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OCCULT FILARIASIS IN CROWS (*Corvus brachyrhynchos brachyrhynchos* BREHM) INFECTED WITH *Splendidofilaria caperata* HIBLER, 1964 (NEMATODA: FILARIOIDEA)

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Abstract: Adult *Splendidofilaria caperata* Hibler, 1964 were found in the tunica media of the pulmonary arteries of 21% of 341 crows (*Corvus brachyrhynchos brachyrhynchos* Brehm) wintering in southern Ontario, Canada. Microfilariae of *S. caperata* were not observed in the peripheral blood or in skin snips of infected crows. Microfilariae were rarely found in lung blood and then only in small numbers. Microfilariae were apparently overcome in the wall of the pulmonary artery; chronic inflammation was associated with their presence in arterial tissue. Crows apparently acquire *S. caperata* from insects that have fed on birds in which a microfilaraemia does develop.

INTRODUCTION

Adult *Splendidofilaria caperata* Hibler, 1964 inhabit the wall of the pulmonary artery of the host. The species was reported in 41% of 124 magpies (*Pica pica hudsonia* (Sabine)) (type host) in Colorado,^{12,13} 3% of 70 starlings (*Sturnus vulgaris vulgaris* L.) in Colorado^{4,5} and 21% of 341 crows (*Corvus brachyrhynchos brachyrhynchos* Brehm) in Ontario.⁶ Infection in magpies results in a microfilaraemia. Also, although chronic inflammation occurs in pulmonary arteries of magpies with numerous (≥ 15) nematodes there is little reaction in arteries with few (≤ 5) nematodes.¹² In contrast to infections of *S. caperata* in magpies, those in crows are always occult (=cryptic or amicrofilaraemic).⁶ It was of interest, therefore, to study the lesions observed in the arteries of crows with natural infections of *S. caperata*.

METHODS AND MATERIALS

Crows were attracted to offal placed in a field in Essex County, southern Ontario, during the winters of 1977-78 and 1978-79. Crows were then shot by hunters concealed in nearby blinds. Frozen car-

casses were collected from hunters and examined for *S. caperata*. Adult nematodes were fixed in 70% glycerine alcohol. Wet preparations of lung blood were examined for microfilariae of *S. caperata*. These were prepared by adding a drop of blood expressed from a piece of lung tissue onto a glass slide containing physiological saline or by teasing a small piece of lung tissue in saline on a glass slide. A petroleum jelly-ringed coverslip was then placed over the mixture and the slide examined with the aid of a compound microscope.

Crows attracted to offal were live-trapped in late January of both winters with a rocket net-trap. They were subsequently maintained in outdoor pens at the University of Guelph for periods of up to 10 months. Blood was taken from the brachial vein of each crow within one week of capture and examined for microfilariae by the haematocrit capillary tube method.²⁰ In addition, wet preparations of 0.04 ml of blood were examined with a compound microscope for microfilariae. Blood samples were collected between 0900 h and 0300 h.

Each month for 10 months, some of those crows captured live were killed by decapitation and examined for *S.*

caperata. Wet preparations of lung blood were examined for microfilariae. Skin snips taken from crows infected with gravid *S. caperata* were teased in physiological saline on a glass slide and the slide then examined with a compound microscope for microfilariae.

Tissues for histologic study were fixed in 10% buffered formaldehyde solution and embedded and sectioned at 7 μ m following standard histologic techniques. Most sections were stained with Ehrlich's haematoxylin and eosin. Some sections were stained with Verhoeff's elastin stain or periodic acid Schiff reagent. Sections were taken at 100 μ m intervals along the lengths of the heart, aorta and pulmonary arteries of some infected crows.

RESULTS

The overall prevalence of *S. caperata* was 21% in 341 crows collected at different times.

Forty-eight of the 72 crows infected with *S. caperata* were examined with special care to determine the location and sex of nematodes present (Table 1). Prevalence in male crows (21%) was not significantly different ($p \leq 0.05$) from that in female crows (20%). Nematodes were always found in the wall of the right or left pulmonary artery. Extremities of

nematodes did not protrude into the lumen of the artery. Male and female nematodes were present in 22 (46%) of these 48 crows. Intensity was 6.9 (range 1-82).

Females in an artery were never gravid if males were absent from that artery even though males sometimes were present in the opposite artery. Female nematodes always contained microfilariae if males accompanied females in an artery. Microfilariae were never found in peripheral blood or skin snips, regardless of the hour or month in which blood was collected or crows were killed. Microfilariae were found in the lung blood, but only in small numbers, in 4 of the 22 crows (see Table 1) harbouring both male and female *S. caperata*.

Grossly, infected arteries were enlarged. Such arteries were unusually difficult to tease. Tortuous channels within the arterial wall commonly extended throughout the length of the artery. These channels were particularly obvious from the inner surface of the artery. Live adult *S. caperata* could be observed within some of these channels. On transverse section the lumen of the artery appeared dilated and the wall abnormally thick.

Histologically, the tunica media of normal arteries was composed of regular layers of smooth muscle lamellae and

TABLE 1. Results of examination of 48 *Corvus brachyrhynchos brachyrhynchos* Brehm collected in Essex County, southern Ontario, infected with *Splendidofilaria caperata* Hibler, 1964.

Location	Number of Crows Harboursing			Total (%)
	♂ and ♀ nematodes	♀ nematodes only	♂ nematodes only	
nematodes in right pulmonary artery	5	12	4	21 (44)
nematodes in left pulmonary artery	1	5	4	10 (21)
nematodes in both right and left pulmonary arteries	16	1	-	17 (35)
Total (%)	22 (46)	18 (37)	8 (17)	48 (100)

elastin and collagen fibres (Fig. 1). Arteries infected with adult *S. caperata* contained numerous large oval cavities lined with a layer of fibrocytes and within the tunica media (Fig. 2). Cavities were often separated by narrow septa composed of collagen fibres, elastin fibres and smooth muscle cells. The smooth muscle lamellae and the elastin and collagen fibres of the tunica media often were abnormally separated by spaces containing thin collagen strands.

Cross sections of male and female nematodes were present in some cavities within the tunica media. In general, large clear areas generally separated nematodes from the periphery of cavities.

Microfilariae were present in the uteri and vagina of female nematodes in arteries which also contained male nematodes. Microfilariae, however, were observed only rarely in the cavities occupied by adult nematodes or in the

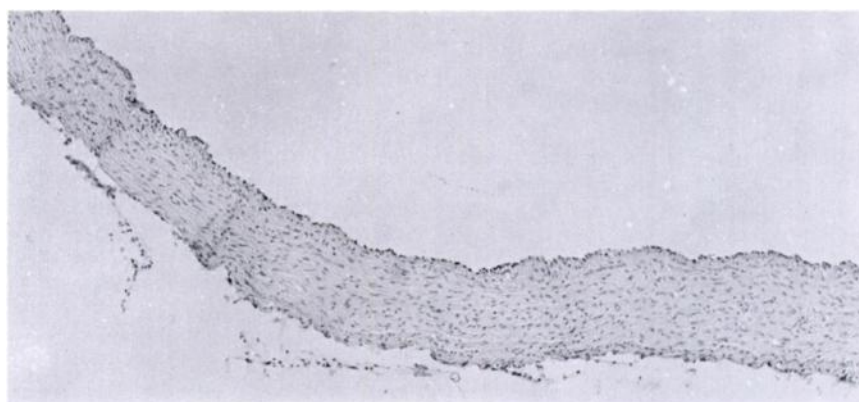


FIGURE 1. Transverse section, normal pulmonary artery of *Corvus brachyrhynchos* Brehm (H & E stain; $\times 100$).



FIGURE 2. Transverse section, pulmonary artery of *Corvus brachyrhynchos* Brehm infected with *Splendidofilaria caperata* Hibler, 1964. Note cavities in the tunica media containing cross sections of adult female *S. caperata* (H & E stain; $\times 50$).

intima. Microfilariae, some surrounded by mononuclear cells and heterophils, occasionally were found in the tunica media of arteries containing adult nematodes (Fig. 3). Microfilariae were more commonly observed in the adventitia. Such microfilariae were frequently surrounded by mononuclear cells and heterophils and occasionally were observed at the centre of microgranulomas. Some microfilariae in the adventitia appeared degenerate (Fig. 4).

In arteries containing gravid female nematodes, the intima commonly had an irregular appearance due to the presence of lymphocytes, plasma cells and heterophils (Fig. 5). Intimal oedema occasionally was observed. The tunica media contained scattered mononuclear cells and heterophils. Inflammatory cell infiltrates appeared to surround small lymphatic and blood vessels in the adventitia (Fig. 5). Lympho-reticular nodules, diffuse collections of mononuclear cells, heterophils and giant cells

and dense collagen were also present in the adventitia (Figs. 6, 7).

Infiltrates in the adventitia and tunica media were more pronounced in arteries containing numerous gravid female nematodes than in arteries containing few female nematodes. The inflammatory lesions observed in transverse sections taken from one region of an infected artery did not always resemble those taken from a different region of the same artery. Microfilariae were unequally distributed in the arterial wall.

Occasionally, the arterial wall adjacent to cavities contained loose myxoid (Fig. 8) or dense eosinophilic material (Fig. 6). Numerous lymphocytes and heterophils and a few plasma cells, giant cells and erythrocytes, in addition to fibrocytes and collagen, occasionally were present in some areas presumably previously occupied by cavities (Fig. 7).

Inflammatory cells were not observed in arteries containing only male or only female *S. caperata*.

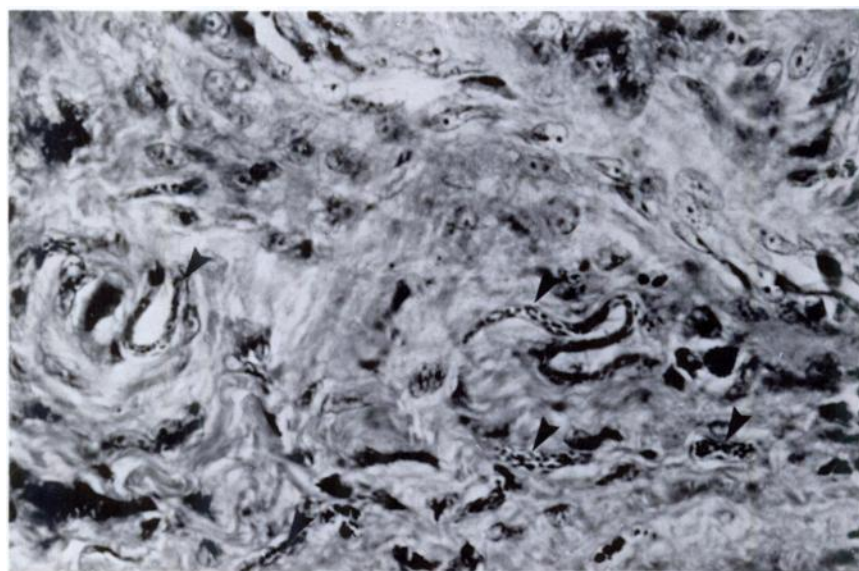


FIGURE 3. Microfilariae (arrows) of *Splendidofilaria caperata* Hibler, 1964 in tunica media of pulmonary artery of *Corvus brachyrhynchos* Brehm (H & E stain; $\times 750$).

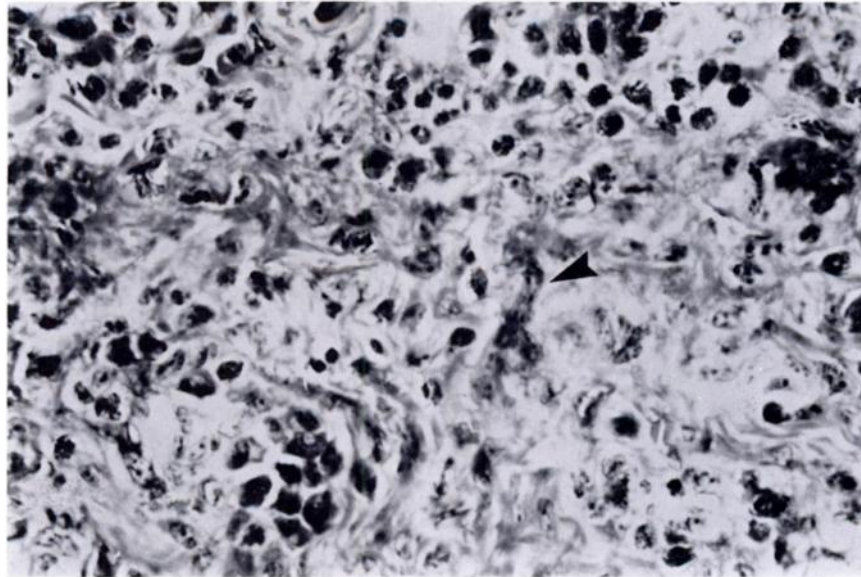


FIGURE 4. Degenerating microfilaria (arrow) of *Splendidofilaria caperata* Hibler, 1964 among inflammatory cells in adventitia of pulmonary artery of *Corvus brachyrhynchos* Brehm (H & E stain, $\times 600$).



FIGURE 5. Cross section of female *Splendidofilaria caperata* Hibler, 1964 in pulmonary artery of *Corvus brachyrhynchos* Brehm. Note irregular appearance of intima due to presence of inflammatory cells. Also, localized collections of inflammatory cells in adventitia (H & E stain; $\times 90$).



FIGURE 6. Section of pulmonary artery of *Corvus brachyrhynchos* Brehm containing cross sections of adult *Splendidofilaria caperata* Hibler, 1964. Note lymphoreticular nodules in adventitia and dense eosinophilic material adjacent to cavities in tunica media (H & E stain; $\times 90$).

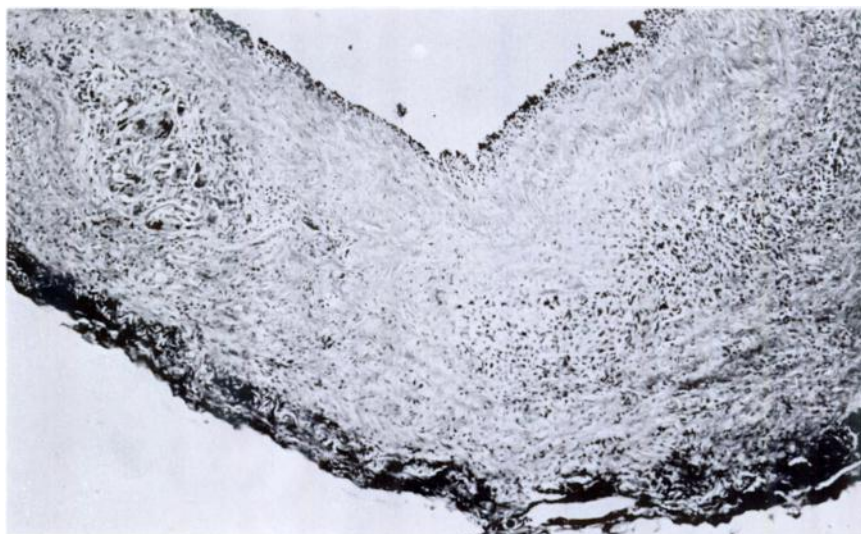


FIGURE 7. Section of pulmonary artery of *Corvus brachyrhynchos* Brehm infected with *Splendidofilaria caperata* Hibler, 1964. Note dense collagen in the adventitia. Also, inflammatory cells, including giant cells, in areas presumably previously occupied by cavities in tunica media (H & E stain; $\times 100$).

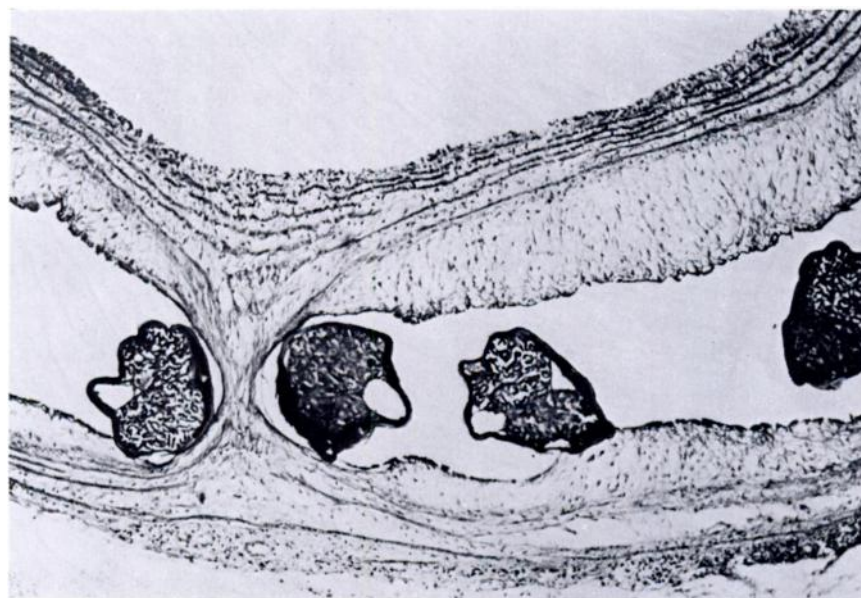


FIGURE 8. Section of pulmonary artery of *Corvus brachyrhynchos* Brehm containing female *Splendidofilaria caperata* Hibler, 1964. Note loose myxoid material adjacent to cavities in tunica media (PAS stain; $\times 90$).

DISCUSSION

The absence of microfilariae of *S. caperata* in the peripheral blood and skin of the crow (*C. b. brachyrhynchos* Brehm) harbouring adult *S. caperata* cannot be attributed to circadian or seasonal fluctuations in the numbers of microfilariae. Young-of-the-year crows might, however, have had a short transient microfilaremia before they were examined in November. A short transient microfilaremia precedes the amicrofilaremic period in occult infections of *Dipetalonema viteae* (Krepkogorskaya, 1933) in golden hamsters^{10,17,18} and *Litomosoides carinii* (Travassos, 1919) in albino rats.^{2,3}

Channels in the tunica media of pulmonary arteries of crows were associated with the presence of adult *S. caperata*. Such channels were not present in the tunica media of pulmonary arteries of uninfected crows. In addition,

vasa vasorum are not present in the tunica media of birds.^{14,16}

Extremities of adult *S. caperata* were not observed in the lumen of the pulmonary arteries and there is no evidence microfilariae are deposited into the blood. Also, channels containing nematodes apparently did not open into the lumen. Microfilariae might normally reach the blood by penetrating the walls of the channels, the tunica media and the intima. They might also pass through the adventitia and reach the blood and lymphatic vessels.

Occult infections of *S. caperata* might occur in crows because microfilariae are overcome in the wall of the pulmonary artery. The presence of microfilariae in the arterial tissue was associated with chronic inflammation in the arterial wall. Dead or damaged microfilariae are highly antigenic^{7,8,18} and their presence would account for the inflammation observed.

Helin *et al.*¹¹ suggest that inflammation of the adventitia is common in arteries subjected to physical damage. Inflammation in the adventitia of arteries infected with gravid *S. caperata* might also be associated with physical damage caused by migrating microfilariae.

Splendidofilaria caperata produces inflammation in the pulmonary artery of both magpies and crows. Inflammation in magpies might be caused by adult nematodes,¹² whereas, in crows microfilariae apparently are the cause of arterial inflammation. Inflammation was not observed in crows harbouring only male or only female *S. caperata*. *Splendidofilaria algonquinensis* (Anderson, 1955) also occurs in channels within the wall of the pulmonary artery of the host.^{1,15} In addition, in house sparrows (*Passer domesticus domesticus* L.) *S. algonquinensis* (= *S. passerina*) was found free in the lumen of the artery. Microfilariae of *S. algonquinensis* occur in the blood of infected sparrows and inflammation was not observed in the wall of the pulmonary arteries.¹⁵

Huizinga *et al.*¹⁵ reported fibrosis in the pulmonary arteries of sparrows infected with *S. algonquinensis*, similar to that observed in crows infected with *S.*

caperata. In the present study, the channels in the arterial wall were also occasionally filled with collagen-like or loose myxoid material. This reparative process might occur while live nematodes are present in the channels. Complete closure, however, occurs only if nematodes are absent.

Elliot⁹ found adult filarioids in the walls of the pulmonary artery and numerous microfilariae in the blood of crows collected near Gravenhurst, Ontario. He did not describe the adult nematodes but the species was likely *S. caperata*. Elliot's description of the microfilariae shows that they belonged to *Chandlerella chitwoodae* Anderson, 1961 and not *S. caperata*; numerous microfilariae of *C. chitwoodae* were always observed in crows infected with adult *S. caperata*.⁶

Splendidofilaria caperata occurs commonly in crows but because the infection is occult, crows must acquire the parasite from insects that have fed on other birds in which a microfilaraemia does develop. *S. caperata* has not been reported from other birds in Ontario. Magpies, the type host, do not occur in this region. Thus, it is still not known which bird species are required for the maintenance of the parasite in Ontario.

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