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Helminth (Cestoda, Nematoda) and coccidian (Apicomplexa: Eimeriidae) parasites of the eastern small-footed myotis, *Myotis leibii* (Chiroptera: Vespertilionidae) from Arkansas, with a description of a new species of *Eimeria*

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Abstract

During May and July 2016, 32 eastern small-footed myotis (*Myotis leibii*) were collected from five counties of northwestern Arkansas and their faeces examined for coccidian parasites. Four of 32 (13%) *M. leibii* harboured an eimerian that we describe here as new. Oocysts of *Eimeria sassei* sp. n. were ovoidal to ellipsoidal with a bi-layered wall and measured (length $\times$ width, L $\times$ W) 18.3 $\times$ 15.2 μ m, with an L/W ratio of 1.2. A micropyle and oocyst residuum were absent but 1–2 polar granules were present. Sporocysts were ovoidal, 9.6 $\times$ 6.3 μ m, with an L/W ratio of 1.5. A pronounced, button-like Stieda body was present but substieda and parastieda bodies were absent. A sporocyst residuum was present as distinct aligned or dispersed granules. One bat that we found dead was examined for helminth parasites. It harbored the tapeworm, *Vampirolepis* sp. and a nematode, *Seuratum cancellatum*. This is the first coccidian as well as the initial helminths reported from *M. leibii*. In addition, this is the seventh species of coccidian parasite documented from Arkansas bats.

Keywords

Apicomplexa; Coccidia; taxonomy; oocysts; Chiroptera; Vespertilionidae; *Eimeria sassei*; Arkansas; *Myotis leibii*; *Seuratum cancellatum*; *Vampirolepis* sp

Introduction

The eastern small-footed myotis, *Myotis leibii* Audubon and Bachman, 1842 is among the smallest and rarest vesper bats in eastern North America (Robbins et al. 1977). This bat is ranked S1 (critically imperiled) in Arkansas (NatureServe 2015). It ranges from Ontario, Canada and New England southward to Georgia and Alabama and westward into Oklahoma

(Best and Jennings 1997; Reid 2006). In Arkansas, *M. leibii* has been reported from scattered localities in the Interior Highlands (Ouachitas and Ozarks) (Sealander and Heidt 1990).

Although information is available on the ecology and natural history of *M. leibii* (Best and Jennings 1997), nothing, to our knowledge, has been reported on any coccidian from this bat and there are no reports of cestodes or nematodes from the species. However, limited information is available on trematode parasites of *M. leibii* from Canada (Stafford 1905). Here, we provide a description of an eimerian from *M. leibii* from Arkansas as well as document, for the first time, two helminth parasites from this host in the state.

Materials and Methods

During May and July 2016, four juvenile and 28 adult *M. leibii* (13 males, 19 females [including nine pregnant, one lactating, and two post lactating]) were taken by hand (with the use of a yardstick) from the crevices between sections of concrete guardrail on top of bridges in Carroll ($n = 4$), Franklin ($n = 5$), Madison ($n = 6$), Newton ($n = 2$) and Washington ($n = 15$) counties, Arkansas. Captured bats were allowed to defecate and fresh faecal pellets were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$). Samples were shipped to CTM and examined for coccidia by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30). Measurements were taken on 26 sporulated oocysts using a calibrated ocular micrometer and reported in micrometres (μm) with means followed by the ranges in parentheses; photographs were taken using Nomarski interference-contrast optics. Oocysts were ~78 days old when measured and photographed. Descriptions of oocysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: oocyst length (L) and width (W), their ratios (L/W), micropyle (M), oocyst residuum (OR), polar granule(s) (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), sporocyst (SP), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR), sporozoites (SZ) anterior (ARB) and posterior (PRB) refractile bodies, and nucleus (N).

A single adult female *M. leibii* collected by D. Blake Sasse in May 2016 from the St. Hwy 74 bridge in Washington County that died unexpectedly was frozen; it was shipped to CTM and examined for helminth parasites. A midventral incision was made from anus to throat, the gastrointestinal tract was removed, split longitudinally, its contents placed in a Petri dish containing 0.9% saline, and examined using a stereomicroscope. A tapeworm was fixed in 70% (v/v) ethanol, stained with acetocarmine, and mounted in Canada balsam. A nematode was fixed in ethanol and examined as a temporary mount in glycerol.

A host voucher (alcoholic specimen) was accessioned into the Mammal Collection of Henderson State University (HSU), Arkadelphia, Arkansas, USA as HSU 946. Photosyntypes of sporulated oocysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska USA.

Results

Four of 32 (13%) *M. leibii* (all pregnant females) collected on 13 May 2016 from Franklin (one), Madison (two), and Washington (one) counties were found to be passing coccidian oocysts that we describe herein as new. In addition to the new coccidian, a single immature tapeworm, *Vampirolepis* sp. (HWML 102050) and a female nematode, *Seuratum cancellatum* Chitwood, 1938 (HWML 98344) were found in the intestinal tract of an adult female *M. leibii* collected on 13 May 2016 from Washington County.

Eimeria sassei sp. nov. (Figs. 1–4)

Description of sporulated oocyst: Oocyst with 2 sporocysts; shape ovoidal to ellipsoidal; rough (mamillated) bi-layered wall, tan in colour, ~ 1.1 (1.0–1.2) thick, outer 0.8 (0.7–0.9) rough, inner 0.3 (0.3–0.4), smooth; L × W: 18.3 × 15.2 (16–21 × 14–17); L/W: 1.2 (1.1–1.3); M and OR absent; 1– (rarely) 2 PG, present.

Description of sporocyst and sporozoites: SP ovoidal; smooth uni-layered wall, ~ 0.5 thick; L × W: 9.6 × 6.2 (7–11 × 6–7); L/W: 1.5 (1.2–1.8); SB pronounced, button-like, SSB and PSB absent; SR: composed of distinct medium-sized coarse granules aligned along perimeter of SP or rarely dispersed; SZ: (not measured) sausage-shaped with spheroidal ARB and PRB; single N slightly posterior to midpoint.

Taxonomic summary

Type host: Eastern small-footed myotis, *Myotis leibii* Audubon and Bachman, 1842 (Mammalia: Chiroptera: Vespertilionidae), pregnant female band #7965. Collected and released on 13 May 2016. This bat was originally banded as a juvenile on 16 July 2015.

Type specimen: Photosyntype of sporulated oocyst deposited in the HWML as No. 102075.

Type locality: Madison County, Arkansas, USA (Eastling 429601, Northling 3964198).

Prevalence: In four of 32 (13%) of the type host. 0/4 (0%) Carroll County, 1/5 (20%) Franklin County, 2/6 (33%) Madison County, 0/2 (0%) Newton County, 1/15 (7%) Washington County.

Sporulation: Exogenous. All oocysts were passed unsporulated or partially sporulated and became fully sporulated within five days at ~23°C.

Prepatent and patent periods: Unknown.

Site of infection: Unknown, oocysts recovered from faeces.

Etymology: The specific epithet is given in honor of D. Blake Sasse, Nongame Mammal/Furbearer Program Leader, Arkansas Game and Fish Commission, for his longtime work on Arkansas mammals. Mr. Sasse also collected all of the bats examined in this study.

Remarks: Six coccidians have been described and/or reported previously from Arkansas bats (Table 1): *Eimeria catronensis* Scott and Duszynski from northern long-eared myotis, *Myotis*

septentrionalis Trouessart, 1897 (McAllister et al. 2004); *Eimeria dowleri* McAllister and Upton from red bats, *Lasiurus borealis* Müller, 1776 (McAllister and Upton 2009); *Eimeria heidti* McAllister, Burt, Seville and Robison from tri-colored bats, *Perimyotis subflavus* F. Cuvier, 1832 (McAllister et al. 2011); *Eimeria macyi* Wheat from *P. subflavus* (McAllister et al. 2001); *Eimeria mcdanieli* McAllister, Seville, Arlen and Connior from *P. subflavus* (McAllister et al. 2014); and *E. sealanderi* McAllister and Upton from *L. borealis* (McAllister and Upton 2009) (Table 2). Nearly all of these possess oocysts and sporocysts that are much larger than the new species and/or they possess an M (*E. catronensis*), an OR (*E. sealanderi*) or a SSB (*E. macyi*), all of which are absent in *E. sasseyi* (Table 2).

Eimeria tumlisoni McAllister, Seville and Roehrs from *M. septentrionalis* from Oklahoma is most similar to *E. sasseyi* (McAllister et al. 2012). However, there are several morphological and mensural differences that distinguish *E. tumlisoni* from *E. sasseyi* as follows: (i) oocysts of *E. tumlisoni* are subspheroidal vs. ovoidal to ellipsoidal in *E. sasseyi*, (ii) the L/W ratio of *E. tumlisoni* sporocysts are greater (average 1.8) than in *E. sasseyi* (average 1.5), (iii) *E. tumlisoni* SR are dispersed vs. distinctly aligned along the sporocyst in *E. sasseyi*, and (iv) the *E. tumlisoni* oocyst wall is much thicker (1.3–1.7 vs. 1.0–1.2) than the new species (McAllister et al. 2012). All of the other coccidians reported from North American bats of the genus *Myotis* are different than *E. sasseyi* (see Duszynski 2002).

Discussion

Tapeworms of the genus *Vampirolepis* are commonly reported from bats in North America (Rausch 1975; Vaucher 1992). This tapeworm was previously reported from Rafinesque's big-eared bat, *Corynorhinus rafinesquii* Lesson, 1827 from Arkansas (McAllister et al. 2005). The ascarid, *S. cancellatum* was originally described from the Mexican greater funnel-eared bat, *Natalus mexicanus* Miller, 1902 from the Yucatán, México (Chitwood 1938). This parasite shows little host specificity as it has been reported from several other bats of the genera *Antrozous*, *Corynorhinus*, *Eptesicus*, *Eumops*, *Myotis*, *Parastrellus*, and *Tadarida* from Texas, USA (Specian and Ubelaker 1976). In Arkansas, McAllister et al. (2004) previously reported *S. cancellatum* from *M. septentrionalis*. These two helminths are the initial cestode and nematode reported from *M. leibii* and the second time they have been reported from an Arkansas bat. There is limited information on trematode parasites of *M. leibii* as Stafford (1905) reported two species of *Lecithodendrium* and *Plagiorchis vespertilionis* (Müller) from specimens collected from Canada.

Compared to rodents, the largest class of mammals, comparatively little is known about the coccidians of second largest class of mammals, the bats of the world (Duszynski 2002). We document the first coccidian parasite reported from *M. leibii*. A list of the coccidians reported from Arkansas bats are given in Table 1. Of the 16 species of bats that occur in the state (Sealander and Heidt 1990), only four species (25%) have been reported to harbor coccidians. Of the other 12 species from Arkansas that have not yet been reported to be passing coccidia in the state, the following have been reported to be infected in other localities, including big brown bats, *Eptesicus fuscus* (Palisot de Beauvois, 1796), hoary bats, *Lasiurus cinereus* (Palisot de Beauvois, 1796), and little brown myotis, *Myotis lucifugus* (LeConte, 1831) (Duszynski 2002). However, other bats in the state have been

surveyed including Brazilian free-tailed bats, *Tadarida brasiliensis* (L. Geoffroy, 1824) and *C. rafinesquii* but were not found to be passing coccidians when examined (McAllister et al. 2005, 2006).

It is interesting to note that of the infected bats ($n = 4$), all were collected on the same date in May 2016. However, none of the 21 bats collected from the same sites over three days in mid-July 2016 were found to be passing oocysts. This may suggest that collections of bat fecal samples should be made during different months (and seasons) throughout the year to increase the possibility of finding individuals infected with coccidia.

The current study involved hosts collected from non-cave habitat due to a moratorium on collecting bats from caves and mines of the state to prevent the spread of devastating white-nose syndrome (caused by the fungus *Pseudogymnoascus destructans*, Frick et al. 2010), recently confirmed in Arkansas (<https://www.whitenosesyndrome.org/news/white-nose-syndrome-confirmed-arkansas>). Indeed, additional studies, similar the current one, can only occur with the assistance of state and federal officials who are able to legally and properly obtain fecal specimens from bats and pass them on to researchers. We may then, and only then, be able to extend our knowledge of the coccidia of many species of bats that, to date, have not yet been reported to harbor these parasites.

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Figs. 1–3.

Nomarski interference-contrast photomicrographs of oocysts of *Eimeria sassei* n. sp. **1.** Oocyst showing oocyst wall (OW), polar granule (PG) and sporocyst residuum (SR). **2.** Oocyst showing PG, Stieda body (SB) and sporocyst (SP). **3.** Oocyst showing bi-layered OW and SB. Scale bars = 10 μ m for all figures.

Fig. 4.
Composite line drawing of oocyst of *Eimeria sassei* n. sp. Scale bar = 10 μ m.

Table 1

Coccidians reported from Arkansas bats.

Coccidian	Host	Prevalence*	Reference
<i>Eimeria catronensis</i>	<i>Myotis septentrionalis</i>	4/20 (20%)	McAllister et al. (2004)
<i>E. dowleri</i>	<i>Lasiurus borealis</i>	2/3 (67%)	McAllister and Upton (2009)
<i>E. heidti</i>	<i>Perimyotis subflavus</i>	2/20 (10%)	McAllister et al. (2011)
		2/11 (18%)	McAllister et al. (2014)
<i>E. macyi</i>	<i>P. subflavus</i>	2/5 (20%)	McAllister et al. (2001)
<i>E. mcdanieli</i>	<i>P. subflavus</i>	2/11 (18%)	McAllister et al. (2014)
<i>E. sassei</i>	<i>Myotis leibii</i>	4/32 (13%)	This study
<i>E. sealanderi</i>	<i>L. borealis</i>	2/3 (67%)	McAllister and Upton (2009)

* Prevalence = number infected/number examined.

Table 2

Comparison of the sporulated oocysts of seven eimerians reported from Arkansas bats.

<i>Eimeria</i> spp.	Type host and other host	Localities	Oocyst shape, size, features ^{*,†}	Sporocyst shape, size, features ^{*,†}	References
<i>E. catronensis</i>	<i>Myotis lucifugus</i>	New Mexico, USA	Ovoidal 22.2 × 14.8; L/W 1.5 18–25 × 14–17	Football-shaped 9.1 × 6.6; L/W 1.2 8–11 × 5–7	Scott and Duszynski, 1997
	<i>Myotis septentrionalis</i>	Arkansas, USA	M, PG: both + OR: –	SB, SR: both + SSB, PSB: both –	McAllister et al. 2004
<i>E. dowleri</i>	<i>Lasius borealis</i>	Arkansas, USA	Subspheroidal to ellipsoidal 24.7 × 22.0; L/W 1.1 23–26 × 20–23 M, OR: both – PG: +	Ovoidal 13.4 × 9.2; L/W 1.5 12–14 × 8–9 SB, SR: both + SSB +, PSB: –	McAllister and Upton, 2009
<i>E. heidi</i>	<i>Perimyotis subflavus</i>	Arkansas, USA	Ovoidal to ellipsoidal 26.1 × 20.5; L/W 1.3 23–31 × 18–23 M, OR: both – PG: +	Ovoidal 13.0 × 8.8; L/W 1.6 11–15 × 7–13 SB, SR: both + SSB, PSB: both –	McAllister et al. 2011
<i>E. macyi</i>	<i>P. subflavus</i>	Alabama, USA	Subspheroidal to broadly ellipsoidal 19.0 × 17.6; L/W 1.1 16–21 × 15–19 M, OR: both – PG: +	Ovoidal 11.0 × 7.0; L/W 1.6 10–12 × 6–8 SB, SR: both + SSB +, PSB: –	Wheat, 1975 McAllister et al. 2001
<i>E. mcdanieli</i>	<i>P. subflavus</i>	Arkansas, USA	Ellipsoidal to elongate 28.3 × 17.9; L/W 1.6 25–30 × 16–20 M, OR: both – PG: +	Ovoidal 12.6 × 8.3; L/W 1.5 11–14 × 8–9 SB, SR: both + SSB +, PSB: –	McAllister et al., 2014
<i>E. sassei</i>	<i>Myotis leibii</i>	Arkansas, USA	Ovoidal to ellipsoidal 18.3 × 15.2; L/W 1.2 16–21 × 14–17	Ovoidal 9.6 × 6.2; L/W 1.5 7.1 × 6–7	This study

<i>Eimeria</i> spp.	Type host and other host Localities	Oöcyst shape, size, features ^{*,†}	Sporocyst shape, size, features ^{*,†}	References
<i>E. sealanderi</i>	<i>L. borealis</i> Arkansas, USA	M, OR: both –	SB, SR: both +	McAllister and Upton, 2009
		PG: +	SSB, PSB: both –	
		Subspheroidal to ellipsoidal	Ovoidal	
		16.7 × 14.4; L/W 1.1	8.9 × 5.7; L/W	
		15–18 × 13–16	8–10 × 5–6	
		M: –	SB, SR: both +	
		OR, PG: both +	SSB +, PSB: –	

^{*} Measurements in µm.

[†] Descriptions of oöcysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: oöcyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oöcyst residuum (OR), polar granule(s) (PG), sporocyst (SP) length (L) and width (W), their ratio (L/W), Stieda body (SB), sub-Stieda body (SSB), para-Stieda body (PSB), and sporocyst residuum (SR).