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PATHOLOGY AND EPIZOOTIOLOGY OF DIOFILARIA SCAPICEPS (LEIDY, 1886) (NEMATODA: FILARIOIDEA) IN SYLVILAGUS FLORIDANUS (J. A. ALLEN) AND LEPUS AMERICANUS ERXLEBEN

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ABSTRACT: Dirofilaria scapiceps was found between the synovial sheath and tendons, i.e., within the tendon sheath, in the ankle region of eastern cottontail rabbits (Sylvilagus floridanus) and snowshoe hares (Lepus americanus). In cottontail rabbits, tendons and sheaths appeared normal and all worms were adults. Only one (4%) of 24 infected rabbits contained dead worms. All female worms were gravid in rabbits killed in late winter or early spring. Microfilaremias in rabbits were high (approximately 30–100 microfilariae/60 μl blood) and of long duration (at least 8–28 mo), and rabbits were considered normal hosts of D. scapiceps. In some snowshoe hares, tendons and sheaths also appeared normal; however, in other hares a chronic proliferative tenosynovitis, characterized by fibroin exudate, hyperplasia and hypertrophy of the intima and inflammatory cell (predominantly lymphocytes and plasma cells) infiltration of the intimal and fibrous layers of the synovial sheath led to encapsulation of worms. Dead subadult, dead adult, and live adult worms were found in the ankles of hares; 86 (46%) of 186 infected hares contained some or only dead worms. Fibrosis commonly occurred around dead worms. Dead subadults were also found in subcutaneous connective tissues over the trunk of the body. Degenerate embryos and amorphous material were observed in uteri of some female worms in hares killed in late winter or early spring. Few (1–5 microfilariae/60 μl blood) or no microfilariae were observed in the peripheral blood of hares and microfilaremias were of short duration (less than 8 mo). Microfilariae in hares are probably trapped and destroyed in the chronic inflammatory lesions in the tendon sheaths since normal, degenerate, and calcified microfilariae were observed in the capsules around adult worms. Some microfilariae might also be destroyed in lymph nodes. Although D. scapiceps can be maintained within snowshoe hare populations, hares are considered abnormal hosts of D. scapiceps. Dirofilaria scapiceps may have spread from cottontail rabbits to snowshoe hares relatively recently.

INTRODUCTION

Dirofilaria scapiceps is a mosquito-transmitted filarioid nematode of rabbits and hares in North America (Bartlett, 1983, 1984a). Adults are generally found in the ankle region, although rarely they occur near the knee joint. Bartlett (1984b) described development in experimentally infected eastern cottontail rabbits and snowshoe hares. This paper presents epizootiologic and pathologic evidence that the cottontail rabbit is a more suitable host for D. scapiceps than the snowshoe hare.

MATERIALS AND METHODS

Eastern cottontail rabbits were live-trapped in Ontario and snowshoe hares were live-trapped in Ontario and Alberta, during November–February 1980–1981. Animals were transported to the University of Guelph and maintained in outdoor cages (Bartlett, 1984a). Four heparinized hematocrit capillary tubes (60 μl capacity) of blood were collected from the marginal ear vein of each animal every 3 wk from early February to mid-May. Tubes were examined for microfilariae using the microhematocrit centrifuge technique (Woo, 1971). The number of microfilariae observed at the interface was counted in those capillary tubes of blood collected in May. Some rabbits and hares survived until autumn and their blood was re-examined in October. Animals which did not die of natural causes were killed by injection of an overdose of sodium pentobarbital or by cervical dislocation. All carcasses were examined for D. scapiceps (see Bartlett, 1983); nematodes recovered were fixed in hot glycerin-alcohol and examined in glycerin.

Worms from other rabbits and hares (Bartlett, 1983) were also examined and the results...
are reported herein. Blood from these rabbits and hares was not examined; however, some ankles and popliteal lymph nodes were fixed in 10% buffered formalin for histologic study. Ankles were decalcified in 10% formic acid for 2–3 wk, transferred to 2% aqueous lithium carbonate for 24 hr, then stored in 70% ethanol (Humason, 1972). Tissues were sectioned at 7 μm and stained with eosin and Delafield's haematoxylin. The presence of collagen was confirmed by its refraction of polarized light.

Infections were considered sterile if male and female worms were not found together in one ankle.

Specimens of D. scapiceps were deposited in the U.S. National Parasite Collection in Beltsville, Maryland 20705, USA: 1) from cottontail rabbits—USNM No. 77346; 2) from snowshoe hares—USNM Nos. 77342–77345.

RESULTS

Examination of peripheral blood and adult worms

Cottontail rabbits: Microfilariae were observed in peripheral blood of 5 of 15 cottontail rabbits examined. Microfilariae were present in all four capillary tubes of blood collected at each sampling time from each microfilaricmic rabbit during February to May. In May the number of microfilariae in each tube was approximately 30–100. In October microfilariae were present in peripheral blood of four rabbits which had been microfilaricmic in the spring and which survived until autumn. One of these rabbits survived for an additional 20 mo and microfilariae were present in its blood throughout this time. At necropsy, adult D. scapiceps were found in six of the 15 rabbits. Male and female worms were found in the five microfilaricmic rabbits; the sixth rabbit had a sterile infection.

Sterile infections occurred in four (17%) of a further 24 infected cottontail rabbits collected in Ontario by Bartlett (1983). Dead adult worms were present in the ankle of only one rabbit. Subadult worms (see Bartlett, 1984b) were not found. Provided male worms were present, all female worms from rabbits killed in late winter or early spring contained microfilariae.

Snowshoe hares: Microfilariae were observed in peripheral blood of seven of 21 snowshoe hares examined. However, these microfilariae were present in only some of the four capillary tubes of blood collected at each sampling period from each microfilaricmic hare during February to May. In May the number of microfilariae in each positive tube was 1–5. In October microfilariae were not observed in peripheral blood of three hares which had been microfilaricmic in the spring and which survived until autumn. At necropsy, adult D. scapiceps were found in 18 of the 21 snowshoe hares. Male and female worms were found in 13 of these hares, including the seven microfilaricmic hares. The five remaining hares had sterile infections.

Sterile infections occurred in 41 (22%) of a further 186 infected snowshoe hares collected in Ontario by Bartlett (1983). Dead adults were found in the ankles of 86 (46%). Many of these dead worms were calcified. Other worms which were judged to have been dead at the time the host was collected did not clear in glycerin, as did worms which were judged to have been alive. In addition, dead subadults were found in the ankles and subcutaneous tissues of the trunk of many hares. Microfilariae were found in some female worms from hares killed in late winter or early spring; uteri of others were empty or contained degenerate embryos.

Gross observations

Cottontail rabbits: Worms were free within the delicate connective tissue sheaths around tendons along the front and lateral surfaces of the distal third of the tibiofibula and also immediately above the joint capsule of the ankle.

Snowshoe hares: Worms in some hares were within capsules around tendons along the front and lateral surfaces of the distal third of the tibiofibula and also immediately above the joint capsule of the ankle.
Worms in other hares were free within the delicate connective tissue sheaths around tendons in these same locations, i.e., they were not encapsulated. Unencapsulated worms were more common among hares collected in Alberta in November than among hares collected in Ontario in February–May. Capsules containing nematodes generally also contained an amber fluid or spongy white material, likely fibrin.

Dead subadult and adult worms in the ankles were free in the lumen of capsules containing live worms, adhered to the sides of these capsules, or encapsulated by themselves. Dead subadults were also found adherent to the outer surface of capsules and to the periosteum of the lateral and medial malleoli of the distal tibia and fibula, the medial side of the talus and the lateral side of the calcaneous. Dead subadults in the subcutaneous tissues of the trunk were either free or encapsulated.

**Histologic observations**

*Cottontail rabbits:* Worms were in the synovial space between the tendon sheath and the tendons (Fig. 1). Tendons and most regions of the sheaths appeared normal, i.e., margins of tendons contained only fibroblasts and tendon sheaths consisted of 1) a thin, smooth intimal layer of cuboidal or squamous synovial cells one to three deep, and 2) an outer meshwork of loose connective tissue containing small blood vessels (Fig. 2). Rarely, small regions of intima had a slightly ruffled surface and the cells were somewhat enlarged; the connective tissue below these regions contained a few scattered lymphocytes and plasma cells (Fig. 3). Microfilariae were occasionally present in spaces near adult worms, but were never noted in popliteal lymph nodes.

*Snowshoe hares:* Worms were in the synovial space between the tendon sheath
and the tendons. When worms were not encapsulated, tendons and sheaths resembled those in cottontail rabbits. When worms were encapsulated (Fig. 4), the capsule wall consisted of thickened layers of the tendon sheath, i.e., the intima was hyperplastic and its synovial cells hypertrophic and the outer connective tissue layer was infiltrated by numerous lymphocytes and plasma cells and a few heterophils or eosinophils, macrophages, and giant cells (Fig. 5). In some hares, variable amounts of dense collagen were present in the outer connective tissue layer below the zone of inflammatory cells. In addition, variable numbers of mononuclear, polymorphonuclear, and giant cells were commonly present in the intima. In some places villous proliferations of intima extended into the capsular lumen and adhered to tendons. Variable amounts of fibrinous exudate were present in many capsules. This exudate commonly contained scattered macrophages and fibroblasts (Fig. 4). In some places it had become organized to the tendon sheaths (Figs. 6, 7) and tendons (Fig. 8), sometimes forming fibrinous adhesions between them. Neovascularization had occurred in the more organized regions of granulation tissue.

Microfilariae of normal appearance were occasionally noted free in the capsule lumen. Other microfilariae in the lumen appeared degenerate and were surrounded by fibrinous exudate (Fig. 9). In some areas of granulation tissue these microfilariae had become calcified (Fig. 10). Microfilariae were also occasionally noted in the capsular wall; some of these microfilariae appeared normal, others degenerate (Fig. 11). Microfilariae occasionally

**Figure 3.** Mild inflammation of tendon sheath in cottontail rabbit. Note slightly ruffled surface of intima and slight enlargement of synovial cells, also scattered mononuclear inflammatory cells in outer connective tissue layer. W = margin of worm. Bar = 50 μm.

**Figure 4.** *Dirofilaria scapiceps* in synovial space between tendons (T) and inflamed tendon sheath in ankle region of snowshoe hare. E = fibrinous exudate. Bar = 500 μm.
Capsule wall in snowshoe hare showing hyperplastic intima (I) containing hypertrophic synovial cells and outer connective tissue layer (CT) containing numerous mononuclear inflammatory cells. Mononuclear cells also present in lumen (L) of capsule. Bar = 50 μm.

Figure 6. Fibrinous exudate becoming organized to wall of capsule in snowshoe hare. W = worm. Bar = 300 μm.

noted in the popliteal lymph nodes were surrounded by numerous lymphocytes and a few granulocytes.

Capsules containing only dead nematodes fitted snugly around the worms and consisted of numerous hypertrophic intimal cells, macrophages, lymphocytes and fibroblasts (Fig. 12). A few plasma cells and giant cells were also present. Dense connective tissue was present at the periphery of the capsule.

Lymphocytes and plasma cells had invaded the epineurium of nerves adjacent to areas of chronic inflammation described above.

**DISCUSSION**

*Dirofilaria scapiceps* occurred in the synovial space between tendons and ten-
subadults migrate to the ankles through the subcutaneous tissues (Bartlett, 1984b).

The chronic inflammation of tendon sheaths observed in many snowshoe hares was characterized by synovial hypertrophy, hyperplasia, villous proliferation, inflammatory cell infiltration, and fibroplasia and it led to encapsulation of worms. Authors who have stated that adult D. scapiceps occur in the "tarsal bursa" of hares may have misinterpreted capsules as part of the bursa proper. Hypertrophy, hyperplasia, and villous proliferation of the intima are normal sequelae of chronic, low-grade irritation of synovial membranes (Jubb and Kennedy, 1970; Jaffe, 1972; Castor, 1975). An increased volume of synovial fluid and a fibrinous exudate may also occur (Jubb and Kennedy, 1970; Castor, 1975). The observed dominance of lymphocytes and plasma cells in the inflammatory cell infiltrate in the tendon sheath suggests, in addition, a chronic immunologic response to the presence of the worms.

Adhesions that formed between tendons and tendon sheaths in some infected snowshoe hares might hinder normal tendon flexibility. This may explain the "reluctance" of infected hares "to move," as reported by Barrett and Dau (1981).

Low numbers or absence of microfilariae in the peripheral blood of snowshoe hares infected with D. scapiceps have been noted previously. Highby (1943) commented on the low prevalence and intensity of larval stages of D. scapiceps in mosquitoes which had fed on infected snowshoe hares (cf. high prevalences and intensities in mosquitoes fed on infected cottontail rabbits (Bartlett, 1984a)). Bookhout (1971) noted the absence of microfilariae in blood smears taken during the
summer from snowshoe hares in an area where necropsies had revealed a high prevalence (64.2%) of *D. scapiceps*. Zarnke (1976) found "larval nematodes," presumed to be microfilariae of *D. scapiceps*, in cultures of various tissues from 13 wild-caught snowshoe hares but was unable to find microfilariae in the blood.

The microfilaremia of *D. scapiceps* (as determined in cottontail rabbits) does not exhibit circadian fluctuations (Bartlett, 1984b); thus, such a phenomenon does not account for the absence or rarity of microfilariae in snowshoe hares. Rather, this may be due to entrapment and destruction of microfilariae in the lumina and walls of capsules surrounding adult worms. Synovial cells possess phagocytic capabilities and may form giant cells (Castor, 1975); those giant cells observed probably formed around degenerate microfilariae. However, some microfilariae may escape from the ankle region via the lymphatics, as numerous lymphatic vessels are present in the tendon sheath (Jaffe, 1972). The efferent lymphatics drain into the popliteal lymph nodes and some microfilariae might become trapped in this location.

The presence of degenerate embryos and amorphous material in the uteri of some female *D. scapiceps* in snowshoe hares suggested that some females might not produce microfilariae. The hares harboring these worms were killed in late winter or early spring and ample time would have elapsed after infection for females to produce microfilariae (Bartlett, 1984b). Similar "reproductive detritus" was reported by Spratt (1972) in the uteri of some *Dirofilaria roemeri* (Linstow, 1905) in grey kangaroos (*Macropus giganteus* Shaw). Spratt suggested such detritus "may be due to the lengthy presence of the parasite in an abnormal host" and pointed out that Beaver and Orihel (1965) reported similar material in the uteri of infertile *Dirofilaria* spp. from man.
In spite of the high prevalence and intensities of *D. scapiceps* in cottontail rabbits and snowshoe hares (Bartlett, 1983) approximately 20% of infections in these animals were sterile (present study). Sterile infections included those in which male worms only were present in one ankle and female worms only in the other ankle, since fertilization occurs after worms migrate to the ankle (Bartlett, 1984b) and worms apparently do not move from one ankle to the other.

As outlined previously, cottontail rabbits are herein considered normal hosts of *D. scapiceps* and snowshoe hares abnormal hosts. Normal and abnormal wildlife hosts of filarioid nematodes have been recognized previously. Spratt (1972) considered that eastern wallaroos (*Macropus robustus* Gould) were normal hosts and grey kangaroos and red-necked wallabies (*M. rufogriseus banksianus* (Quoy and Gaimard)) were abnormal hosts of *Dirofilaria roemeri*. Spratt (1975) reported that infections with *D. roemeri* in red kangaroos (*Macropus rufus* (Desmarest)) exhibited some features of infections in normal hosts and some of infections in abnormal hosts. Bartlett and Anderson (1981) and Bartlett (1982) considered magpies (*Pica pica* (L.)) and starlings (*Sturnus vulgaris* L.) to be normal hosts of *Splendidofofilaria caperata* Hibler, 1964 and crows (*Corvus brachyrhynchos* Brehm) to be abnormal hosts.

Bartlett (1983) reported *D. scapiceps* in snowshoe hares in areas where this hare is the only lagomorph species present. Thus, *D. scapiceps* can be maintained within snowshoe hare populations in spite of the rarity or absence of microfilariae in peripheral blood. In contrast, the abnormal hosts of *D. roemeri* and *S. caperata* apparently acquire infections only in areas
where the normal hosts serve as reservoirs of infection (Spratt, 1972, 1974; Bartlett and Anderson, 1981). *Dirofilaria scapiceps* may be maintained in snowshoe hare populations in areas where high mosquito densities and some microfilaricidal hares are present. In addition, microfilariae of *D. scapiceps* are generally abundant in the fluid in capsules surrounding gravid female nematodes and might enter capillaries in areas of granulation tissue or capillaries in adjacent subcutaneous tissues. Thus, mosquitoes which feed in the ankle region might ingest these microfilariae. Variations in mosquito density, mosquito feeding behavior, and hare density likely cause the marked variations in the prevalence of *D. scapiceps* in snowshoe hares in different localities in the boreal forest reported by Bartlett (1983).

The apparently poor host–parasite relationship of snowshoe hares and *D. scapiceps* suggests that it may have developed relatively recently. The parasite may have secondarily spread from the cotton-tail rabbit, which is an animal of South America, Central America, and southern North America, to the snowshoe hare, which occurs largely in northern North America. *Dirofilaria scapiceps* is known from lagomorphs only in North America (Bartlett, 1983), and this raises the possibility that the parasite evolved in the New World. Further work is required to confirm the absence of the parasite in *Sylvilagus* spp. in central and South America.

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**LITERATURE CITED**


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CORRECTION . . .

The following corrections should be made to the article:


Page 1: The fourth author, Clarence Bagshaw, is associated with the Savannah River Ecology Laboratory, P.O. Drawer E, Aiken, South Carolina 29801, USA.

Table 1: The four birds listed as "Mergus spp." were collected from the coastal region of Rhode Island, and were not from Aiken, South Carolina, as implied by the section on Materials and Methods.

Page 4: ACKNOWLEDGMENTS should include the following statement: “This study was also supported in part by a contract (DE-AC09-76SR00819) between the U.S. Department of Energy and the University of Georgia.”