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**Isospora boughtoni VOLK, 1938 AND Isospora sp. (PROTOZOA:
EIMERIIDAE) FROM AN OPOSSUM *Didelphis marsupialis***

On January 17, 1969, an adult male opossum, *Didelphis marsupialis*, was brought to the USDA Regional Parasite Research Laboratory, Auburn, Alabama, for parasitologic examination. The opossum had been struck and killed by an automobile near the Laboratory sometime during the previous night. Sugar flotation of a fecal sample revealed the

presence of coccidial oocysts, which after sporulation were identified as two species of *Isospora*. Feathers of a bird, identified as a brownheaded cowbird, *Molothrus ater*, were also present in the intestinal contents. This report describes the two *Isospora* species, one of which may be of avian origin.

Materials and Methods

The intestinal contents of the opossum were mixed with water and flushed through a 60 mesh sieve, concentrated by sedimentation, suspended in a 2.5% potassium dichromate solution, and placed in a thin layer in a petri dish. The oocysts were then allowed to sporulate for one week at room temperature. After

sporulation was completed, the sample was placed in fresh potassium dichromate and stored in a refrigerator until examined. After sugar flotation, the sporulated oocysts were studied with a Leitz Ortholux microscope equipped with planapochromatic objectives.

Results

Isospora boughtoni Volk, 1938

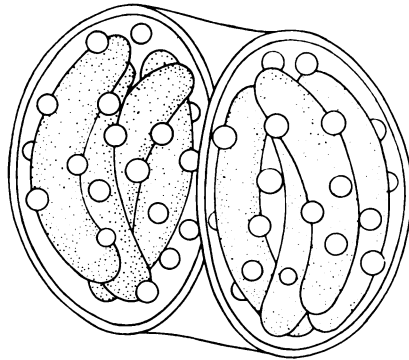
Description: Sporocysts usually outside of oocyst; very few intact oocysts present. Intact sporulated oocyst (Fig. 1) contains two sporocysts, each with four sporozoites. Oocyst wall a thin, delicate membrane which is easily ruptured. Sporocysts within the oocysts irregular in position, causing the oocysts to be distorted. Because of this distortion, measurements of sporulated oocysts would be misleading. Micropyle, oocyst residuum, and polar granule absent.

Sporocysts ellipsoidal with one side more convex than the other. Stieda body absent. Fifty sporocysts measured 10 to 12 by 6 to 8 μ with a mean of 11.1 by 7.2 μ . Sporocyst residuum present as 20 to 30 coarse granules scattered throughout the sporocyst. Sporozoites slightly curved, sausage-shaped, with both ends approximately the same size; unable to distinguish anterior end from posterior end. Sporozoites lie length-wise along the long axis of the sporocyst. Sporozoite refractile bodies absent. A light spherical area could be seen at one end of some of the sporozoites.

Remarks: *Isospora boughtoni* was described by Volk (1938, J. Parasit. 24: 547-548) from the American opossum from the vicinity of Athens, Georgia. Our measurements of the sporocysts are larger than those reported in the original description. We were also unable to distinguish the three bodies seen by Volk in the sporozoites, although a light area having the appearance of a vacuole was seen in some of them.

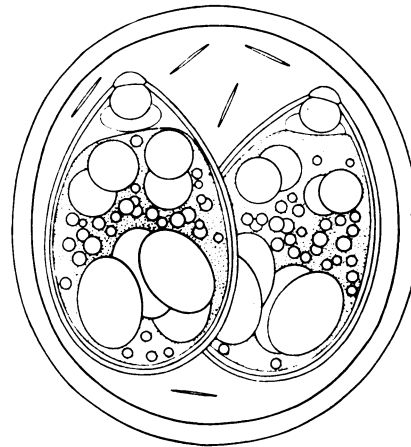
This is evidently the second report of *I. boughtoni* from the opossum. Other Eimeriidae reported from *Didelphis* are *Eimeria didelphydis* Carini, 1936 and *E. gambai* Carini, 1938, from the South American opossum *Didelphis aurita*.

Volk found the endogenous stages, including sporulated oocysts, in the upper small intestine of the opossum. He did not report finding individual sporocysts, but he did mention the irregular shape of the oocysts due to the delicate oocyst wall tightly enclosing the two sporocysts. Very few intact oocysts of *I. boughtoni* were found in the material we examined. Since we did not find this



5 μ

FIGURE 1. Sporulated oocyst of *Isospora boughtoni*.



10 μ

FIGURE 2. Sporulated oocyst of *Isospora* sp.

species of coccidia until the feces had been at room temperature for one week, it is not known if the oocysts were ruptured while in the intestinal tract or during preparation for study.

Although it is more common for the oocysts of mammalian coccidia to be passed in the feces in the unsporulated state, there are reports of oocysts being sporulated and also ruptured when passed. Pellérdy (1965, *Coccidia and Coccidiosis Akademiae Kiado, Budapest, Hungary*) listed species of *Isospora* from mammals (*I. bigemina*, *I. canivelocis*, and *I. hominis*) in which individual sporocysts were often found in the feces of the host. Levine and Ivens (1965, *J. Parasit.* 51: 859-864) found free sporocysts, which they considered to be *I. rivolta*, in the feces of 3% of 139 dogs examined in Illinois.

Isospora sp.

Description: Sporulated oocysts usually subspherical, sometimes spherical or ellipsoidal. Two sporocysts, each with four sporozoites, in the sporulated oocyst (Fig. 2). Oocyst wall single, approximately 1 μ , light brown, tending to be slightly darker on the inner surface. Wall often appears two-layered, but proved to be single by crushing oocysts with pres-

sure on coverslip. Micropyle and oocyst residuum absent. One hundred oocysts measured 18 to 26 by 18 to 24 μ with a mean of 21.6 by 20.5 μ ; their length-width ratios ranged from 1.0 to 1.3 with a mean of 1.05. Polar body normally 5 to 15 splinter-like fragments, often scattered throughout oocyst. A dumbbell-shaped polar body, 3.2 by 1.6 μ , present in 10% of oocysts. This polar body sometimes appeared to be fragmenting, or had splinter-like fragments associated with it.

Stieda body at the pointed end of ovoid sporocyst. Substiedal body present directly beneath the Stieda body. Another structure, herein termed "satellite body", associated with substiedal body (Figs. 2, 3). Satellite body occurs in the majority of sporocysts, although it may be obscured by the contents of the sporocyst. One hundred sporocysts measured 13 to 17 by 8 to 11 μ with a mean of 15.1 by 9.8 μ . Sporocysts usually lie lengthwise in the long axis of the oocyst. Sporocyst residuum composed of discrete coarse granules. Outline of sporozoites usually not distinct. Each sporozoite with two refractile bodies, a larger posterior and a smaller anterior. Sporozoites tend to lie in one direction in the sporocyst; the

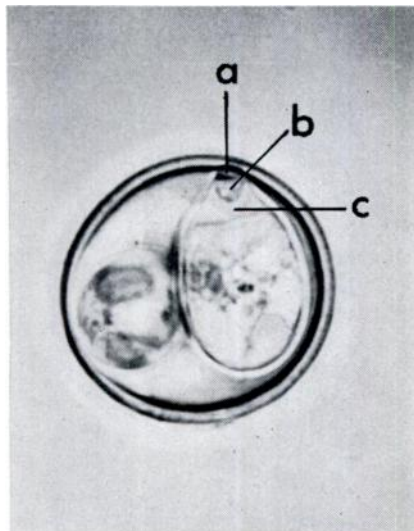


FIGURE 3. Photomicrograph of a sporulated oocyst of *Isospora* sp. with a sporocyst showing the Stieda body (a), the substiedal body (b), and the satellite body (c). X 1,800.

large refractile bodies of the sporozoites normally oriented at the large end of sporocyst. Sporocyst residuum and sporozoites enclosed by a fine membrane.

Remarks: Because of the opossum's omnivorous eating habits, the possibility that oocysts in its feces may have originated in another host must be considered. We hesitate to name this *Isospora* as a new species from the opossum because of the occurrence of feathers of a brown-headed cowbird (*Molothrus ater*) in the intestinal contents of the opossum, and the close resemblance of the oocysts of this species of *Isospora* to some of those described from birds. It is possible that the oocysts were from the bird eaten by the opossum. We were unable to look for intestinal stages of the coccidia in the opossum because of postmortem changes. Boughton et al. (1938, Ohio J. Sci. 38: 149-163) listed an *Isospora* sp. from the eastern cowbird (*Molothrus ater*) but gave no description of it. Further observations of the coccidia occurring in the opossum and in the cowbird

must be done before any conclusions can be reached regarding the correct host of this parasite.

The possibility that coccidial oocysts found in the feces of a host may be of spurious origin, especially in omnivores and carnivores, has received little attention to date. Levine and Mohan (1960, J. Parasit. 46: 733-741) described an *Isospora* sp. from cattle in Illinois and compared it with *Isospora lacazei* from the English sparrow. They observed that the two forms were practically identical and that the oocysts found in the bovine feces may well have been those of *I. lacazei*.

The oocysts found in the feces of the opossum closely resemble the description of *Isospora lacazei* given by Levine and Mohan and by Anwar (1966, J. Protozool. 13: 84-90) except that these authors did not report the presence of a satellite body. Observations of oocysts identified as *I. lacazei* from several species of birds from the vicinity of Auburn, Alabama, revealed that the *Isospora* sp. from the opossum differed from *I. lacazei* in several ways. The *Isospora* sp. had a satellite body, its sporocyst residuum was composed of larger granules, and the outline of its sporozoites was much less distinct.

This report is apparently the first describing a satellite body in a coccidial oocyst. This structure is closely associated with the substiedal body but appears to have a different consistency. We have not as yet observed the satellite body in any of the species of *Isospora* from the birds we have necropsied. Schwalbach, (1959, Arch. Protistenk. 104: 431-491) stressed the importance of the presence or absence of the Stieda body and the substiedal body for identifying the various species of *Isospora* from birds. Davis, (1967, J. Protozool. 14: 573-585) found these two structures in two of three species of *Isospora* from white-footed mice in California. Ernst et al. (1968, J. Protozool. 15: 430-432) also reported them in the sporocysts of *Eimeria utahensis* from kangaroo rats. The satellite body adds still another structure to this complex.

Schwalbach used the term "Mikropyl-enknopf" for the Stieda body and "Mikropylenpfropf" for the substiedal body. Anwar used "apical cap" and "Stieda body", respectively, for these two structures. Since micropyle has long been the

name for a structure on the oocyst wall, and Steida body is normally used instead of apical cap, we prefer to follow Davis and Ernst et al., by using Stieda body and substiedal body for these two structures.

Summary

The sporulated oocysts of two species of *Iso spora* from one opossum, *Didelphis marsupialis*, are described. Oocysts of *Iso spora boughtoni* have a thin wall that is easily ruptured, and the sporocysts are usually found outside the oocysts. In intact oocysts the sporocysts are irregularly arranged, and the oocyst wall is distorted. The sporocysts (mean 11.1 by 7.2 μ) are ellipsoidal, and one side is more convex than the other. The sporocyst residuum is composed of 20 to 30 granules.

The second species is designated *Iso spora* sp. because it may be of avian rather than opossum origin. Feathers of a brown-headed cowbird, *Molothrus ater*, were found in the intestinal contents of the opossum. The single-walled, subspherical oocysts (mean 21.6 by 20.5 μ) contain a polar body composed of 5 to 15 splinter-like fragments. A satellite body, herein described for the first time, is closely associated with the substiedal body. The sporocyst residuum is composed of coarse granules.

We express our appreciation to Dr. J. L. Dusi, Auburn University, for identifying the feathers found in the opossum's intestine.

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