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FILARIAL WORMS OF COLUMBIAN BLACK-TAILED DEER IN CALIFORNIA
1. Observations in the vertebrate host[1]

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Abstract: An 8-year survey of filarial worm infections in black-tailed deer *(Odocoileus hemionus columbianus)* in a northern California study area revealed that the great majority of deer became infected with three filariids. The footworm *(Wehrdikmansia cervipedis)* and the arterial worm *(Elaeophora schneideri)* showed increased prevalence with host age but just the opposite was seen with the abdominal worm *(Setaria yehi)*. Most fawns were infected with *S. yehi* as were almost half of the yearlings but the parasite was relatively scarce in deer over 2 years of age.

INTRODUCTION

Columbian black-tailed deer *(Odocoileus hemionus columbianus)* have been studied for about 20 years at the University of California field station at Hopland (Mendocino Co.). Parasitological investigations have been concerned mainly with the potential for exchange of parasites between deer and livestock.[21] During the period 1964 to 1971, research efforts were focused on the parasitic nematodes transmitted by arthropods. This report contains observations on three species of filarial worms commonly found in deer on the station and indeed, in the western United States:[8,14,25] namely, the footworm *(Wehrdikmansia cervipedis)*, the arterial worm *(Elaeophora schneideri)* and the abdominal worm *(Setaria yehi)*. A subsequent paper will deal with transmission studies and observations on the vectors of these parasites.

The 1890 hectare Hopland field station has a relatively large resident deer population, generally numbering between 500 and 1,000 animals.[7] They share the station with at least several times this number of sheep and a few cattle and horses. The topography can be summarized as rolling hills interspersed with ravines, with elevations ranging from 200 m to 1,000 m. This is a summer drought area and vegetation types include grassland (27%), dense woodland (22%), chaparral (15%), and mixed woodland-grass types (36%).[18]

METHODS

Most of the deer in this survey were killed in connection with other projects over approximately 8 years; some were taken during yearly public hunts, and a small percentage represent kills of sick or injured animals. Separation into various sex and age classes was by methods described by Robinette et al.[25] At necropsy, the major arteries of the head (common carotid and internal maxillary)

were examined for *E. schneideri*. During the last 3 years of the survey (1969-1971), cephalic artery examinations were supplemented by searching for microfilariae in skin snips taken from the head and ears. The snips were soaked in physiological saline (0.85%) during the first year, but thereafter Earle's solution was used. Snips were examined after several hours of soaking at room temperature and again the next day. This procedure increased the percentage of animals recorded as positive for arterial worms (e.g., almost doubling the number of positives among deer over 3 years old). Footworms were generally detectable upon skinning the carcass, most commonly occurring subcutaneously near the tibio-tarsal joints. Microfilariae of *W. cervipedis* were found only in skin snips from the ears. The body cavity and viscera were examined for abdominal worms. With captive deer the peripheral blood was examined for microfilariae of *S. yehi*, either in 20- to 60-mm^3 blood films, after a concentration technique,[30] or after squashing the abdomens of blood-sucking flies caught just after engorgement. Worms were fixed in AFA and preserved in 70% alcohol with 5% glycerine.

RESULTS

Sex-related differences in susceptibility of deer to filarial infection were not apparent in this study which was largely based on parasite prevalence data. But even when quantitative data regarding individual infection intensity were available, there did not appear to be significant differences between the sexes in each host age class. For this report data from male and female deer were pooled for each age group.

Arterial worms were found in 65 of 170 deer examined (38.2%) but this is a minimal figure since only about half of the examinations included skin snips as well as a search of the head arteries. When both techniques were applied, 64 of 82 (78%) deer over 3 years old were positive while relatively few additional positives were detected among younger

deer examined for both adult worms and microfilariae. As indicated in Fig. 1 (which represents a summary of all the data on *E. schneideri*) the prevalence of the parasite increased with host age, arterial worms occurring in more than half of the deer over 3 years of age. The parasite was rare in animals less than a year old. One positive fawn (aged 9-10 months) was examined in early March, 1966, and four full-sized *E. schneideri* (2♂, 2♀) (lengths: 680, 645, 1,050, 1,020 mm) were recovered. The females were not gravid. Another positive fawn (aged 10 - 11 months) was killed in mid-April of the same year and 14 full-sized worms (6♂, 8♀) were found. Two of the females had uterine microfilariae. The number of adult worms found in the heads of older deer was generally less than 10 but as many as 32 were recovered from an individual animal. The worm male to female sex ratio was 1:1.8 (78 worms). No illness or gross lesions were obviously associated with infection. Multiple skin samples were taken from different body regions of six deer killed in August, 1970, that were positive for both footworms and arterial worms. From 36 to 45 superficial skin snips (each 6- to 8 mm^2 and weighing 9.8-17.6 mg) were taken from representative parts of each animal (ears, head, neck, torso, limbs, tail) and soaked in Earle's solution. Examinations were made after 2-4 and 18-24 hours. Microfilariae of *E. schneideri* occurred almost exclusively in skin from regions of the head, mainly the poll, face, and to a lesser extent, the ears. Several samples from the neck and one from the rear leg contained a few microfilariae. However, even in areas of concentration, *E. schneideri* microfilariae were generally sparse (10 to 50 per sample) compared with footworm microfilariae which were usually abundant at this time of the year in their only site of predilection, the ears.

Footworms were present in 90 of the 156 deer examined (57.7%) and, of those over 3 years old, 86.5% were positive. *W. cervipedis* was found in only two deer under 1 year of age (Fig. 1). Both of these were killed in early March, 1967, and they harbored respectively

three and seven non-gravid female worms of nearly adult length (120-150 mm). In addition to examining multiple skin snips from the aforementioned six positive deer, a careful search for adult footworms was made of the entire hides and carcasses after skinning. Particular care was paid to the skin and subcutaneous tissues in the region of the head. The ears were macerated and soaked overnight in Earle's solution, followed by 8 hours of digestion in pepsin-HCL.[6] A total of 208 adult worms was found, numbering 59, 53, 40, 34, 14, 8 in individual animals. All worms were from the limbs and they were predominantly non-gravid females. Only seven male worms were found (3.3%), none being detected in two deer. Uterine microfilariae were seen in 29 females (14.3%), at least one gravid worm occurring in each deer. Adult worms were concentrated about the hock joints and a few occurred in the upper limbs. Approximately three times as many worms were found in the

rear legs as in the front legs. Although adult worms were not found in the head or trunk regions of the six animals examined in detail, they were not uncommonly seen in other deer in subcutaneous tissues of the flanks, shoulders, belly, and brisket. During this survey, no lesion or obvious debility appeared to be associated with footworms, even when they were fairly abundant (i.e., over 50). Microfilariae were found almost exclusively in the skin of the ears. Apart from an occasional microfilaria in head skin, samples from other parts of the body were negative, even those taken from areas next to the ears or the skin overlying gravid females in the limbs. Ear snips taken in the summer and fall usually yielded 100 to 500 microfilariae after soaking in saline overnight but counts between 1200-1500 were often recorded. There was considerable variability in numbers of microfilariae emerging from different snips taken from the same ear but there was no indication that this was
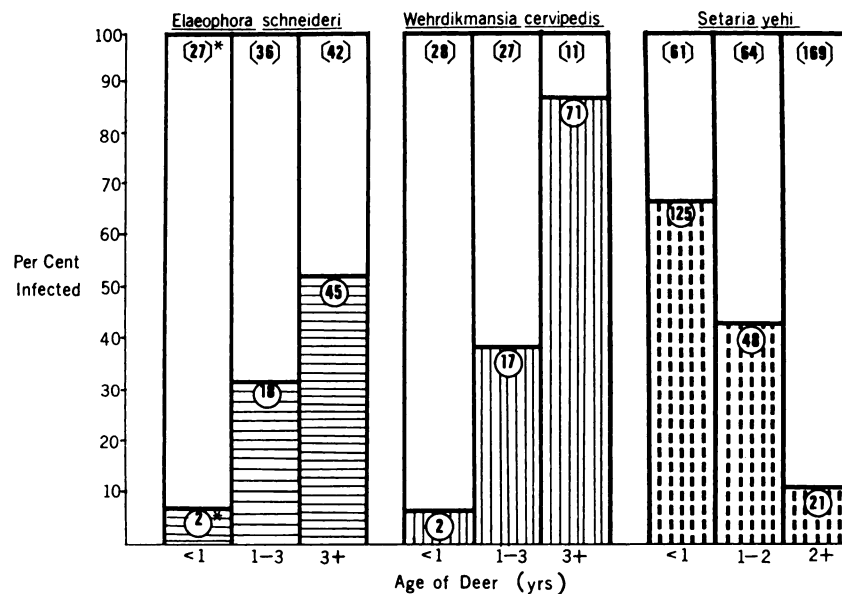

Fig.1 Filarial worm infections in various age classes of black-tailed mule deer

* ◯ = number of deer positive

( ) = number of deer negative

correlated with the inner vs outer aspects or proximal vs distal parts of the ear. Some microfilariae emerged after soaking when ear snips were taken 6 days after the death of the deer host (the carcass had been held at 4-8 C).

In striking contrast to the increased prevalence with host age seen with foot-worms and arterial worms, *S. yehi* infections were most common in young deer, becoming exceptional in deer over 2 years of age (Fig. 1). At least 67.2% of the fawns and 42.8% of the yearlings were infected while only 11.1% of the older deer harbored viable abdominal worms. Dead, encapsulated, or calcified worms were scarce in fawns, more frequent in yearlings, and common in 2 year olds. Deer over 3 years of age rarely showed any signs of the parasite. Relatively few infected deer had more than 30 abdominal worms, the heaviest worm burden encountered being 88 (with a male to female ratio of 1:1.2). Heavier infections generally occurred in weak, debilitated fawns and yearlings and more than half of the positive older deer were diagnosed as being sick or in poor condition.

At Hopland, deer are born mainly in May and June.[7] Patent *S. yehi* infections in fawns were first detected in October and by November gravid worms were commonly found in young deer. The blood of six captive fawns (2 ♂, 4 ♀) was examined for microfilariae at irregular intervals for more than a year, sometimes by blood films, more frequently by examining the contents of freshly engorged blood-sucking arthropods. All were positive during their first year from November through July, with microfilaremias varying irregularly between 3 and 45 per 20 mm.[3] Two of the females had negative blood in September of their 2nd year (and thereafter) and a third doe became negative in October. One of the males was negative for adult worms in December of the 2nd year when it was accidentally killed. Two yearlings positive in November were not examined again until May when they were killed. No adult worms or microfilariae were found at necropsy.

DISCUSSION

Our study area may be regarded as hyperenzootic for these several filarial worms of deer. High prevalence rates were consistently found year after year. The great majority of deer that lived beyond two summers in this area became infected with all three species. However, there was a striking age-related difference between when deer acquired abdominal worm infections and when arterial worm and footworm infections became prevalent (Fig. 1). Immunological as well as ecological factors seem to be significantly involved.

Transmission of abdominal worms was primarily by the tree-hole breeding mosquito, *Aedes sierrensis*, which generally reached peak numbers soon after the birth of most fawns and was by far the dominant mammal-feeding mosquito on the station (data from concurrent transmission studies will be presented in the next paper of this series). All age classes of deer were subject to repeated and relatively heavy mosquito attack during late spring and early summer. During this time, most deer were clustered into "family" units consisting of the mother (usually negative for *Setaria*), generally a pair of yearlings (usually infected) and a pair of newborn fawns (rarely uninfected when examined in the fall and winter months). Older males tended to be at higher elevations on the station where the vector was less common.

That host age *per se* (i.e., "age immunity") was not a prime factor in restricting *S. yehi* to younger animals was indicated by several observations. Some older deer (over 2 years old) did harbor viable *S. yehi*, particularly if they were ill or otherwise stressed. Although it was unusual to find *S. yehi* in healthy, older deer, our records included ten instances where this seemed to be the case. Seven of these positive deer were bucks taken at higher elevations where frequency of contact with the parasite was thought to be less, due to fewer tree-hole mosquitoes; and three were mature does whose home range was known to be restricted to high chaparral areas adjacent to the

station where mosquitoes were sparse or absent.

It is highly probable that most deer in the study area were repeatedly exposed to infective stages of *S. yehi,* on perhaps a nightly basis for several months or more. The fact that there was rarely a build-up in parasite numbers in deer points to the development of a strong acquired immunity against superimposed infection. Few healthy fawns had even a dozen abdominal worms while those in poor or sickly condition predictably had heavier worm burdens. Microfilaremia levels in fawns seemed to remain fairly steady in winter and spring but fell off during their second summer on the station. The apparent failure of challenge *S. yehi* to develop in animals already harboring adult worms suggests that protective immunity was expressed primarily against the infective larvae or early developing stages in deer, reflecting probably the relative vulnerability of these stages to immunological attack and perhaps their relative importance as immunogens. Scott *et al.*[20] experimentally demonstrated immune effects against the infective stages of the cotton rat filariid, and Otto[21] has reviewed other examples of protective immunity in filarial infections. That the presumably "immunologically privileged" adults of *S. yehi* in deer are eventually affected by immune processes is suggested by the gradual reduction in microfilaremia during the approximate year of patency and the increased number of dead, encapsulated or calcified worms seen in 1 to 3 year-old deer.

The increasing prevalence of arterial worms and footworms with host age stands in contrast to the pattern seen with *S. yehi.* With footworm infections, a relatively brief transmission season is probably the main factor determining the host age relationship. As will be detailed elsewhere, the only vector of footworms that we have been able to incriminate in our study area was the blackfly, *Prosimulium impostor.* Adults of this fly consistently appeared early in Spring and were gone by the time most of the fawns were born in May and June. Thus, most deer probably had no chance for contact with this parasite until they were yearlings. And even then, many apparently were not infected, probably because of a relatively low vector density, but after several transmission seasons the great majority of deer harbored footworms. These worms were difficult to remove intact and infections were not routinely assessed by worm counts and measurements. It was apparent, however, that worm burdens did not increase linearly with host age. It was not unusual in late fall to find a variable mix of viable, partially grown and full-sized worms along with calcified adults, sometimes with parts still alive, in the same older deer. Partial calcification of viable worms has been observed in other subcutaneous filarial infections, e.g., *Onchocerca volvulus* and *O. gutturosa.*[22]

The finding of nearly adult-sized (12-15 cm.), non-gravid, female footworms in two 8-10 month old fawns in March is suggestive of a relatively long prepatent period, on the assumption that the worms were almost as old as their hosts because of the limited time for transmission. No male worms were found but this was frequently the case even in older infections in which most of the females contained uterine microfilariae. Full-sized females were generally gravid in early spring and ceased producing microfilariae later in the year. The evidence for seasonality in microfilariae production by footworms will be presented elsewhere. After discovering that footworm microfilariae localized only in the ear skin, Hibler[17] searched for adult worms in the head region on the assumption that microfilariae would most likely accumulate in the skin overlying gravid worms; and in at least one deer, males and gravid females were found under the skin inside the ear, near the base of the conchal cartilage. We carefully skinned and searched the heads of 14 deer with moderate numbers of footworms (10-75) in the limbs and with the ear skins teeming with microfilariae, without finding adult worms in the head, even after soaking teased tissues from six of these in Earle's solution or after partial digestion of head and ear skin in pepsin-HC1.

Uterine microfilariae occurred in at least some females in the limbs of each deer. In heavy infections, adult worms have been reported to occur subcutaneously in many parts of the body, including the head.[15,20] However, our failure to detect microfilariae in the skin, body fluids, or other tissues adjacent to gravid worms in the limbs, as was the experience of Herman and Bischoff,[16] suggests a remarkable polarization in sites of predilection in deer, the adults occurring primarily in the limbs and the microfilariae almost exclusively in the ears at the opposite end of the body. Our examination of hundreds of deer in California leads us to believe that this is the normal situation in this area. It is possible that strains of *W. cervipedis* may localize in different body regions in different geographic areas, a well-documented phenomenon with the closely related filariid of man, *Onchocerca volvulus*. Such variation has frequently been attributed, at least in part, to the biting habits of different vector species.[5,9,11] Nelson et al.[23] observed what appeared to be directional behavior by microfilariae of *Onchocerca gutturosa* when they were injected by various routes into rodents. Most of the microfilariae migrated to the distal parts of the ears and nose. The possibility was suggested that microfilariae may migrate along a temperature gradient to the coolest regions of the body.[12] But this was regarded as unlikely in the normal cow host where *O. gutturosa* microfilariae also exhibited a striking directional behavior, accumulating preferentially in the skin of the lower body near the umbilicus even though the adult worms were localized in the upper body around the nuchal ligament and in the connective tissues between the spleen and rumen. It would also be difficult to account for footworm migrations in the deer simply on the basis of responses to temperature gradients even though the extremities are mainly involved; the infective larvae and microfilariae essentially moving in opposite directions from one extremity to the other. The principal (and possibly sole) footworm vector in our area feeds primarily if not exclusively on the ears

of deer. Thus, infective stages are introduced into the aural area but most of the worms mature in regions far removed from this site. We found no evidence that adults localized near the site of inoculation nor any indication that microfilariae accumulated near the sites of production. Indeed, the lack of correspondence between the preferred biting site of the vector (i.e., site of inoculation) and subsequent adult worm localization could hardly be more extreme than in the case of the deer footworm. No footworm microfilariae were detected in skin from the limbs, tail, or muzzle and only occasionally were a few seen in head skin apart from the ears. The distribution of microfilariae within the skin of the outer ear was generally spotty; no consistent relationship being apparent between microfilarial density and particular parts of the ear even though the vector showed a decided preference for the inner aspects of the ears. The most obvious and important correlation was, however, between the biting habits of the vector and the distribution of microfilariae in the definitive host, unquestionably an adaptation for ensuring transmission. Even in *O. volvulus* infections there is some indication that adult worm localization may not be influenced as much by the site of inoculation as had previously been thought, in spite of the early admonition of Strong.[27] For example, Duke[10] reported that *O. volvulus* tended to localize in the hip region of the chimpanzee regardless of where it was introduced.

Arterial worm infections were rarely detected in deer during their first year of life. Thereafter, prevalence increased with host age. We have definitely underestimated the prevalence of this parasite by including data based only on examinations of the main cephalic arteries for adult worms (Fig. 1). The parasite is known to sometimes occur in other arteries of the body and intra-arterial migration may occur after death of the host.[1]

Two species of tabanid fly have been incriminated as vectors.[2] One *(Hybomitra procyon)* occurs in the study area as an adult only in the spring, before the majority of fawns are born; the other

(Tabanus monoensis) is present only during mid-summer and fall. Although the latter could transmit the parasite to fawns, this apparently is an uncommon occurrence. Observations on the biting behavior of tabanid flies in this area have revealed that fawns usually are not attacked by these flies. The finding of adult-sized male and female worms, the latter non-gravid in one but gravid in another 9-10 month old fawn, .indicates a prepatent period of at least 6 months and perhaps up to 9 months in deer. A comparable estimate of prepatent period was made in elk by Adcock and Hibler.[1] Microfilariae of *E. schneideri* were seen in the skin of infected deer at all seasons of the year but they seemed to be relatively more abundant in mid-summer and fall.

Like most observers of these parasites, we found nothing to suggest that they were of primary pathologic significance to deer that were otherwise healthy.

Setaria yehi were likely to be more numerous in debilitated deer but this appeared to be a secondary effect. However, in abnormal hosts, these parasites may not be so benign. This has been documented only for *E. schneideri*,[18] but at least one species of *Setaria* is known to cause neurological disturbances when introduced into abnormal hosts.[3,19] Although we know little of their host preferences at present, the vectors of *S. yehi* and *E. schneideri* are essentially large-mammal feeders and hosts other than deer may be well exposed to their infective stages. We have no information about other hosts at risk from *W. cervipedis* in our area; footworms having been found only in deer. In less suitable hosts perhaps one might anticipate nodular lesions if the speculations of Anderson[4] prove to be valid, namely that nodule formation due to subcutaneous filarial worms may reflect a less than optimal host-parasite balance.

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