J. Helminthol. Soc. Wash.
61(1), 1994, pp. 12–16

Caryospora tremula and *Sarcocystis* sp. from Turkey Vultures, *Cathartes aura*: Descriptions of Oocysts and Sporocysts and Attempted Transmission to Rodents

DAVID S. LINDSAY,[1] STEVE J. UPTON,[2] CHRISTINE A. SUNDERMANN,[3] RICHARD D. MCKOWN,[4] AND BYRON L. BLAGBURN[1]

[1] Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, Alabama 36849-5519,
[2] Division of Biology, Ackert Hall, Kansas State University, Manhattan, Kansas 66506,
[3] Department of Zoology and Wildlife Sciences, Auburn University, Auburn, Alabama 36849, and
[4] Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas 77843

ABSTRACT: Oocysts of *Caryospora tremula* (Allen, 1933) were observed in the feces of 3 turkey vultures, *Cathartes aura,* 1 from Kansas and 2 from Alabama. Oocysts from all birds were structurally similar. Oocysts were subspherical to ellipsoidal and measured 33.4 × 28.0 μm. Oocysts enclosed a single spherical sporocyst that measured 20.4 × 20.1 μm. Each sporocyst contained 8 sporozoites that measured 16.3 × 5.3 μm. Sporozoites contained anterior and occasionally posterior granular refractile bodies. Free *Sarcocystis* sporocysts were observed in the feces of 1 of the 2 turkey vultures from Alabama. The sporocysts were fully sporulated, contained 4 sporozoites, and measured 11.4 × 8.9 μm. Developmental stages of *C. tremula* or *Sarcocystis* sp. were not observed in mice or cotton rats inoculated orally with oocysts and sporocysts.

KEY WORDS: Apicomplexa, *Caryospora tremula, Sarcocystis* sp., oocyst, sporocyst, transmission studies, turkey vulture, *Cathartes aura.*

Oocysts that were structurally similar to those of *Caryospora tremula* (Allen, 1933) Hoare, 1934, were observed in the feces of 3 turkey vultures, *Cathartes aura.* One turkey vulture also was excreting sporocysts of a species of *Sarcocystis.* Because *C. tremula* has not been reported for nearly 50 yr and because the original description was incomplete, we present a redescription of the oocysts of this species. In addition, we report the structure of sporocysts of a *Sarcocystis* species from 1 turkey vulture and our attempts to transmit these coccidians to mice and cotton rats.

Materials and Methods

The turkey vulture (vulture 1) from Kansas was submitted for treatment of a fractured wing after being struck by a car to Kansas State University, College of Veterinary Medicine, Manhattan, Kansas, in September 1991. The 2 turkey vultures (vultures 2 and 3) from Alabama were submitted for treatment to the Southeastern Raptor Rehabilitation Center (SRRC) at the College of Veterinary Medicine, Auburn University, Alabama, in June and October 1992. Raptors at the SRRC are fed food that has been frozen and then thawed. Feces from all birds were examined by flotation in Sheather's sugar solution, and feces containing oocysts or sporocysts were mixed in 2.5% (w/v) potassium dichromate solution in a thin-layer (<5 mm) in plastic Petri dishes and sporulated at room temperature (22–24°C). Fecal samples were examined from vultures 2 and 3, once or twice weekly, over a 2-mo period. Additionally, single fecal samples were examined from 2 black vultures (*Coragyps atratus*) that were residents at the SRRC. Oocyst cultures were examined daily with Nomarski interference-contrast or bright-field microscopes to determine when sporulation had occurred. Oocysts were concentrated by flotation in Sheather's sugar solution and counted in a hemacytometer prior to experimental transmission studies. Oocysts and sporocysts were stored at 4°C and were less than 2 mo old when used. Oocysts and sporocysts were measured with a calibrated ocular micrometer. All measurements are in micrometers and expressed as means ± standard deviation, followed in parentheses by the range and number (*N*) of stages measured.

The oocyst and sporocyst shape indices (length/width ratios) were compared using analysis of variance (ANOVA). Significant differences were considered to be present if $P < 0.05$ was detected by ANOVA.

Seven female Hsd:ICR mice and 4 female cotton rats were used for experimental oral inoculations with *C. tremula* oocysts and *Sarcocystis* sp. sporocysts. Three of the mice intramuscularly received 4 mg of methylprednisolone acetate (MPA; The Upjohn Company, Kalamazoo, Michigan) in an attempt to enhance infectivity of the inoculum. Mice received 3×10^4 *C. tremula* oocysts, and cotton rats received 2×10^4 *C. tremula* oocysts. The number of *Sarcocystis* sp. sporocysts present in the inoculum was not determined. Two female Hsd:ICR mice and 2 male cotton rats were not inoculated and served as controls.

Inoculated MPA-treated mice were killed and examined at necropsy 21, 35, and 49 days postinoculation (PI); inoculated mice not treated with MPA and inoculated cotton rats were killed and examined at necropsy 7, 21, 35, and 49 days PI; and control mice and cotton rats were killed and examined 35 and 49 days PI. A portion of cerebral cortex was collected from each rodent and examined as an unstained smear with

Table 1. Measurements and shape indices (length/width ratios) of *Caryospora tremula* oocysts and sporocysts from turkey vultures.*

	Vulture 1 sporulated	Vulture 2 unsporulated	Vulture 2 sporulated	Vulture 3 sporulated
Oocyst length	34.0 ± 1.18	33.3 ± 1.46	34.3 ± 1.38	32.1 ± 1.69
	(32.0–36.2)	(30.0–36.0)	(32.0–37.0)	(30.0–38.0)
Oocyst width	29.0 ± 1.03	27.5 ± 1.45	28.6 ± 1.47	26.8 ± 0.87
	(27.6–32.0)	(25.0–30.0)	(25.0–32.0)	(25.0–28.0)
Oocyst index	1.17 ± 0.04	1.21 ± 0.05	1.20 ± 0.04	1.20 ± 0.08
	(1.08–1.23)	(1.10–1.31)	(1.10–1.28)	(1.07–1.52)
Sporocyst length	20.4 ± 0.73	NA†	20.8 ± 0.60	20.0 ± 0.54
	(18.6–21.6)	NA	(19.0–22.0)	(19.0–21.0)
Sporocyst width	ND‡	NA	20.3 ± 0.61	19.8 ± 0.58
	ND	NA	(19.0–21.0)	(19.0–21.0)
Sporocyst index	ND	NA	1.02 ± 0.03	1.01 ± 0.02
	ND	NA	(1.00–1.11)	(1.01–1.05)

* Measurements are expressed in micrometers as means ± standard deviation, with the ranges in parentheses. All measurements are based on 25 observations.

† NA = not applicable to unsporulated oocysts.

‡ ND = not determined for sporocysts of this isolate because sporocysts were considered to be spherical.

light microscopy. The remaining brain, eyes, and portions of tongue, heart, thigh (semitendinosus and semimembranosus), gastrocnemius, diaphragm, abdominal muscles, lung, thymus, liver, spleen, pancreas, kidney, adrenal gland, stomach, ileum, cecum, ear, and facial tissue were fixed in 10% neutral-buffered formalin solution. Tissues were processed by routine histological methods and stained with hematoxylin and eosin for light microscopic examination.

Portions of skeletal muscle were obtained from the carcass of each rodent (1–2 g for mice, 3–6 g for cotton rats) and digested in hydrochloric acid–pepsin solution (0.52 g pepsin, 0.50 g NaCl, 1.4 ml concentrated HCl, and 98.6 ml H_2O) for 10 min. The suspension was washed once in Hanks' balanced salt solution (HBSS) by centrifugation, resuspended in HBSS, and examined with bright-field microscopy for parasites.

Results

Measurements of *C. tremula* oocysts from each turkey vulture are presented in Table 1. A general composite description is presented here.

Caryospora tremula (Allen, 1933) Hoare, 1934

Apicomplexa: Eimeriorina

DESCRIPTION OF OOCYSTS (Figs. 1–4): Oocysts subspherical to ellipsoidal, occasionally ovoidal, 33.4 ± 1.65 × 28.0 ± 1.50 (30–38 × 25–32, N = 100); shape index (length/width) 1.2 ± 0.06 (1.07–1.52, N = 100); oocyst wall bilayered and smooth; outer layer 1.0–1.4 thick; inner layer 0.6–0.8 thick; micropyle and polar granule absent; small, spherical oocyst residuum rarely present; sporocysts spherical or occasionally subspherical, 20.4 ± 0.7 × 20.1 ± 0.7 (18.6–21.6 × 18.6–21.6, N = 75); shape index 1.01 ± 0.02 (1.00–1.05, N = 75), with smooth single-layered wall 0.8–1.0 thick; sporocyst residuum present consisting of hundreds of diffuse granules on day 2 of sporulation, as a compact mass on days 3, 4, and occasionally 5 of sporulation, and usually as scattered granules on and beyond day 5 of sporulation; 8 sporozoites present in each sporocyst, 16.3 ± 0.73 × 5.2 ± 0.29 (15.2–18.4 × 4.8–5.6, N = 25), arranged parallel or randomly in sporocyst; anterior ends of sporozoites tapering slightly; each sporozoite usually with single, spherical to ellipsoidal posterior granular refractile body 3.0 × 2.8 and rarely spherical anterior granular refractile body 2.5; nucleus prominent, centrally or posteriorly located.

HOST: *Cathartes aura* Wield "turkey vulture" (Falconiformes: Cathartidae)

LOCALITY: Type locality is Washington, D.C. Other localities (present study) Kansas and Alabama, U.S.A.

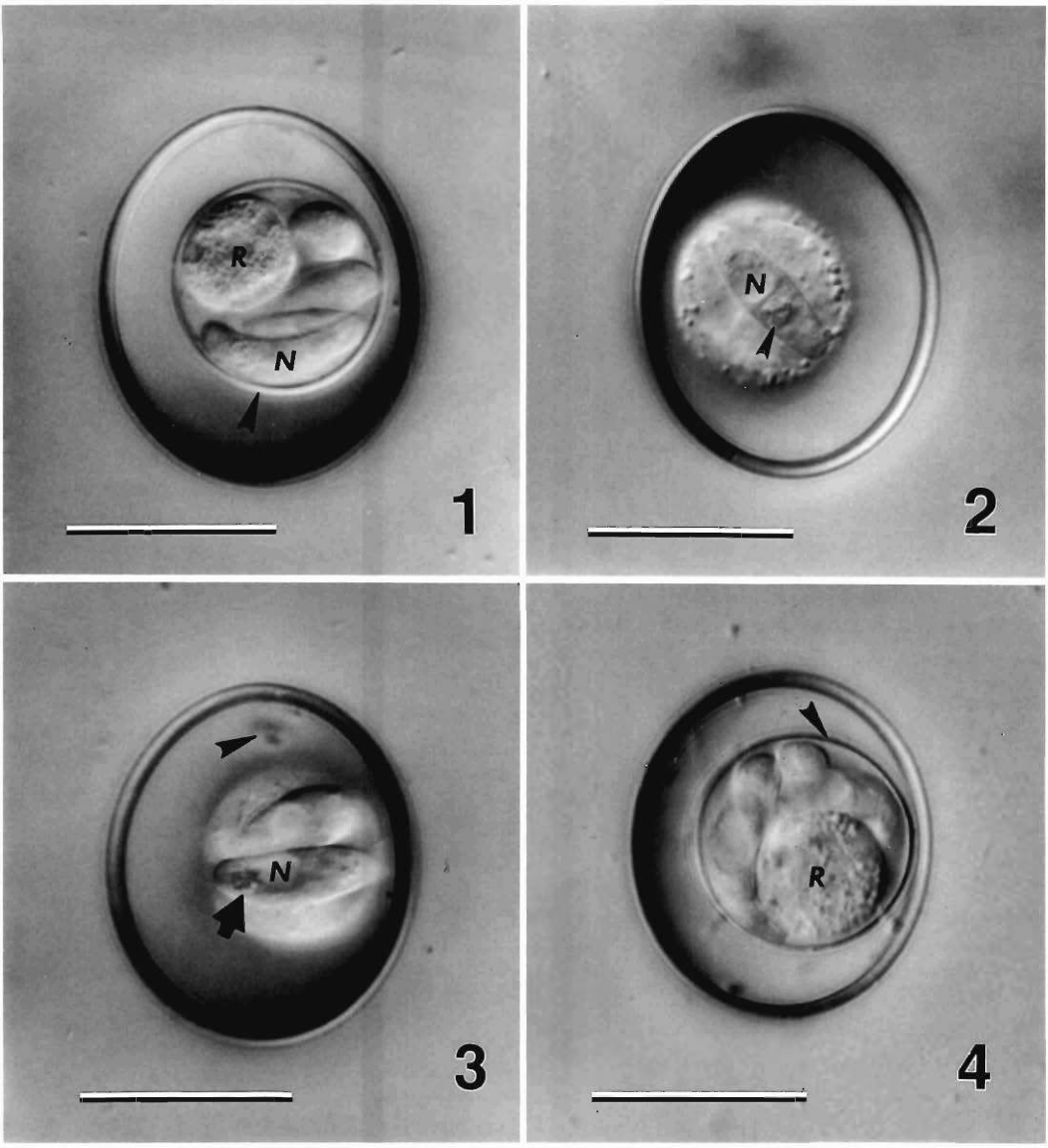
LOCATION IN HOST: Oocysts were originally described from the intestine of a turkey vulture. Oocysts in this study were recovered from feces.

SPORULATION: Some oocysts had completed sporulation within 48 hr; all had sporulated within 4 days at room temperature (22–24°C).

TRANSMISSION TO SECONDARY HOSTS: No stages of *C. tremula* were observed in mice or cotton rats.

COMMENTS: No significant differences (P > 0.05) were detected in the oocyst or sporocyst shape indices among the 3 *C. tremula* isolates.

Exposure of *C. tremula* oocysts to 2.5% po-



Figures 1–4. Sporulated oocysts of *Caryospora tremula* from a turkey vulture from Kansas. Bars = 20 μm. 1. Oocyst with a sporocyst with a compact sporocyst residuum (R). Note the sporocyst wall (arrowhead) and the centrally placed sporozoite nucleus (N). 2. Oocyst with a sporocyst with a dispersed sporocyst residuum. Note the nucleus (N) of a sporozoite and the granular refractile body (arrowhead). 3. Oocyst containing a small oocyst residuum (arrowhead). The sporozoite in the field of focus contains a nucleus (N) and a small granular refractile body (arrow). 4. Oocyst with a sporocyst that contains sporozoites in cross or tangential sections. Note the sporocyst wall (arrowhead) and the compact sporocyst residuum (R).

tassium dichromate solution for periods of 1 wk or more apparently caused the oocyst wall to become fragile because oocysts with collapsed walls surrounding the sporocysts were observed after flotation in sugar solution. Free *C. tremula* sporocysts were occasionally observed in sugar flotations and probably represent complete collapse and removal of the oocyst wall.

Oocysts of *C. tremula* were present in all fecal samples examined from vultures 2 and 3 over

the 2-mo period. Oocysts of *C. tremula* were not observed in the fecal samples from the 2 black vultures.

Sarcocystis sp.

Apicomplexa: Eimeriorina

DESCRIPTION OF SPOROCYSTS (Fig. 5): Sporocysts elongate with 1 side slightly flattened, 11.4 ± 0.65 × 8.9 ± 0.60 (10.0–12.0 × 8.0–10.0, $N = 25$); residuum composed of dispersed, discreet granules; 4 sporozoites present; no refractile bodies observed in sporozoites.

HOST: *Cathartes aura* Wield "turkey vulture" (Falconiformes: Cathartidae)

LOCALITY: Alabama, U.S.A.

LOCATION IN HOST: Unknown. Sporocysts in this study were recovered from feces.

TRANSMISSION TO INTERMEDIATE HOSTS: No stages of *Sarcocystis* were observed in mice or cotton rats.

COMMENTS: The turkey vulture (vulture 2) was excreting low numbers of sporocysts for the 2-mo observation period. No oocysts were seen.

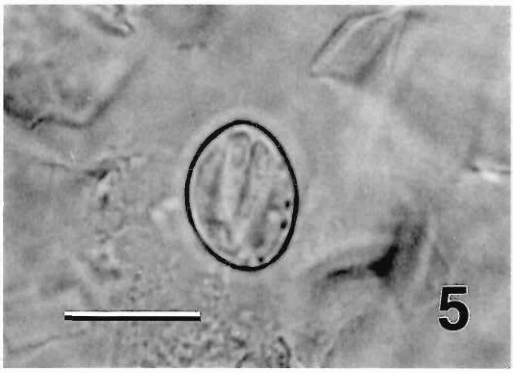


Figure 5. Sporocyst of *Sarcocystis* species from a turkey vulture from Alabama. Note the sporozoites and dispersed granules that compose the sporocyst residuum. Bar = 10 μm.

Discussion

Oocysts of *C. tremula* were originally reported as ellipsoidal and 33–35 × 28–30 in diameter and sporocysts as 23.5–25 in diameter (Allen, 1933). In the present study, oocysts were 30–38 × 25–32 and sporocysts were 18.6–21.6 × 16.6–21.6. The original measurements of *C. tremula* oocysts by Allen (1933) fall within the range of oocysts in our study, but the sporocyst measurements are larger by 2–6 μm. We observed refractile bodies in sporozoites and a small amount of oocyst residual material in *C. tremula* oocysts, which Allen (1933) did not mention in her description.

Allen (1933) originally created a new genus (*Eumonospora*) to accommodate the new coccidian, which she termed *Eumonospora tremula*. This genus was quickly synonymized with *Caryospora* by Hoare (1934), which was accepted by the original author (Allen, 1934).

Several members of the genus *Caryospora* use rodents as secondary hosts in their life cycles (Stockdale and Cawthorn, 1981; Cawthorn and Stockdale, 1982; Wacha and Christiansen, 1982; Upton et al., 1984). Severe disease and death can occur in cotton rats and mice inoculated with oocysts of *C. bigenetica* Wacha and Christiansen, 1982 (Wacha and Christiansen, 1982; Lindsay et al., 1988; Upton and Barnard, 1988). Severe disease occurs in mice infected with oocysts of *C. simplex* Leger, 1904 (Upton et al., 1984). Both *C. bigenetica* and *C. simplex* have viperiid snakes as the primary hosts. *Caryospora bubonis* Cawthorn and Stockdale, 1981, of the great horned owl, *Bubo virginianus,* is transmissible to mice (Stockdale and Cawthorn, 1981; Cawthorn and Stockdale, 1982); however, no other species of *Caryospora* from avian hosts have been shown to have secondary hosts (see Lindsay and Sundermann, 1989; Upton et al., 1990; Upton and Sundermann, 1990).

Acknowledgments

We thank Natasha S. Rippey and Cindy Tily for technical assistance and David Kralovanic and other student members of the Southeastern Raptor Rehabilitation Center, College of Veterinary Medicine, Auburn University, for assistance with vultures.

Literature Cited

Allen, E. A. 1933. *Eumonospora tremula* gen. et. sp. nov., a coccidium from the intestine of the turkey buzzard, *Cathartes aura septentrionalis* Wied. Transactions of the American Microscopical Society 52:192–194.

———. 1934. *Eumonospora tremula* (Allen, 1933) emended to *Caryospora tremula* (Allen, 1933) Hoare, 1934. Transactions of the American Microscopical Society 53:293.

Cawthorn, R. J., and P. H. G. Stockdale. 1982. The developmental cycle of *Caryospora bubonis* Cawthorn and Stockdale 1981 (Protozoa: Eimeriidae)

in the great horned owl, *Bubo virginianus* (Gmelin). Canadian Journal of Zoology 60:152–157.

Hoare, C. A. 1934. On the systematic status of *Eumonospora tremula* Allen, 1933 (Coccidia: Eimeriidae). Transactions of the American Microscopical Society 53:6–7.

Lindsay, D. S., and C. A. Sundermann. 1989. Recent advances in the biology of the coccidian genus *Caryospora*. Journal of Veterinary Parasitology 3:1–5.

———, ———, **B. L. Blagburn, S. J. Upton, and S. M. Barnard.** 1988. Fatal *Caryospora bigenetica* (Apicomplexa: Eimeriidae) infections in cotton rats, *Sigmodon hispidus*. Journal of Parasitology 74:838–841.

Stockdale, P. H. G., and R. J. Cawthorn. 1981. The coccidian *Caryospora bubonis* in the great horned owl (*Bubo virginianus*). Journal of Protozoology 28:255–257.

Upton, S. J., and S. M. Barnard. 1988. Development of *Caryospora bigenetica* (Apicomplexa: Eimeriidae) in experimentally infected mice. International Journal for Parasitology 18:15–20.

———, **T. W. Campbell, M. Weigel, and R. D. McKown.** 1990. The Eimeriidae of raptors: review of the literature and description of new species of the genera *Caryospora* and *Eimeria*. Canadian Journal of Zoology 68:1256–1265.

———, **W. L. Current, J. V. Ernst, and S. M. Barnard.** 1984. Extraintestinal development of *Caryospora simplex* (Apicomplexa: Eimeriidae) in experimentally infected mice, *Mus musculus*. Journal of Protozoology 31:392–398.

———, **and C. A. Sundermann.** 1990. *Caryospora*: biology. Pages 187–204 *in* P. L. Long, ed. Coccidiosis of Man and Domestic Animals. CRC Press, Boca Raton, Florida.

Wacha, R. S., and J. L. Christiansen. 1982. Development of *Caryospora bigenetica* n. sp. (Apicomplexa, Eimeriidae) in rattlesnakes and laboratory mice. Journal of Protozoology 29:272–278.