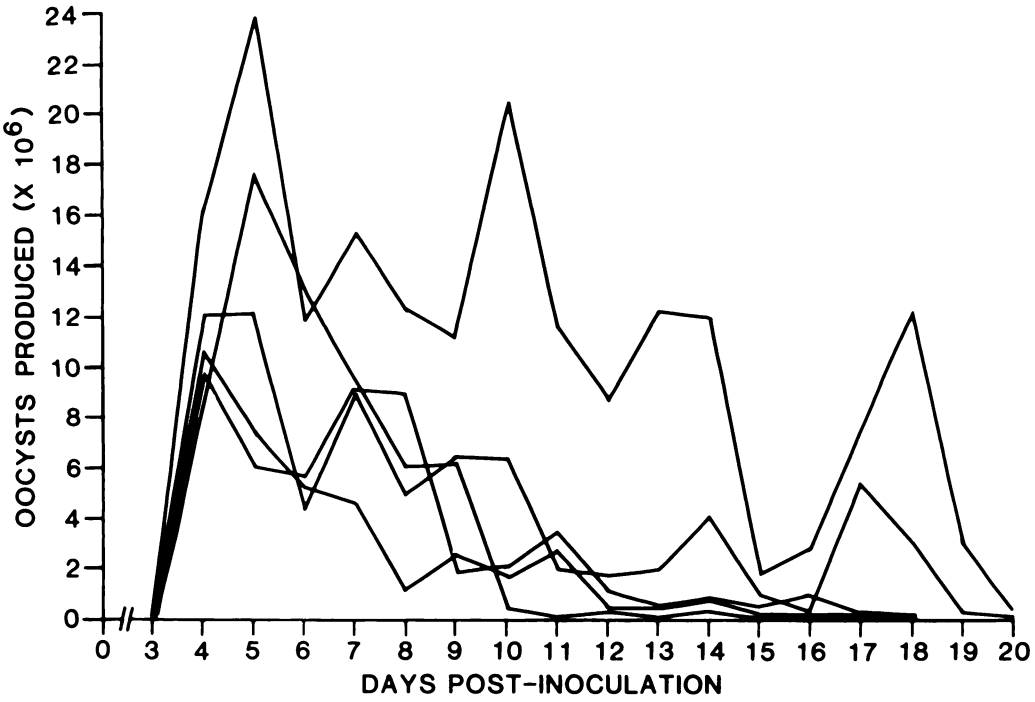


FIGURE 9. Daily oocyst production of *Eimeria lettyae* sp. n. in five individual bobwhites each given 1 × $10^3$ sporulated oocysts.

contained a large refractile body. The oocyst lacked a micropyle, oocyst residuum, and polar granules although a refractile granule was sometimes seen in the oocyst wall slightly to one side of the end (Fig. 8). Oocysts were relatively fragile and easily ruptured by pressure on a cover slip.

TABLE 2. Oocyst production of *Eimeria lettyae* sp. n. from five northern bobwhites, 2½ wk old. Feces collected 4 to 10 days postinoculation.

| Inoculation dose/bobwhite | Oocysts recovered/ bobwhite (×$10^6$) | Oocyst return/oocyst given (×$10^3$) |
|---|---|---|
| 1 × $10^2$ | 59 | 594 |
| 5 × $10^2$ | 189 | 267 |
| 1 × $10^3$ | 174 | 174 |
| 5 × $10^3$ | 91 | 18 |
| 1 × $10^4$ | 178 | 17 |
| 1 × $10^4$ | 178 | 17 |
| 5 × $10^5$ | 223 | 0.4 |
| 1 × $10^6$ | 72 | 0.07 |

*Sentinel bobwhite:* All uninoculated control bobwhites caged separately from inoculated bobwhites remained negative. Uninoculated sentinel bobwhites caged with inoculated bobwhites were negative at 96 and 192 hr PI. At 288 hr PI one of three sentinel bobwhites had a few developing gametes in the small intestine.

#### Prepatent period

The prepatent period in five bobwhites varied from 88 to 91 hr. One became patent at 88 hr, two at 89 hr, and two at 91 hr.

#### Oocyst production

The time of peak oocyst production was variable among individual bobwhites given 1 × $10^3$ oocysts each (Fig. 9). All bobwhites showed numerous oocysts in the feces by 4 days PI. The peak of oocyst production, however, lasted through as short a time as 7 days PI, to as long as 14

TABLE 3. Cross-immunity of two isolates of *Eimeria lettyae* sp. n. and *E. dispersa* in northern bobwhites.*

| Immunization: source of *E. lettyae* sp. n. | Challenge isolate | | Post-challenge oocysts recovered/ bobwhite ($\times 10^6$) | % Protection |
|---|---|---|---|---|
| | Species | Source | | |
| None | *E. lettyae* | Pennsylvania | 264 | 0 |
| None | *E. lettyae* | Florida | 466 | 0 |
| Pennsylvania | *E. lettyae* | Pennsylvania | 0.15 | 99.9 |
| Pennsylvania | *E. lettyae* | Florida | 11 | 97.6 |
| Florida | *E. lettyae* | Pennsylvania | 3 | 98.9 |
| Florida | *E. lettyae* | Florida | 0.08 | 99.9 |
| None | *E. lettyae* | Pennsylvania | 205 | 0 |
| None | *E. dispersa* | Alabama | 66 | 0 |
| Pennsylvania | *E. lettyae* | Pennsylvania | 2 | 99.0 |
| Pennsylvania | *E. dispersa* | Alabama | 63 | 4.5 |

* Bobwhites immunized with three oral doses of $1 \times 10^4$ oocysts of *E. lettyae* sp. n. given 4 days apart, challenged 21 days later with $1 \times 10^5$ oocysts/bobwhite of *E. lettyae* sp. n. or *E. dispersa*. Feces collected 5–9 days post-challenge.

days PI. Second and third peaks of production were sometimes seen. Oocyst production in any individual bobwhite did not again reach $1 \times 10^6$ oocysts/bobwhite/day after 19 days PI; however, all bobwhites continued to produce some oocysts through 67 days PI. One bobwhite was still producing oocysts on day 76 PI, the last day feces were examined.

When dose of inoculum was varied, the total number of oocysts recovered on days 4–10 PI was relatively constant for doses of $5 \times 10^2$–$5 \times 10^5$ oocysts/bobwhite (Table 2). Maximum oocyst return in this experiment was $594 \times 10^5$ oocysts/oocyst given with a dose of $1 \times 10^2$ oocysts. In some single oocyst isolations over $2 \times 10^6$ oocysts were recovered from a single bobwhite.

#### Sporulation time

All bobwhites given oocysts sporulated for 14 or 16 hr remained negative for coccidia based on examination of feces and intestinal scrapings. Bobwhites given oocysts sporulated for 18 or 20 hr had feces positive for oocysts 5 days later.

#### Cross-transmission

No parasites were found in feces or tissue of chickens, turkeys, pheasants, chukar partridges, or Japanese quail given oocysts of *E. lettyae* sp. n. All bobwhites given the same inoculum were positive. Attempts to infect turkeys with the isolate of *E. lettyae* sp. n. from Florida were also unsuccessful.

#### Cross-immunity

Immunizing bobwhite with the Pennsylvania or Florida strains protected against challenge with the homologous or reciprocal strain (Table 3). Immunizing with *E. lettyae* sp. n. gave no protection against challenge with *E. dispersa*. Immunizing bobwhite by 25 oral inoculations of $10^2$ or $10^3$ sporulated oocysts of *E. lettyae*/time did not eliminate oocyst production following challenge with $10^5$ oocysts/bobwhite.

## TAXONOMIC SUMMARY

### *Eimeria lettyae* sp. n.

*Diagnosis:* Oval oocysts averaging $21.1 \times 17.2$ μm with some as short as 16.4 μm. Micropyle, residuum, and polar granules lacking. Refractile granule sometimes present in oocyst wall. Oocysts not distinctly different from *E. dispersa*. Sporozoites measuring $10.7 \times 4.8$ μm with stieda and substiedal bodies. Five asexual generations in the bobwhite. Initial infection in the duodenum, extending to the

jejunum with later generations of meronts and finally to the ileum. Development of all generations above the nucleus of the host epithelial cell. Meronts located in the epithelium on sides and tips of villi rather than in the crypts. Gametes found throughout the digestive tract from the duodenum to large intestine including ceca. Prepatent period 88 to 91 hr. Oocyst production for extended periods from a single infection (up to 76 days). Sporulation in 18 to 20 hr at 25 C. Not infective for Japanese quail, turkeys, chicken, ring-necked pheasants, or chickens.

*Type host:* Northern bobwhite, *Colinus virginianus* (L.). Hapantotype slides in U.S. National Parasite Collection, Beltsville, Maryland, accession number 78748, and in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, D.C.

*Geographic range:* Cooperstown, Pennsylvania and Leon County, Florida.

*Etymology:* Named for the author's wife, Letty Williams Ruff.

## DISCUSSION

### Species identification

*Eimeria lettyae* sp. n. is distinctly different from the two other named species of *Eimeria* that can infect the northern bobwhite, *E. colini* and *E. dispersa*. The oocysts of *E. colini* are larger (24.8 × 20.9 μm). The shortest *E. colini* oocyst reported by Fisher and Kelley (1977) was 22.4 μm whereas *E. lettyae* sp. n. (present study) ranges down to 16.4 μm and averages only 21.1 μm in length. *Eimeria colini* possesses a micropyle whereas *E. lettyae* sp. n. does not. Comparisons of life cycle stages and area of development between the two species are not possible as these were not described for *E. colini*. The variability in oocyst size, even within single oocyst isolates (Table 1), emphasizes the unreliability of oocyst measurements as the sole basis for species identification. Similarly, characteristics of the oocyst, sporocyst, sporozoites, meronts, and gametes are generally not, by themselves, sufficient for species identification of avian coccidia.

*Eimeria dispersa* is closer to *E. lettyae* sp. n. in oocyst size. Tyzzer (1929) reported that oocysts of *E. dispersa* from bobwhites measured 22.8 × 18.8 μm; Venard (1933) reported oocysts of 23.2 × 18.7 μm from quail. *Eimeria dispersa* also lacks polar granules. Although *E. lettyae* sp. n. and *E. dispersa* infect similar regions of the intestine, the latter species readily infects a variety of gallinaceous birds (Tyzzer, 1929) whereas the former is specific for the bobwhite. *Eimeria dispersa* has two generations of meronts whereas *E. lettyae* sp. n. has five. In addition Reid (1978) states that *E. dispersa* has a prepatent period of 144 hr in the bobwhite, much longer than the 88–91 hr of *E. lettyae* sp. n.

*Eimeria lettyae* sp. n. could be the same species reported as *E. acervulina* by Venard (1933) except that he found polar inclusions. It could also be the same as the unnamed species of Prostowo and Edgar (1970) although they found only two generations of meronts that were slightly larger than the 3rd and 4th generation meronts described in the present paper.

### Development

It is unclear whether the first meronts found with light microscopy at 32–40 hr PI are 1st or 2nd generation meronts of *E. lettyae* sp. n. This question develops from the progressive changes seen in the IFA studies. The gradual reduction in the number of visible sporozoites between 12 and 24 hr could come either from the movement of sporozoites out of the intestine to other tissues or the maturation of the sporozoite/trophozoite to young meronts that were not reactive with the monoclonal antibody used in the IFA stain. The specific monoclonal antibody used reacts with the refractile body of the sporozoite

of all coccidia of chickens and turkeys examined to date. However, the monoclonal antibody does not cross-react with the 1st generation merozoites of the coccidia of chickens and turkeys (Augustine and Danforth, pers. comm.). The reason for the sudden appearance of large numbers of sporozoites and/or merozoites at 48 hr when only a few were seen at 40 hr is unclear. It is not known if the fluorescing parasites are reappearing sporozoites and 1st generation meronts or if the 1st generation meront develops elsewhere and the fluorescing parasites are 1st generation merozoites and 2nd generation meronts. Regardless, the occurrence of fluorescing merozoites in the meronts demonstrates that sporozoite antigen reactive with the monoclonal antibody is still found in at least one later generation of meronts and merozoites of *E. lettyae* sp. n.

#### Oocyst production

Oocyst production by *E. lettyae* sp. n. is unusually numerous for a species infecting birds. For example, in our laboratory *E. acervulina*, one of the most prolific species infecting chickens, will produce a maximum of 600–700 $\times$ $10^6$ oocysts/chicken; at a maximum rate of less than 0.2 $\times$ $10^6$ oocysts recovered/oocyst given. Less prolific species such as *E. tenella* and *E. maxima* produce less than 50 $\times$ $10^6$ oocysts/chicken. This is from White Leghorns weighing approximately 250 g at the time of inoculation. Although the bobwhite in these studies were only $\frac{1}{5}$ as big (approximately 50 g) they produced a maximum of over 200 $\times$ $10^6$ oocysts/bobwhite with a maximum rate of over 2 $\times$ $10^6$ oocysts recovered/oocyst given. The five asexual generations are more than are generally found in avian species of *Eimeria* and are probably a factor in the large reproductive capabilities of *E. lettyae* sp. n. Another factor is the continued passage of oocysts by infected bobwhites for extended periods. A similar long-term passage of *E. dispersa* oocysts from the bobwhite was reported in Tyzzer (1929) as a personal observation of H. L. Stoddard. Late cycling seems to occur either by some 5th generation merozoites forming additional 5th generation meronts, or meronts indistinguishable from those of the 5th generation, instead of gametes, or by delayed maturation of 5th generation meronts. Late production of oocysts from an additional asexual generation has been described in *E. meleagrimitis* (Ruff et al., 1980). Reinfection cannot be entirely eliminated as the source of late oocyst passage. Although eight uninoculated sentinel bobwhites caged with inoculated bobwhites remained negative, one bobwhite had a few developing gametes when tissue sections were examined at 288 hr PI. Because oocysts can sporulate in 18 hr, moving bobwhites to clean cages every 24 hr, as was done in this experiment, does not insure that reinfection was prevented.

#### Cross-transmission and cross-immunity

The cross-transmission studies showed that *E. lettyae* sp. n. is very host specific. The cross-immunity studies showed that isolates from Pennsylvania and Florida were the same species suggesting that *E. lettyae* sp. n. has a wide geographic range. The same studies showed that the isolate from Pennsylvania was not *E. dispersa*. Some oocysts were passed after challenge of immunized quail, even those immunized 25 times; thus, infected bobwhites do not develop solid immunity and can continue to serve as a source of infection to other bobwhites for several months.

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