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SURVEY FOR *Sarcocystis* IN THE BROWN-HEADED COWBIRD *(Molothrus ater)*: A COMPARISON OF MACROSCOPIC, MICROSCOPIC AND DIGESTION TECHNIQUES

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Abstract: Adult cowbirds from the Houston, Texas area were examined for *Sarcocystis* by three methods. Macroscopically, 53 of 253 (20.9%) birds examined were positive. Microscopic examination of abdominal muscle from 62 of the 200 negative birds showed another 4 (6.4%) to be infected. Pepsin digestion, the most sensitive technique for macroscopically negative birds, showed 7 of the 62 (11.2%) to be infected.

INTRODUCTION

Sarcocystis recently has been shown to be part of a coccidian life cycle involving a predator as a definitive host and its prey as an intermediate host[6]. Briefly, after the intermediate host ingests sporocysts from feces of the definitive host, the parasite multiplies intracellularly by schizogony, then enters muscle cells and again multiplies. The resulting cyst contains zoites capable, when eaten, of initiating sporocyst production in the intestine of the definitive host. Muscle cysts commonly found in cattle in the United States usually are only visible microscopically but many other species, such as those found in ducks and various passerine birds, are visible macroscopically. Digestion is a sensitive way of finding zoites of microscopic cysts in cattle, hogs and sheep[4]. It also should be applicable to finding zoites of muscle cysts which are not yet large enough to be seen macroscopically, but perhaps containing zoites which can withstand digestion and hence are infectious. In the course of transmission studies using an avian species of *Sarcocystis*, we surveyed brown-headed cowbirds *(Molothrus ater)* from the Houston, Texas area for the prevalence of the parasite. In addition to macroscopic evidence, we compared microscopic examination of abdominal wall samples and pepsin digestion of negative birds for evidence of infection.

MATERIALS AND METHODS

Cowbirds were trapped in the Houston, Texas area. They were killed by thoracic constriction and transported to the University of Texas Medical Branch, Galveston, Texas, where they were skinned, eviscerated and examined macroscopically using a lamp with a magnifying lens attached. Some of the birds negative for cysts were examined further by two methods. First the thin abdominal muscle was removed and examined by transillumination by pressing the tissue between two slides and examining at 10-70X with a dissecting microscope. Secondly, the eviscerated carcass with head and feet removed was placed in a Waring Blender, covered with digestive fluid (pepsin 0.75% w/v, NaCl 0.75% w/v, HCl 1%

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v/v in water) and homogenized for 30 seconds. Enough digestive fluid to equal ca 10X the weight of the bird was added and the material stirred on a magnetic stirrer at 37 C for 30 min. The homogenate was strained through gauze and the filtrate centrifuged at 2000 rpm for 10 min. A drop of sediment was examined for the zoites with phase optics ≧2 min at 400X with a Zeiss Photomicroscope.

RESULTS

Fifty-three of 253 (21%) cowbirds were grossly positive for *Sarcocystis* (Table 1). A higher proportion of the 65 females had cysts (24.5%) than the 188 males (19.6%). The distribution of muscle cysts in cowbirds differed from that observed in ducks[2]. In cowbirds the cysts were more common in the upper and lower leg and back muscles while in ducks they were more common in breast and thighs. Cowbird cysts were spindle shaped and measured 2.5-7 X ca 0.1-0.5 (4.2 x ca 0.3) mm. No cysts were seen in heart muscle.

Microscopic examination of the abdominal muscle from 62 grossly negative birds showed four additional positive birds (6%). One of these microscopic cysts measured 2.35 X 0.15 mm (measured at 100X). Three of the four microscopically positive birds also were positive by the digestion technique. A cyst from the bird microscopically positive but negative by digestion was observed to have rounded zoites rather than the banana-shaped organisms typical of the mature cyst. This suggests that the cyst organisms in this bird may not have matured sufficiently to withstand digestion.

The most sensitive method for finding *Sarcocystis* in grossly negative birds was the digestion technique; an additional seven birds (11%) were infected. Zoites were seen more clearly by phase microscopy than by bright field. They were pointed at one end and measured ca 8 X 2 μm in a wet preparation (400X). No motility was seen. Zoites in Giemsa's-stained smears were 5-7.5 X 1.5-3 μm, with a mean size of 6.4 X 2.1 μm (1000X).

TABLE 1. Detection of **Sarcocystis** in cowbirds **(Molothrus ater)** by three methods (No. positive/No. examined).

Date Collected	Gross Examination	Microscopic Examination[1]	
		Abdominal Muscle Press	Pepsin Digestion
28 January, 1977	9/35	—	—
3-4 February	9/41	2/16	1/16
10-11 February	5/35	1/10	2/10
17 February	5/37	0/16	1/16
24 February	7/26	1/10	3/10
3 March	4/18	0/10	0/10
4-10 March	3/13	—	—
11 March	8/23	—	—
25 March	3/25	—	—
TOTALS (%)	53/253 (20.9)	4/62 (6.4)	7/62 (11.2)

[1]Both muscle press and pepsin digestion were each done on the same birds which were all grossly negative for tissue cysts.

DISCUSSION

In 1960, as a result of a survey of meat for *Toxoplasma*, Jacobs *et al.*[4] suggested that digestion techniques would be a profitable method to use in studying *Sarcocystis*. They were unable to find cysts by microscopic examination of diaphragms from sheep although 98% were positive by digestion. In preliminary trials using the digestive technique on ground beef from various retail outlets; we found zoites of *Sarcocystis* in every sample[1]. Digestion of birds grossly positive for *Sarcocystis* in our study also invariably yielded numerous zoites.

Of 253 cowbirds examined macroscopically, 21% had visible cysts. In a random sample of 62 birds which were grossly negative, 11% were found to have zoites when their tissues were digested in pepsin. If our sample of grossly negative birds is representative of the population sampled, then ca 30% of the cowbirds in the Houston area carry some form of *Sarcocystis*. Zoites seen only upon digestion of grossly negative birds might be from cysts invisible because they were in muscles below the body surface. They also may be from developing cysts, not yet large enough to be seen macroscopically. A third alternative may be that cowbirds have two species of *Sarcocystis*, one with microscopic and one with macroscopic cysts as seen in sheep[6].

The wintering cowbirds sampled in this study had a lower prevalence of *Sarcocystis* than reported by Fayer and Kocan[3] for another icterid bird *Quiscalus quiscula* (93%). We also found *Sarcocystis* to be more prevalent in two species of grackles; all of six *Q. quiscula* captured with cowbirds were positive, as were seven of 16 (44%) *Cassidix mexicanus*.

Apparently there is not a published report of *Sarcocystis* in *M. ater*, but it has been reported from *M. bonariensis* in Uruguay[5]. In 1929, Vogelsang (cited by Kalyakin and Zasukhin[5]) gave the name *S. debonei* to the parasite from *M. bonariensis*. Some *Sarcocystis* have been given two specific names because the sporocyst from the definitive host was given one name in the genus *Isospora* and the muscle cyst in the intermediate host was given another in the genus *Sarcocystis*. Cowbirds and both species of grackles with muscle cysts in our survey infected opossums *(Didelphis virginiana)* which excreted sporocysts[2]. These sporocysts were similar to some described from the opossum and named *Isospora boughtoni* by Volk[7]. Because we do not know if this species of *Sarcocystis* is host specific, we are reluctant to attach or provide a specific name. However, it would be interesting to know if the same species infects all three of these icterid species since they associate together in winter flocks and share many of the same habitats.

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