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SURVEY FOR COCCIDIA AND HAEMOSPORIDIA IN THE LESSER PRAIRIE-CHICKEN (*TYMPANUCHUS PALLIDICINCTUS*) FROM NEW MEXICO WITH DESCRIPTION OF A NEW *EIMERIA* SPECIES

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ABSTRACT: Blood films and fecal samples of the lesser prairie-chicken (*Tympanuchus pallidicinctus*) were examined for parasites when we surveyed specimens captured during a radiotracking study conducted in Chaves County, New Mexico (USA). All birds were captured on the Caprock Wildlife Habitat Management Area, administered by the Bureau of Land Management. Samples were collected in late March, April, and early May 1998–2000. Oocysts were detected in five of 64 (8%) birds sampled and, upon sporulation, were determined to be an *Eimeria* species. This is the first eimerian reported from the lesser prairie-chicken and is described here as a new species. Sporulated oocysts are ellipsoidal, 27.1×22.7 (22–32×18–26) μm, with micropyle absent, but oocyst residuum and polar granule present. Sporocysts are ovoidal, 11.9×7.8 (10–14×6–10); a Stieda body, and sporocyst residuum are present, as is a small, indistinct substieda body. Inspection of blood smears revealed four cases of *Plasmodium* infection of 32 (13%) individuals sampled. The characteristics of this plasmodiid are consistent with the description of *Plasmodium (Giovannolaia) pedioecetii,* previously found in *T. pallidicinctus* (Stabler, 1978).

Key words: Eimeria tympanuchi, lesser prairie-chicken, new species, *Plasmodium (Giovannolaia) pedioecetii, Tympanuchus pallidicinctus.*

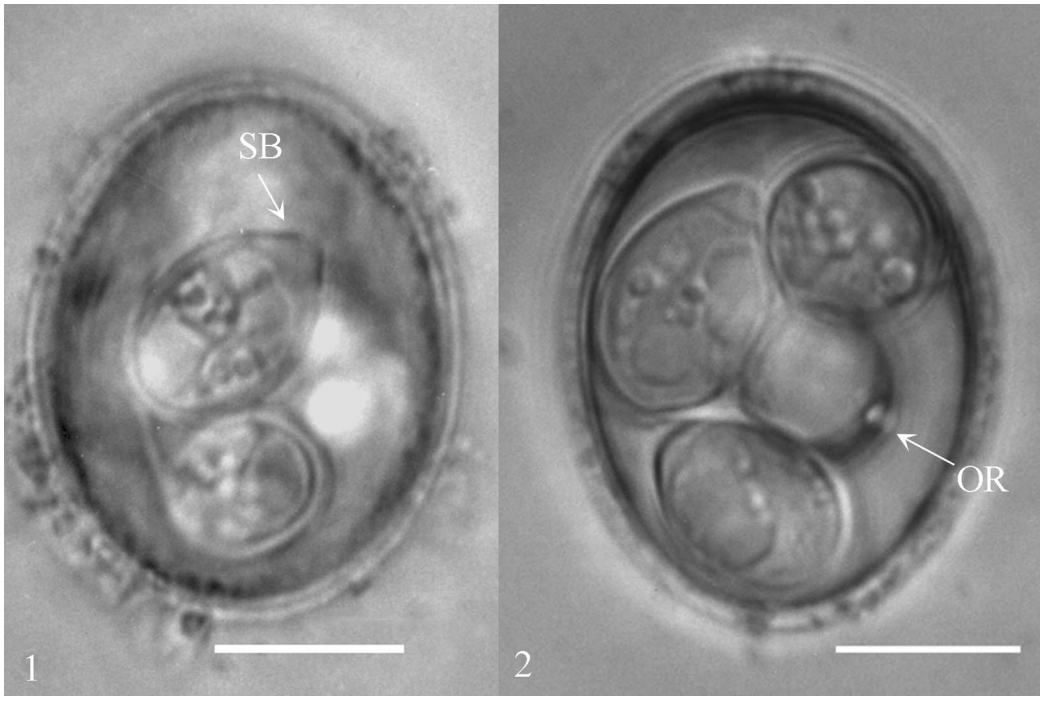
INTRODUCTION

The lesser prairie-chicken *Tympanuchus pallidicinctus* (Phasianidae: Tetraoninae) is a lekking grouse species found in the dry short grass prairie of Colorado, Kansas, New Mexico, Oklahoma, and Texas (USA). In New Mexico, the shinnery oak (*Quercus havardii*) shrublands of the Staked Plain are the principal breeding grounds. A 4 yr trapping and banding study of *T. pallidicinctus* was conducted in Chaves County, eastern New Mexico. In the last 3 yr of the study, blood and fecal samples were collected in the field. This population of prairie-chickens inhabits rangeland managed for wildlife, grazing, and oil and gas development. Recent declines in *T. pallidicinctus* populations are a consequence of habitat alteration and drought (Giesen, 1998). Chemical control of shinnery oak has further reduced available nesting and brood-rearing habitat. The species is a candidate for federal protection as threatened by the United States Fish and Wildlife Service. This study found the eastern New Mexico population of *T. pallidicinctus* positive for *Eimeria* and *Plasmodium* species. Parasite load is not currently perceived as a significant contributor to lesser prairie-chicken mortality. However, if remaining lesser prairie-chicken populations become concentrated into smaller areas of acceptable habitat, parasite transmission rates could rise (Dobson and May, 1986).

MATERIALS AND METHODS

Lesser prairie-chickens were trapped in the wild on the Bureau of Land Management administered Caprock Wildlife Habitat Management Area, Chaves County, New Mexico (33°28′22″N, 103°47′44″W). Members of four leks were sampled for blood and fecal parasites (Johnson and Smith, 1998, 1999). All birds screened for parasites were trapped within 3,200 ha of contiguous habitat. This constitutes a small percentage of the extant New Mexico population. We trapped birds in circular, welded-wire, walk-in traps connected by chicken-wire drift fences (Toepfer et al., 1987). Samples were taken in late March, April, and early May, from 1998–2000. Average capture time was 6:30 AM (0500–08:15 AM, $n=56$), with only one bird captured after 8:00 AM. Each bird was temporarily detained in a small cardboard box. Fecal samples were collected from holding boxes, or during the banding and release process, and transported to the University of New

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FIGURES 1, 2. Photomicrographs of sporulated oocysts of *Eimeria tympanuchi*. SB=Stieda body, OR=oocyst residuum. Bar=10 μm.

Mexico (UNM; Albuquerque, New Mexico). Morphologic data including weight, tarsus length, wing chord, culmen depth, and length of pinna were taken on each donor bird, as well as age, sex, date, time, and location of capture. The unique identity of each bird was kept via a numbered aluminum leg band provided by the New Mexico Department of Game and Fish.

Fecal samples were placed in separate vials containing 2% aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$). In the laboratory each sample was incubated at room temperature and processed following the methods outlined in Duszynski and Wilber (1997). All samples were examined and oocysts measured and photographed within 1 yr of collection. Photosyntypes of sporulated oocysts are deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland (USA) following Bandoni and Duszynski (1988) and Duszynski (1999).

We drew 10–50 μl of blood from captured birds using brachial venipuncture. Smears of whole blood were made in the field. Each slide was fixed in 100% methyl alcohol to preserve the cells, and later were stained with Giemsa's blood stain. Smears were scanned under oil immersion (1,000×) to detect the presence of blood parasites. Blood films were scanned for 20 min (≥150 fields/film) for hematozoa, including *Plasmodium, Haemoproteus, Leucocytozoon,* trypanosomes, and microfilarie. Prevalence of parasitemias was determined from four randomly selected counts of 500 erythrocytes (n=2,000) per positive slide (Godfrey et al., 1987). Positive slides were read extensively in order to best describe life stage characteristics. All measurements of *Eimeria* and *Plasmodium* species are in micrometers with means followed by ranges in parentheses.

RESULTS

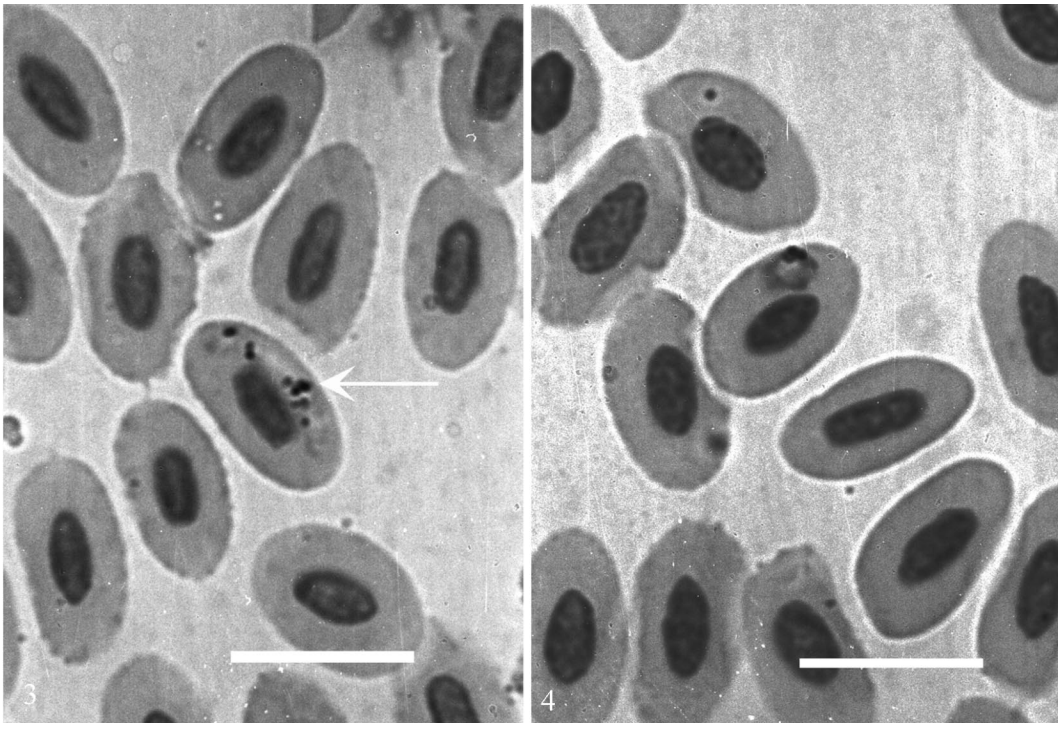
The birds we examined were infected with one *Eimeria* species, described here as new, and with one malarial species, *Plasmodium (Giovannolaia) pedioecetii* first described by Shillinger (1942), emended by Stabler et al. (1973).

Eimeria tympanuchi n. sp. (Figs. 1, 2, 5)

Description

Sporulated oocysts (n=56) ellipsoidal, 27.1×22.7 (22–32×18–26); shape index (SI=length/width)=1.2 (1.0–1.5); wall smooth in optical cross-section, 1.8 (1.0–

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FIGURES 3, 4. Photomicrographs of *Plasmodium (Giovannolaia) pedioecetii* macrogametocyte (3, arrow) and ring stage (4). Bar=10 μm.

2.5) thick, with two layers; outer, lightly striated, ~2/3 of total thickness; micropyle absent; usually one small, refractile polar granule present; two to five oocyst residual bodies present as round, opaque spheres. Sporocysts (n=56) ovoidal, 11.9×7.8 (10–14×6–10) with L : W ratio 1.5 (1.2–2.0); Stieda body present at pointed end of sporocyst, with evidence that a small, indistinct substieda body also may be present immediately below it; sporocyst residuum present, comprised of small uniformly shaped spheres in a cluster, approximately two, partially obscuring sporozoites; sporozoites with two refractile bodies, a larger one at the blunt end and a second, smaller body at pointed end with a nucleus visible between them.

Taxonomic summary

Type host: Tympanuchus pallidicinctus (Ridgway, 1885), lesser prairie-chicken (Phasianidae: Tetraoninae).

Type locality: USA, New Mexico, Chaves County, 33°28′22″N, 103°47′44″W.

Prevalence: 5/64 (8%).

Site of infection: Unknown, oocysts recovered from feces.

Material deposited: Photosyntypes of sporulated oocysts in the USNPC, No. 092386.00.

Etymology: The specific name is derived from the generic name of the host.

Remarks: There are now 15 *Eimeria* species described from grouse and ptarmigan (Phasianidae:Tetraoninae) (Table 1). The sporulated oocysts of *E. tympanuchi* are distinguished from the others by the presence of a distinct oocyst residuum, lacking a micropyle, and the presence of both Stieda and substieda bodies (see Table 1).

Plasmodium (Giovannolaia) pedioecetii (Shillinger, 1942) Stabler et al., 1973 (Figs. 3, 4)

Plasmodium was detected in five of 34 (17%) samples. The samples come from 32

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TABLE 1. Mensural characters of sporulated oocysts of *Eimeria* species known from the subfamily Tetraonimae as given in the original description (NG = measurements, structures not given/mentioned) and/or in subsequent re-descriptions of sporulated oocysts.

Host/*Eimeria* spp.	Oocyst		Sporocyst		Qualitative wall structures		Author(s)
	Length×width (range)	L/W (range)	Length×width (range)	L/W (range)	Thickness (μm)	Present[1]	
Bonasa umbellus							
E. angusta	32.5×17.1 (28–37×15–19)	1.9 (1.7–2.2)	14.8×6.1 (12–17×5–8)	2.4 (2.0–2.9)	1.5–2.0	M, PG, SB, SR	Allen, 1934; Todd and Lepp, 1970
E. bonasae	21.6×20.6 (18–25×18–23)	1.05 (1.0–1.2)	12.8×7.1 (10–14×6–8)	1.8 (1.6–2.0)	1.5	PG, SB, SR	Allen, 1934; Todd and Lepp, 1970
Centrocercus urophasianus							
E. centrocerci	22.6×17.1 (17–25×13–18)	1.3 NG	11.8×7.6 (11–13×7–8)	1.6 NG	?	M(?), PG, SB, SR	Simon, 1939; Honess and Post, 1955
E. pattersoni	20.2×13.5 (18–23×12–15)	1.5 NG	NG	NG	?	M(?)	Honess and Post, 1955
Lagopus leucurus							
E. leucuri	26.6×17.7 (22–31.5×15–20)	1.5 NG	15.4×6.7 (13–18×6–7)	2.3 NG	1.5	M, MC, PG, SB, SR	Stabler et al., 1979
E. oreoecetes	26.0×22.6 (23–29×20–26)	1.2 NG	14.6×8.8 (13–16×8–10)	1.6 NG	2.0	PG, SB, SSB, SR	Stabler et al., 1979
Lagopus mutus							
E. brinkmanni	28.6×18.8 (26–30×18–20)	1.5 (1.4–1.6)	13×7 NG	1.9 NG	NG	PG, SB	Levine, 1953
E. fanthami	28.3×18.8 (27–29×18–20)	1.5 (1.4–1.6)	NG	NG	NG	PG, SB	Levine, 1953
E. lagapodi	24×15 NG	1.6 NG	12×12 NG	1.0 NG	NG	M(?)	Galli-Valerio, 1929
Tetrao tetrix							
E. lyruri	29.6×15.3 (22–37×12–20)	1.9 NG	9.8×3.6 NG	2.7 NG	NG	PG, SR	Galli-Valerio, 1927; Yakimoff and Gousseff, 1936
E. nadsoni	24.9×21.3 (21–29×17–24)	1.2 NG	12.2×10.9 NG	1.1 NG	1.5–2.0	PG, SB(?)	Yakimoff and Gousseff, 1936
E. tetricis	31×15 (30–31×15–15)	2.1 NG	NG	NG	1.2	M, MC(?), SB, SR	Haase, 1939

TABLE 1. Continued.

Host/*Eimeria* spp.	Oocyst		Sporocyst		Qualitative wall structures		Author(s)
	Length×width (range)	L/W (range)	Length×width (range)	L/W (range)	Thickness	Present[1]	
T. urogallus							
E. ventriosa	32×22 (31–33×20–23)	1.4 NG	NG	NG	1.0	M, PG, SB, SR	Haase, 1939
E. yakisevi	20 (24–19.5)	NG	10.8×5.1 NG	2.1 NG	NG		Yakimoff and Gousseff, 1936; Hardcastle, 1943
Tympanuchus pallidicinctus							
E. tympanuchi	27.1×22.7 (22–32×18–26)	1.2 (1.0–1.5)	11.9×7.8 (10–14×6–10)	1.5 (1.2–2.0)	1.8	OR, PG, SR, SB, SSB	This study

[1] Oocyst and sporocyst qualitative structures present in each species. Abbreviations: M=micropyle; MC=micropyle cap; OR=oocyst residuum; PG=1 or more polar granules; SB=Stieda body; SSB=substieda body; SR=sporocyst residuum. If not stated in the original description or the re-description, this structure is presumed to be absent although this is not always certain in some of the older work.

FIGURE 5. Line drawing of a sporulated oocyst of *Eimeria tympanuchi*. Bar=6 μm.

subjects, because two birds were captured and screened in successive years. One individual was found positive in successive years, the only infected bird either recaptured or resighted between years. Therefore, four of 32 possible hosts were infected (13%). Intensity of each case (0.005–0.007, n=5) suggests hosts persisted with chronic infections.

Trophozoites either were ameboid (78%) with one to five ($\bar{x}$=1.5) small pigment granules or were ring-shaped (22%), with central vacuoles and one to four aggregated pigment granules (Fig. 4); 70% of all trophozoites seen were subpolar or polar.

Gametocytes (n=29) appeared immature and measured 7.5×1.9 (6.25–10×1.25–2.5); 25/29 (86%) were found on the lateral edge of the nucleus without apparent alteration of nuclear position or cell wall. Pigment granules, 3.4 (1–11), were often on the edge of developing gametocytes. Mature gametocytes (Fig. 3) were elongate and narrow, with irregular edges and with pigment granules throughout their cytoplasm. The ends of the mature gametocyte usually curled around the polar ends of the nucleus without distortion

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of erythrocyte shape. Only one mature segmenter was seen with nine merozoites visible in a tight cluster. It did not distort the erythrocyte's shape or the position of its nucleus.

The stage of the life cycle most evident was ameboid trophozoites in erythrocytes (60%). The gamont life stages were often immature (66%), hence the observation of mean length and width falls beneath the stated means of Stabler (1978). Mature gametocyte dimensions and morphology are consistent with *P. (Giovannolaia) pedioecetii.* Length/width measurements of a subset of nine mature gametocytes, 9.5×2.1, almost exactly reflects the means reported by Stabler (1978), 9.2×2.0. Gametocytes from the present study, however, were more prone to lateral orientation (85%) than those reported by Stabler (33.8%), in 1978.

DISCUSSION

There are currently 15 *Eimeria* spp. described from the subfamily Tetraoninae, including the new species described here. The systematics of grouse and ptarmigan were recently amended, and this paper follows the American Ornithologists' Union (1998) classification of species within Tetraoninae. The Tetraoninae has six genera including *Tympanuchus,* which has three species: *T. cupido, T. pallidicinctus,* and *T. phasianellus.* The lack of clear genetic differences within this species complex (Ellsworth et al., 1996) and proximity of historic range, suggested a comparison of existing eimerians of closely related species. In a comparison of the characters of sporulated oocysts from Tetraonid hosts, the oocyst residuum of *E. tympanuchi* is novel. There is no report of an oocyst residuum present in any of the *Eimeria* species found in hosts of the subfamily Tetraoninae.

Light infections of the hematozoan *Plasmodium (Giovannolaia) pedioecetii* were found. This species was previously described from lesser prairie-chickens of New Mexico and Texas by Stabler (1978). In this study there was a low prevalence of schizonts and mature gamonts. In a study of *P. (Giovannolaia) pedioecetii* from galliformes, Stabler and Kitzmiller (1976) reported a daily peak in merozoite production from 8:00 AM to noon. The earlier average capture time of birds sampled in our study (0500–08:15 AM) may explain the lack of segmenters and large number of underdeveloped gametocytes.

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