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PATHOGENICITY OF *EIMERIA LETTYAE* RUFF, 1985 IN THE NORTHERN BOBWHITE (*COLINUS VIRGINIANUS* L.)

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ABSTRACT: Sporulated oocysts of *Eimeria lettyae* were administered orally to 5-day-old or 18-day-old northern bobwhites (*Colinus virginianus*, L.). Five-day-old bobwhites were more susceptible based on higher mortality and reduced weight gain. A dose of 5×10^5 oocysts produced 25–43% mortality in 5-day-old bobwhites, but none in 18-day-old bobwhites. A dose of 1×10^6 oocysts/bobwhite produced 83–100% mortality in 5-day-old bobwhites, and 17–83% mortality in 18-day-old bobwhites. Body weight gain was reduced significantly with a dose of 1×10^5 oocysts or greater in 5-day-old bobwhites and with a dose of 5×10^5 oocysts or greater in 18-day-old bobwhites. Infection in all age groups reduced concentrations of plasma pigment and plasma protein, but did not affect packed cell volumes. No grossly visible lesions were present in the intestine although there was a shortening of the villi in the duodenum. In mature bobwhites, infection with *E. lettyae* did not cause mortality, but did reduce egg production and fertility.

INTRODUCTION

In 1981, a game-farm in Pennsylvania experienced an outbreak of coccidiosis in growing northern bobwhites. Signs of disease included loose droppings and mortality. The coccidium involved proved to be a new species subsequently named *Eimeria lettyae* (Ruff, 1985). The studies reported are experimental investigations on the pathogenicity of that species in northern bobwhites.

MATERIALS AND METHODS

Coccidia

The source of oocysts was a culture composed of pooled oocysts from four separate single oocyst isolations of *E. lettyae*, as described by Ruff (1985). Oocysts were harvested from feces, stored at 4 C in 2.5% potassium dichromate, and used before they were 4 mo old. Oocysts were washed and resuspended in tap water before bobwhites were inoculated. All doses of oocysts were based on the number of sporulated oocysts counted with a hemocytometer. Bobwhites were administered oocysts orally into the crop.

Bobwhites

Bobwhites were hatched from eggs produced at the laboratory. They were raised in wire-floored cages and provided unmedicated turkey starter (30% protein) and water ad libitum. All bobwhites were kept on constant lighting. Uninoculated bobwhites were checked periodically by microscopically examining intestinal scrapings and concentrated (sugar floated) feces. These bobwhites remained uninfected.

Experimental design

Bobwhites were divided into groups based on the weight distribution method of Gardiner and Wehr (1950). Blood was collected by cardiac puncture using heparin as an anti-coagulant. Plasma protein was determined by a modified Lowry method (Layne, 1957) and plasma pigment by the method of Wilson (1956). Infections were confirmed by microscopic examination of the intestine at the time of necropsy. Stained sections of infected intestine were examined also. Data were analyzed using analysis of variance and Duncan's multiple range test ($P \leq 0.05$). Design of individual experiments was as follows.

Experiment 1: Thirty bobwhites, 5 days old, were divided into five groups of six bobwhites each. Groups were inoculated with 0, 1×10^3, 1×10^4, 1×10^5, or 1×10^6 sporulated oocysts/bobwhite, respectively. Thirty bobwhites, 18 days old, were treated in a like manner. Individual bobwhite were weighed and mortality was recorded daily for 8 days.

Experiments 2 and 3: Twenty-eight bobwhites, 5 days old, were divided into four groups of seven bobwhites each (Experiment 2). Groups were inoculated with 0, 1×10^3, 5×10^5, or 1×10^6 sporulated oocysts/bobwhite, respec-

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TABLE 1. Mortality (%) in northern bobwhites orally administered sporulated oocysts of *Eimeria lettyae*.[a]

Age at inoculation	Expt.	Inoculation dose (oocysts/quail)					
		0	1×10^3	1×10^4	1×10^5	5×10^5	1×10^6
5 days	1	0	0	0	14	—[b]	100
	2	0	0	—	—	43	100
	3	0	0	—	—	25	83
18 days	1	0	0	0	17	—	83
	2	0	0	—	—	0	17
	3	0	0	—	—	0	50

[a] Mortality through 8 days postinoculation (DPI), Exp. 1; 6 DPI, Exp. 2 and 3.
[b] — = not done.

tively. Twenty-eight bobwhites, 18 days old, were treated in a like manner. Mortality was recorded daily. Six days postinoculation (DPI), each bobwhite was weighed and blood collected. Sufficient blood could not be collected for testing from bobwhites inoculated at 5 days of age. Parameters measured were weight gain, packed cell volume (PCV), plasma pigment, and plasma protein.

The design in Experiment 3 was the same except that there were 12 bobwhites/group and only six bobwhites from each group, selected at random, were bled.

Experiment 4: Nine male and 21 female mature bobwhites, 22 wk old, were divided into three groups of three male and seven female bobwhites each. Daily egg production for each group was recorded for 14 days. Each group was then inoculated with 0, 1×10^4, or 1×10^6 sporulated oocysts/bobwhite, respectively. Daily egg production was recorded for an additional 12 days. In addition, unbroken eggs were set for hatching. Unhatched eggs were broken out and fertility recorded. Production was expressed as eggs/female/day, percent fertile eggs, and percent hatched of fertile eggs.

Experiment 5: Fifteen male and 15 female bobwhites, 22 wk old, were weighed and divided into three groups of five males and five females each. Groups were then inoculated with 0, 1×10^4, or 1×10^6 sporulated oocysts/bobwhite, respectively. Individual bobwhites were weighed 5 days later.

RESULTS

Young bobwhites (5 days old when inoculated) were more susceptible to infection with *E. lettyae* than older bobwhites (18 days old when inoculated) as mortality was greater, sometimes reaching 100%, and occurred with a lower dose of oocysts (Table 1). A dose of 1×10^6 oocysts/bobwhite was generally required to produce mortality in 18-day-old bobwhites whereas 5×10^5 oocysts/bobwhite produced sig-

TABLE 2. Weight (g) of northern bobwhites 6 days after oral administration of sporulated oocysts of *Eimeria lettyae*.[a]

Age at inoculation	Experiment	Initial weight (g)	Inoculation dose (oocysts/quail)			
			0	1×10^3	5×10^5	1×10^6
5 days	1	13.9	26.8[A]	23.7[A]	—[b]	13.6[B]
	2	11.6	23.8[A]	19.6[A]	—	—
	3	15.0	29.5[A]	27.6[A]	17.4[B]	17.2[B]
18 days	1	49.7	65.0[A]	64.4[A]	—	27.3[B]
	2	51.2	69.2[A]	72.0[A]	48.8[B]	48.4[B]
	3	50.2	70.8[A]	68.2[A]	47.8[B]	40.3[B]

[a] Values within a row followed by a common capital letter are not significantly different ($P \leq 0.05$).
[b] — = not done.

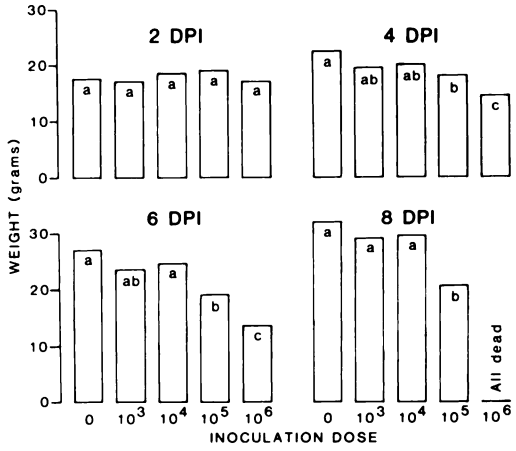

FIGURE 1. Body weight (g) at various days post-inoculation (DPI) in 5-day-old northern bobwhites inoculated with various dosages of sporulated oocysts of *Eimeria lettyae*, Experiment 1. Values within a day followed by a common letter are not significantly different ($P \leq 0.05$).

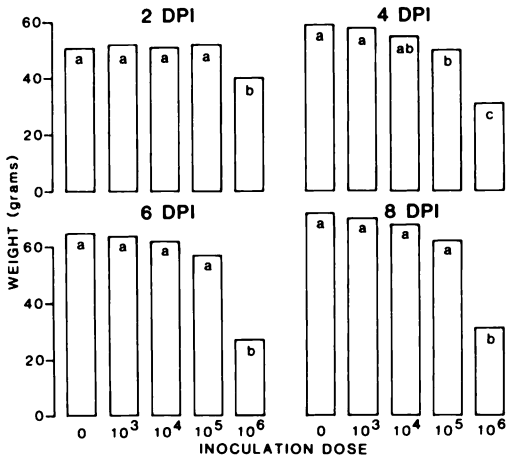

FIGURE 2. Body weight (g) at various days post-inoculation (DPI) in 18-day-old northern bobwhites inoculated with various dosages of sporulated oocysts of *Eimeria lettyae*, Experiment 1. Values within a day followed by a common letter are not significantly different ($P \leq 0.05$).

nificant mortality in 5-day-old bobwhites. Mortality occurred on days 4–6 PI in both age groups. No coccidiosis-induced mortality was seen in mature bobwhites.

Bobwhite inoculated with 5×10^5 or more oocysts exhibited typical outward signs of coccidiosis 4 days later. These signs included listlessness, droopiness, and anorexia. There was, however, almost a complete lack of macroscopic lesions in the intestine other than a slight bleaching. Hemorrhage was not seen and the mucosal surface appeared normal when observed with a dissecting microscope (i.e., no necrosis or disruption of the surface was evident). Watery intestinal contents were sometimes seen, but were by no means consistent.

Microscopic examinations were made of stained tissues from bobwhites given 1×10^6 oocysts. There was no evidence of necrosis or intestinal sloughing in infected tissue. Epithelial cells completely covered the vilar tip. There was, however, a marked shortening of the villi at 4 DPI. Villi in the duodenum of control bobwhites, and those infected for 3 DPI or less, measured 1.18 to 1.44 nm. At 4 DPI, villi in the duodenum measured 0.60 to 0.74 nm. Recovery was evident by 8 DPI when villi measured 0.79 to 1.21 nm.

Infection with *Eimeria lettyae* depressed weight gains of bobwhites (Table 2). This depression was significant with oocyst doses of 1×10^5 or more in 5-day-old bobwhites and 5×10^5 or more in 18-day-old bobwhites. With higher doses of oocysts, there was an actual weight loss by 6 days after inoculation. Depression of weight gain was evident as early as 4 DPI in 5-day-old bobwhites (Fig. 1) and by 2 DPI in 18-day-old bobwhites (Fig. 2) and persisted through 8 DPI. When mature bobwhites in production were weighed over a 5-day period (Experiment 5), there was a slight but significant weight loss even in uninoculated bobwhites; 5 and 12 g in males and females, respectively. The weight loss was, however, greater in males given 1×10^4 or 1×10^6 oocysts and females given 1×10^6 oocysts, a loss of 15, 16 and 22 g/bobwhite, respectively.

Packed cell volume was not affected by infection (Table 3). However, plasma pig-

TABLE 3. Effect of infection with *Eimeria lettyae* on packed cell volumes, plasma pigments and plasma proteins at 6 DPI in northern bobwhites 18 days old at time of inoculation.*

Parameter	Experiment	Inoculation dose (oocysts/quail)			
		0	1×10^3	5×10^5	1×10^6
Packed cell volume (%)	2	29.7[A]	31.5[A]	27.4[A]	32.9[A]
	3	34.7[A]	30.8[A]	31.6[A]	29.7[A]
Plasma pigment (μg/ml)	2	6.30[A]	5.02[A]	1.20[B]	1.25[B]
	3	5.99[A]	4.01[AB]	2.01[B]	1.83[B]
Plasma protein (mg/100 ml)	2	2.43[A]	2.40[A]	2.38[A]	1.94[A]
	3	2.43[A]	2.22[A]	2.34[A]	1.61[B]

* Values within a row followed by a common capital letter are not significantly different ($P \leq 0.05$).

ment decreased with dosages of 5×10^5 or 1×10^6 oocysts. Plasma protein decreased at a dosage of 1×10^6 oocysts although the decrease was significant only in one experiment.

Daily egg production varied from 0.35–0.67 eggs/female/day in uninoculated bobwhites (Table 4). Inoculation with 1×10^4 oocysts/bobwhites caused a drop in egg production beginning at 4 DPI. Egg production decreased at 2 DPI with 1×10^6 oocysts/bobwhite and egg production ceased on days 7 to 10 PI. The decreased egg production with 1×10^4 oocysts/bobwhite was not accompanied by any significant change in percent fertility (64% vs. 67%) although hatchability was reduced slightly (86% vs. 62%, for eggs from uninoculated control and inoculated bobwhites, respectively). No fertile eggs were produced in the first 9 days following inoculation with 10^6 oocysts.

DISCUSSION

Experimental infections with large numbers of oocysts of *Eimeria lettyae* were very detrimental to young bobwhites based on increased mortality, depressed weight gain, and reductions in blood values for pigment and protein. These changes occurred even though no pathological changes were observed in the intestine either macroscopically or microscopically other than the presence of parasites and a shortening of the villi at 4 DPI.

The relatively high mortality (up to 83%) in bobwhites inoculated at 18 days of age is unusual compared with other coccidial infections in bobwhites and Japanese quail (*Coturnix coturnix japonica*). Tyzzer (1929) reported that infections with large numbers of oocysts of *Eimeria dispersa* produced high mortality if infection occurred during the first week of hatching, whereas in bobwhites 2 wk of age or older only stunting and diarrhea developed. Similarly, a dosage of 5×10^5 oocysts of a mixed culture containing 65% *Eimeria uzura* and 33% *Eimeria tsunodai* produced 100% mortality in 3-day-old Japanese quail, but only 8% mortality in 17-day-old quail (Ruff et al., 1984). *Eimeria uzura* alone failed to produce mortality in 3-day-old Japanese quail given 1×10^6 oocysts each. Norton and Pierce (1971) found little effect of *Eimeria bateri* on body weight even at dosages of 1.28×10^6 oocysts/quail. The pathogenicity of *Eimeria colini* (Fisher and Kelley, 1977) in bobwhites is unknown.

The failure of *E. lettyae* to produce grossly visible intestinal lesions precludes developing a lesion scoring system to measure severity of the infections as has been done with coccidia of chickens. The extreme pathogenicity of *E. lettyae* in the absence of consistent visible intestinal lesions is unusual, but not unknown with

TABLE 4. Egg production (eggs/female/day, $\bar{x} \pm$ SEM) of northern bobwhites orally administered sporulated oocysts of *Eimeria lettyae*.

Time period	Inoculation dose (oocysts/quail)		
	0 (Control)	1×10^4	1×10^6
−14 DPI[a]	0.37 ± 0.06	0.42 ± 0.06	0.50 ± 0.05
1–3 DPI	0.53 ± 0.09	0.52 ± 0.16	0.11 ± 0.13
4–6 DPI	0.67 ± 0.24	0.11 ± 0.13	0.19 ± 0.06
7–9 DPI	0.35 ± 0.03	0.20 ± 0.13	0 ± 0
10–12 DPI	0.67 ± 0.16	0.40 ± 0.14	0.14 ± 0.10

[a] DPI = days postinoculation; −14 DPI = 14-day period prior to inoculation.

avian coccidia. *Eimeria mitis* in chickens can produce marked weight depression, malabsorption of nutrients, and decreased plasma pigment concentrations even though that species does not produce gross intestinal lesions (Joyner, 1958; Ruff and Edgar, 1982). Furthermore, with turkey coccidiosis, parasites and malabsorption of nutrients can be found without any evidence of intestinal damage (Ruff et al., 1981).

The shortening of the villi with *E. lettyae* differs somewhat from that seen with *E. acervulina* in chickens. With the latter infection, there is evidence of necrosis at the tips of the villi (Witlock and Ruff, 1977) that was not seen in the present study. The shortening of the villi would, however, reduce the surface area of the duodenum available for nutrient absorption. This malabsorption could contribute to the depressed weight gain as would anorexia. Reduced feed intake was observed in infected bobwhites in these studies, but was not quantitated.

The changes in blood values support the pathogenicity of *E. lettyae*. The lack of change in PCV is not surprising because no evidence of hemorrhage was seen in the intestine. The plasma pigment concentrations were lower in uninoculated bobwhites than the concentrations found in chickens given a similar concentration of carotenoids in the ration. The pigment measured may, therefore, be β-carotene and other forms of vitamin A rather than xanthophyll. Regardless, pigment concentrations were depressed by infection, possibly due to malabsorption of vitamin A and carotenoids from the diet, a common effect of intestinal coccidiosis in other avian species (Ruff, 1978). The reason for the decrease in plasma protein is unknown. It may reflect changes in metabolism induced by the infection or it may result from efflux of protein into the lumen of the intestine because of permeability changes caused by the infection.

The pathologic effects of the infection such as mortality, depressed weight gain, and shortening of the villi in the duodenum occurred by 4 DPI. This would suggest that the major pathogenic life cycle stages were the 5th generation meronts and gametes that immediately precede these major effects (Ruff, 1985).

Eimeria lettyae did not markedly affect weight in mature birds nor did mortality occur. Egg production and fertility, however, were reduced, especially at higher inoculation dosages. The loss of fertility may be related to reduced frequency of mating due to general malaise.

These results, coupled with the prolonged output of large numbers of oocysts reported by Ruff (1985) would suggest that *E. lettyae* constitutes a potential hazard for bobwhite of all ages under pen-rearing conditions. Under wild, free-ranging conditions, *E. lettyae* should pose little problem because relatively high numbers of oocysts are needed to produce severe dis-

ease and individual wild bobwhites would be unlikely to ingest that many oocysts in a short period under natural conditions.

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