This is to certify that the

thesis entitled
An Entomological and Epidemiological study of Equine Onchocerciasis in Michigan

presented by
Willie James Roberts

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Entomology

Harold L. Newson
Major professor

Date September 18, 1986

AN ENTOMOLOGICAL AND EPIDEMIOLOGICAL STUDY OF EQUINE ONCHOCERCIASIS IN MICHIGAN

By

Willie James Roberts

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1986

ABSTRACT

AN ENTOMOLOGICAL AND EPIDEMIOLOGICAL STUDY OF EQUINE ONCHOCERCIASIS IN MICHIGAN

By

Willie James Roberts

A field study was conducted to evaluate the role of Culicoides, particularly C. obsoletus (Meigen) as potential vectors of Onchocerca filarial nematode infections in Michigan equines. Culicoides were collected at the study site using a blacklight trap and from the infected equines by aspirator. Culicoides obsoletus, C. v. variipennis (Coquillett), and C. stellifer (Coquillett) ingested microfilariae after feeding on an infected horse. These three species were also found naturally infected with Onchocerca type larvae. In experimental infection studies, wild caught C. obsoletus and C. v. variipennis were allowed to feed on an infected horse. Second stage filarial larvae were found in the thoracic muscles of C. obsoletus 2 and 11 days post feeding and in C. v. variipennis 6, 7, 8, 10, 11, 18, and 20 days after feeding. Onchocerca type infective stage larvae were found in the head of wild caught and laboratory reared C. v. variipennis, 6 and 25 and 23 days, respectively, after feeding on an infected horse. It is concluded that C. v. variipennis may be a potential vector of equine onchcerciasis in Michigan and that C. stellifer may be a secondary potential vector. The role of C. obsoletus as a potential vector is still unresolved, since no Onchocerca infective stage larvae were found in several hundred dissections of wild caught midges.

To Teresa

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the equine owners; Cindy Jones, Robert F. Bunn, Wayne K. Ross, and Marcy Fisher for the use of their horses and ponies during this study. I would like to thank Drs. Robert Dunstan, Joana Sonea, Wasito, Elizabeth Lyons, Edmund Rosser, Edward Mather, Schillhorn Van Veen, and Christopher Brown of the College of Veterinary Medicine at Michigan State University for their technical support and guidance. I would also like to thank Dr. Richard Merritt, William Morgan, James Kidder, and Dan Kelly for the use of their research facilities.

I would like to express my deepest gratitude to Dr. Harold D. Newson for his academic and emotional support and to Dr. Willis W. Wirth for his assistance in identifying the ceratopogonids specimens collected during this field study. I am also grateful to Drs. Robert F. Ruppel, Roland L. Fischer, and John B. Kaneene for serving on my committee. I would like to thank Bassey Eyo, Kathy Smith, Hastari Wuryastuti, and Anthony D' Angelo for their warmth and friendship.

I would especially like to thank my wife, Teresa for her support and the numerous sacrifices which she endured.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Equine onchocerciasis is a chronic infection in equines caused by filarial nematodes of the genus Onchocerca (Diesing). These filarial infections are rarely recognized as a cause of ill health in equines (Nelson, 1970). However, they have been associated with four pathological conditions: periodic opthalmia, remittent dermatitis, poll evil (atlantal bursitis), and fistulous withers. Some authors consider Onchocerca infections as either a direct or indirect cause of these conditions (Robson, 1918; Hall, 1923; Roberts, 1963; McMullan, 1972; Herd and Donham, 1983). According to Cello (1971), the ocular lesions observed in the opthalmia are nearly identical to the lesions seen in human cases of onchocerciasis. Studies of Onchocerca infections in equines could contribute to the possible control or elimination of the disease in man.

Previous studies of Onchocerca infections have been concerned with prevalence of the microfilariae in the equine host (McCullough et al., 1977; Stannard and Cello, 1975), the morphology of the parasites (Gibson, 1952; Mellor, 1974a) and transmission of the Onchocerca spp. under laboratory conditions (Mellor, 1971; Collins and Jones, 1978). There have been few studies on the natural transmission of Onchocerca to equines. In 1933, Steward incriminated Culicoides (Diptera: Ceratopogonidae) as vectors of equine Onchocerca in England. He concluded that C. nubeculosus (Meigen) was a natural

vector of O. cervicalis (Railliet and Henry) and C. obsoletus and C. parroti (Kieffer) also were probable vectors. Since then there have been few studies on the natural transmission of Onchocerca infections in equines. According to Wirth (1977), this may be due to their small size and difficulty of colonization. However, some authors have attempted to identify potential vectors in the field. In Australia, Ottley and Moorehouse (1983) collected engorged wild ceratopogonids and black flies (Diptera: Simuliidae) from infected horses in the field. They reported that Forcipomyia (Lasiohelea) townsvillensis (Taylor) (Diptera: Ceratopogonidae) and a black fly, Austrosimulium pestilens (Mackerras and Mackerras) may be potential vectors. Foil et al. (1984) collected engorged Culicoides from a pony baited stable trap in Louisiana. Filarial nematodes were found in the thoracic muscles of only C. variipennis (Coquillett) and they concluded that partial development of O. cervicalis occurs in this species.

Recently, equine onchocerciasis was reported from horses in Michigan, but no mention was made of the vector (Schmidt et al., 1982). Two suspected vectors, C. obsoletus and C. variipennis variipennis have been reported from the state. The purpose of this study was to evaluate the role of Culicoides, particularly C. obsoletus as potential vectors of Onchocerca in Michigan equines.

LITERATURE REVIEW

Culicoides Vectors

Two species of Culicoides, C. nubeculosus and C. variipennis have been demonstrated to be vectors of Onchocerca cervicalis under laboratory conditions (Mellor, 1971). He used a membrane feeding technique to infect both species of Culicoides with O. cervicalis microfilariae extracted from the skin of an infected horse. Infective (third stage) larvae developed in 5.5% of 232 C. nubeculosus and 7.7% of 117 C. variipennis sonorensis (Coquillett) females. Mellor (1975) fed laboratory bred C. nubeculosus and C. variipennis sonorensis on the abdomen of a horse with 7 mff/mg of skin. He found 28 of 169 (16.6%) C. nubeculosus and 24 of 329 (7.3%) C. variipennis sonorensis had ingested O. cervicalis microfilariae, thus demonstrating that these two species will feed on infected animals under field conditions. In other studies both midges have been observed to feed on horses under field conditions. (Steward, 1933; Jones et al., 1977).

In England, Steward (1933) reported a 5-6% natural infection rate in field collected C. nubeculosus and he also observed the development of O. cervicalis to the infective stage in these wild flies. In the United States, Foil et al. (1984) collected engorged C. variipennis females from a pony baited stable trap in Louisiana. They found 8 of 114 (7%) C. variipennis infected; one third stage

larvae was observed in a single midge. According to Moignoux (1952), C. nubeculosus is a vector of O. reticulata (Diesing) of horses in Europe. He recorded a 2.1% natural infection rate and a 29% experimental infection rate. Supperer (1953) suggested that this parasite was not O. reticulata but O. cervicalis.

Culicoides nubeculosus and C. variipennis are closely related taxonomically; they belong to the same subgenus Monoculicoides. Culicoides nubeculosus is distributed in Europe and Asia and C. variipennis occurs in North America from Canada to Mexico. Culicoides variipennis is considered to be a species complex in North America, consisting of 5 subspecies. According to Jamnback (1965), the subspecies found in Michigan is C. variipennis variipennis.

Other Culicoides spp. have been reported as possible vectors of Onchocerca species in equines. According to Steward (1933), C. obsoletus and C. parroti may be vectors of O. cervicalis. The midges were found to be infected with microfilariae after feeding on an infected horse. He reported that 9 of 172 (5.2%) C. obsoletus and 1 of 6 C. parroti ingested microfilariae. Apparent development of microfilariae was noted in one C. obsoletus which had a blood meal 3-4 days earlier.

Taxonomy and Biology of Culicoides obsoletus

The C. obsoletus group has a worldwide distribution, the species group is found in Eurasia, North Africa, and North America. In the United States it is comprised of 4 species: Culicoides alachua (Jamnback and Wirth), C. obsoletus, C. sanguisuga (Coquillett), and C. chiopterus (Meigen). Culicoides alachua is

found in northern Florida and South Carolina (Blanton and Wirth, 1979), while the other three species have been reported from Michigan (Jamnback and Wirth, 1963; Williams, 1955). Jamnback and Wirth (1963) and Jamnback (1965) listed the characters used to separate species in the obsoletus group in the United States.

Only the female Culicoides bite. A blood meal is required for the development of the eggs and digestion of the blood meal is completed in 4-5 days at 70°F (Jamnback, 1961). He also noted that the first egg batch may be autogenous. According to Hill (1947), the female midges may take as many as 5 blood meals before laying their eggs. Jamnback (1961) reported that 9 of 17 wild collected C. obsoletus females laid eggs 6-7 days after a single blood meal; the eggs hatched 4-7 days later. Service (1969) noted that the eggs hatched in 3 days. The larvae pass through 4 molts (Lawson, 1951) over a 3 1/2 - 5 months period (Hill, 1947). Jamnback (1965) suggested that C. obsoletus may overwinter in the larval stage. Pupation occurs in the dry portion of the breeding medium (Hill, 1947).

The immatures have been collected from a wide range of habitats. Hill (1947); Kettle and Lawson (1952) found C. obsoletus breeding in sphagnum bogs and marshes. Murray (1957) collected the midges along sandy stream banks, in tree holes, and piles of damp leaves. The temperature of the latter breeding site averaged 71°F. Jones (1961) reported C. obsoletus breeding in a pile of cow manure and bedding, and in decaying cornstalks. Both breeding habitats were exposed to direct sunlight and their surfaces were dry. Hair et al. (1966)

collected the midge from a pile of chicken litter in an open field. The majority of the larvae were found several inches below the dry surface in the moist layer. The midge has been found in alkaline water habitats (Kardatzke and Rowley, 1971), moist straw, decaying spruce leaves (Jamnback,1965), and decomposing banana stems (Birley et al., 1984). Most Culicoides pupae of other species can be collected by flooding the breeding media with water, however, the obsoletus group pupae remain submerged (Jamnback, 1965). Adult C. obsoletus emerge about 5 days after pupation (Hill, 1947; Jamnback, 1965). Jamnback (1961) reported that wild C. obsoletus females lived for 51 days under laboratory conditions.

Little is known of the flight range of adult C. obsoletus. It has been suggested that Culicoides do not usually occur far from their breeding sites, but that the wind may disperse them beyond their normal flight range (Hill, 1947). Birley et al., (1984) collected C. obsoletus about 400 m from a known breeding site, a banana plantation. The prevailing wind was blowing in a westerly direction across the plantation towards the collection site. Shemanchuk (1972) concluded that the wind acts as a passive mode of transport, since wind velocities greater than 10 mph hindered flight of the midge. Jamnback and Watthews (1963) observed that an average wind velocity of 1.5 mph reduced the landing rate of obsoletus group females to zero. The flight activity of C. obsoletus also is influenced by other climatic conditions. Jamnback and Watthews (1963) recorded large numbers of the obsoletus group females landing on a human host at temperatures between 55 and 81^{o} F, and

noted that the midges were inactive at temperatures below 50° F. Shemanchuk (1972) recorded C. obsoletus activity from 46 to 72 ° F with optimum activity occurring between 54 and 63° F. The midges were also active during a light rain.

The adults of C. obsoletus occur from early spring to late autumn. Jamnback (1965) collected them from May-September in New York and Jorgensen (1969) observed them in southeastern Washington until late October. Hill (1947) reported C. obsoletus from early April to late October in England; the population peaked in early June and again in late September. From this she suggested that C. obsoletus has two generations a year. Murray (1957) found the midges from June to September in Virginia and reported that the population peaked twice, from mid June to mid July and from late July to mid August. Service (1969) found the midges until November in southern England and recorded only a single population peak in May. Schmidtmann et al. (1980) considers C. obsoletus to be multivoltine in central New York. In Michigan, Williams (1955) collected the midge from late June to early August; they were most abundant in July.

The adult midges have been reported to be active throughtout the day. According to Hill (1947), C. obsoletus is most abundant in the early evening hours, beginning 3 1/2 hours before sunset. Others have observed midge activity during the daylight hours in shaded areas or on cloudy days (Service, 1969; Twinn, 1931), and in wooded areas (Williams, 1951). Murray (1957) reported large numbers active between 2 and 5 a.m. in the morning. Williams (1955)

suggested that light intensity has a major influence on the activity of C. obsoletus.

Culicoides obsoletus has a wide host range, it feeds on horses, cattle, sheep, man (Twinn, 1931; Jamnback, 1965; Schmidtmann et al.,1980) and birds (Bennett, 1960). Schmidtmann et al. (1980) collected C. obsoletus from the belly, back, brisket, and neck of ponies in New York but the majority of the females were found on the belly. Mellor (1974c) reported that the obsoletus group females occur on all parts of the horse, but they were most numerous along the ventral abdominal midline. They have been reported to attack vertebrate hosts located from 0-25 ft above the ground (Bennett, 1960; Service, 1969). According to Service (1969), the majority of C. obsoletus occur below 10 ft from the ground.

Taxonomy and Biology of O. cervicalis

In 1910, Railliet and Henry reviewed the genus Onchocerca and separated Onchocerca cervicalis from the type species Onchocerca reticulata. The taxonomy of O. cervicalis has caused much controversy; some authors still consider O. cervicalis as a synonym of O. reticulata. According to Sandground (1934), the only morphological difference between the two species is the length of the left spicule in the adult male. Others consider the two parasites as separate species. Le Roux (1950) reported that the "rugae" are more closely spaced in O. reticulata than in O. cervicalis. He also thought O. cervicalis might be a probable synonym of O. gutturosa (Neumann). Bain (1975) re-examined both parasites and suggested that distinct morphological differences exist in the

adult female cuticle. Lichtenfels (1975) used several characters to differentiate between the two species: the anatomical location of the adults in the host; length of the left spicule in the male; and the morphology of the female cuticle. The site of development of the adult parasites has been commonly used by authors to distinguish between the two species.

The taxonomy of the immature stages of Onchocerca spp. in equines is as controversial as the adult stage. Some authors have separated the two species based on their differences in size (Supperer,1953; Lichtenfels,1975). According to Gibson (1952) and Mellor (1974a), the size of the microfilariae is of little taxonomic importance unless the fixation and staining method is indicated, since the size of the microfilariae is affected by these procedures. The location of fixed internal morphological structures: the first nucleus; anterior edge of the nerve ring; excretory pore; excretory cell; rectal cell; anal pore; last nucleus; and length of the tail from the anterior end of the microfilariae has been used to distinquish between Onchocerca spp. (Steward, 1933; Gibson, 1952; Collins, 1973; and Mellor, 1974a). Moignoux (1952) reported that the microfilariae of both O. cervicalis and O. reticulata develop in C. nubeculosus and the larvae are morphologically similar. According to Gibson (1952), the morphology and arrangement of the caudal nuclei can be used to identify O. reticulata (= cervicalis), but Mellor (1974a) did not observe these same characteristics in the caudal nuclei of O. cervicalis microfilariae extracted from the skin of infected horses in England.

The adults of O. cervicalis are whitish, thread-like nematodes. The cuticle

is transversely striated with external spiral thickenings, which are interrupted in the lateral field; there are 3 to 4 transverse striations between each ring (Railliet and Henry, 1910; Lichtenfels, 1975; Bain, 1975). The adult female parasites are 35 to 38 cm long and 0.2 to 0.35 mm wide, and males are 6 to 7 cm long and 0.07 to 0.10 mm wide (Steward, 1933). The viviparous females produce large numbers of unsheathed microfilariae which range in size from 160 to 240 u long and 2 to 5 u wide (Steward, 1933; Levine, 1968). In microfilariae stained with nuclear stains, dense staining bodies or nuclei are arranged in a column formation, 1 to 3 abreast (Mellor, 1974a). The adults of O. cervicalis are usually found in the ligamentum nuchae of the equine (Mellor, 1973a). This large neck ligament consists of a funicular and lamellar portion (M' Fadyean, 1902). The parasite is found predominately in the funicular portion between the last cervical and the fourth thoracic vertebrae (Mellor, 1973a). Ottley et al. (1983) found adult O. cervicalis in both the ligamentum nuchae and the scapular cartilage of a horse in Australia. The microfilariae are found mainly in the skin, at a depth of 1.6 to 1.8 mm from the surface; no periodicity of the microfilariae occurs in the skin (Steward, 1933). The microfilariae are rarely found in the blood, however, Thomas (1963) reported finding an unknown Onchocerca species in the blood of equines in South Africa.

According to Mellor (1974a), two sizes of O. cervicalis microfilariae occurs in the horse: a uterine, large size and a skin, small size. He suggested that the skin microfilaria is the mature form. Mellor (1973a) reported that the microfilariae are distributed unevenly in the skin and throughout the body of the

horse; they congregated in isolated clusters in the skin instead of being evenly distributed. Rabalis and Votava (1974) found large numbers of microfilariae along the flank and the umbilicus proper of the horse. Collins (1973) reported finding the highest concentration of microfilariae in the skin near the withers and the ventral region posterior to the forelegs of the horse. Mellor (1973a) recorded 95% of the microfilariae from the skin along the ventral abdominal midline.

The method of distribution of the microfilariae in the skin of equines is poorly understood. It is thought that the microfilariae migrate through the skin to various areas of the definitive host. Mellor (1974b) suggested that the microfilariae are guided by a stimulus, such as a chemical gradient or a combination of co-evolutionary factors between parasite and vector. El Sammani and Hussein (1983) suggested that the microfilariae of O. raillieti (Bain, Muller, Khamis, Guilhon, and Schillhorn Van Veen) may migrate to the preferred biting site of the vector.

The longevity of O. cervicalis microfilariae in the skin is unknown, however, the microfilariae of Onchocerca volvulus (Leuckardt) may survive in human skin for 1-3 years (WHO, 1966).

Onchocerca cervicalis microfilariae continue their development in an intermediate host, Culicoides spp. (Steward, 1933). The midge ingests the microfilariae from the skin of the equine, along with blood from ruptured capillaries. According to Mellor (1975), the microfilariae are either ingested from the area near the damaged skin tissue or they are attracted to the vector

by a chemical attractant in the midge's saliva. The ingested microfilariae and blood meal pass to the midgut of the midge. The microfilariae can be found in the midgut 1-3 days after a blood meal (Steward,1933; Mellor, 1975). However, Mellor (1975) found O. cervicalis microfilariae in the haemocoel and thoracic muscles of C. variipennis and C. nubeculosus approximately 5 and 15 minutes, respectively, after blood feeding. He suggested that the microfilariae enter the haemocoel by penetrating through the midgut wall. Development of the microfilariae continues in the thoracic muscles but at an unequal rate (Steward, 1933). After 7 days, the microfilariae have reached the "sausage form" or 2nd stage larvae; they are 133-240 u long by 17-27 u wide. By the 16th day, they begin to increase in length and decrease in width. The infective larvae (third stage) may be found in the head of the midge on the 22nd day and in the proboscis 24-25 days after an infected blood meal. The infective (L_3) larvae measure 600-700 u long by 18-21 u wide (Steward, 1933). The infective larvae enter the equine host when the midge feeds again. The parasite may emerge from the membranous area at the base of the proboscis during the feeding process (Steward, 1933).

Temperature has been shown to influence the rate of development of O. cervicalis in the Culicoides vector. Steward (1933) made no mention of the temperature at which he observed the development of O. cervicalis to infective (L_3) larvae in C. nubeculosus. Mellor (1975) observed the complete development of O. cervicalis in C. nubeculosus and C. variipennis in 14 to 15 days at 69.8 to

73.4°F, but he did not indicate the anatomical location of the infective (L_3) larvae in these flies. Infective stages were not observed in the heads of the midges. Collins and Jones (1978) reported that O. cervicalis developed to the infective (L_3) stage in C. variipennis in 5 days at 80°F and 12 days at 71°F and were distributed in the head, thorax, and abdomen of the flies. Moignoux (1952) observed the complete development of O. reticulata in C. nubeculosus in 22 days at 77-86°F. He mentioned that the parasite was also able to complete development at 60.8°F, but did not state the developmental time at this lower temperature. The activity of the L_3 larvae in the equine host is unknown. It is assumed that they migrate to the ligamentum nuchae and there continue their development to the adult stage (Mellor, 1974b). The length of time from innoculation of the infective stage until the microfilariae occur in the skin has not been determined for O. cervicalis but for O. volvulus in man it takes 4-5 months (McMullan, 1972).

Distribution and Prevalence of Equine Onchocerciasis

Onchocerca spp. infections in equines have a worldwide distribution. They have been reported from Europe, Asia, North America, Central and South America, Australia, Africa, and the Middle East (Bain et al., 1976; Ottley et al., 1985). In the United States, Onchocerca infections in equines were first observed by Van Volkenberg in 1921. He also found parts of another nematode parasite in the ligamentum nuchae, but without the spiral thickenings seen on the cuticle of Onchocerca. According to Lichtenfels (1975), O. cervicalis may be

the only Onchocerca sp. occurring in North American equines. Two methods have been used to diagnose Onchocerca infection in equines: demonstration of the adult parasite in the ligamentum nuchae; and/or the presence of the microfilariae in the skin (Levine, 1968). Lloyd and Soulsby (1978) found 74 of 121 (61%) equines from the eastern United States infected with O. cervicalis microfilariae. Mc Cullough et al. (1977) reported that 29 of 57 (50.8%) horses biopsied in Maryland were positive for O. cervicalis microfilariae. Cummings and James (1985) found 341 of 664 (51.4%) horses from the southeastern and midwestern United States with cutaneous O. cervicalis microfilariae. Stannard and Cello (1975) found 48 of 100 (48%) horses from California, Oregon, Nevada, and Arizona infected with O. cervicalis microfilariae. Microfilariae were extracted from tissue samples collected from the eye, ventral abdominal midline, and the lower eye lids of the infected horses. Polley (1984) collected skin biopsies from areas adjacent to the umbilicus of 623 horses from western Canada and the northwestern United States and reported a microfilarial prevalence rate of 25.8%. Collins (1973) found O. cervicalis microfilariae in 52 of 232 (22%) horses in southeastern Louisiana. In Michigan, Schmidt et al. (1982) collected a section of the ligamentum nuchae from 83 horses and reported finding adult O. cervicalis in 31 (37%) of the ligament sections examined.

DESCRIPTION OF THE STUDY AREA

The study was conducted at two sites in Clinton County, Michigan (Figure 1). Site A is a 19 acre farm located 1/4 mile north and 2 miles west of the Capitol City Airport (Figures 1, 2). A case of equine onchocerciasis had been reported from this farm in 1977. The farm consists of a house, garage, storage shed, stable, pasture, and a maple-beech woodlot. The owners of the farm are horse breeders; they usually have 7-10 horses on their property throughout the year. There are also 5 chickens, 5 cats, 3 nesting pairs of barn swallows, 2 peacocks, and 2 dogs on the farm. Except for a stallion, the horses are rarely kept in the stable and are allowed to roam on the pasture day and night.

The pasture is divided into 3 sections: a north, south, and west section. The west pasture is the largest, covered by an abundance of low growing scrub vegetation and horse manure. The contour of the pasture is irregular. It slopes 5-20 degrees along a 634 ft by 528 ft perimeter, then levels off near the entrance of the woodlot. The temperature is usually several degrees cooler and the wind is not as strong in this depression as at the surface of the ridge. The maple-beech woodlot covers 6.3 acres and contains two temporary ponds located in the woodlot. The north and south pastures are grass covered savanna-like, except for a single apple and black walnut tree in the north and south pastures, respectively. The horse bedding and manure removed from the horse stalls is dumped in several large piles in the south and west pastures. A

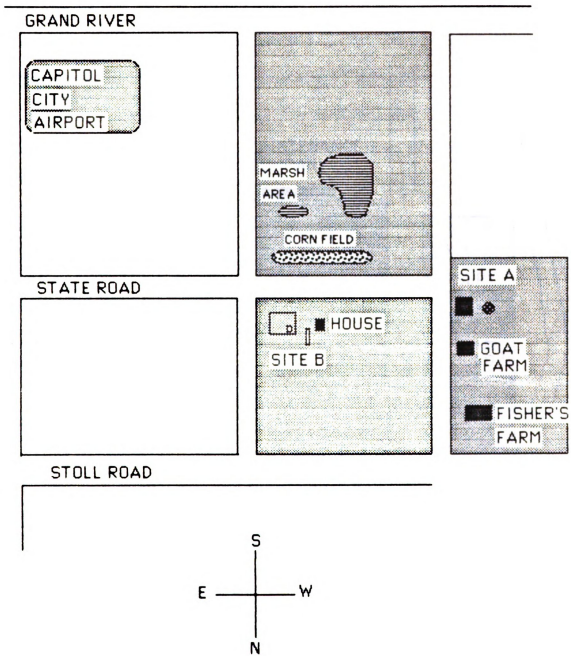

Figure 1. Location of site A and B.

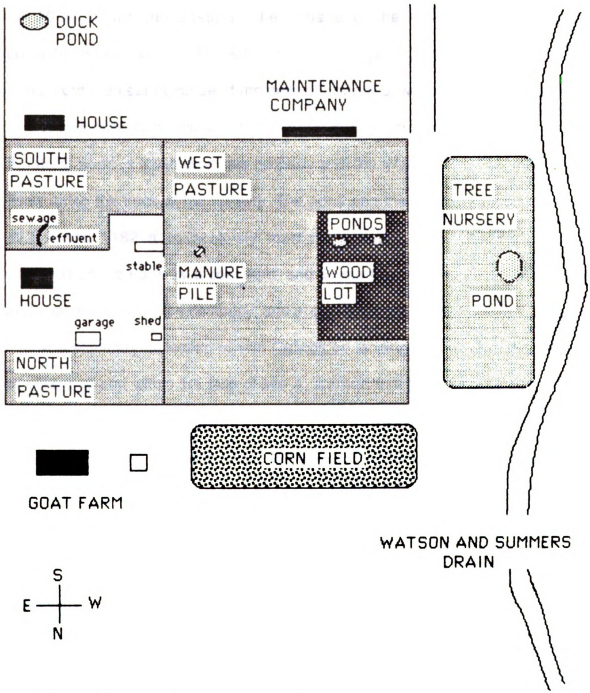

Figure 2. Study site A.

drain pipe from the farm house extends into the south pasture and its effluent is discharged on the ground surface, which also contains a mixture of horse manure, maple leaves, black walnut leaves and nuts. The sewage effluent flows for 20-30 ft into the pasture. The surface of the soil has a sandy loam consistency and a black foul smelling bottom layer. This area remains moist throughout the year because of the daily discharge of water from the drain pipe.

A tree nursery is adjacent to the western edge of site A. A 211 ft by 370 ft natural pond is located on this property and the Watson and Summers drain flows along the back of the nursery. The pond and the drains are located about 422 ft and 1162 ft respectively from the woodlot. The site (Figure 2) is bordered on the north by a corn field and a goat farm, on the south by a house and maintenance service company, and a dirt road on the east.

A preliminary collection of wild Culicoides landing on the ventral abdomen of horses at and within 1/4 mile of site A, showed that C. obsoletus occurred in the area (Table 1).

Site B (Figure 3) is a 2 1/2 acre residence located 1 mile east of site A. It consists of a house, storage shed, garden plot, stable, and a pasture. Both the pasture and stable are covered with a thick mat of pony manure. The owners have 5 ponies which are mainly used for pleasure; all of the ponies were foaled in Michigan. The ponies usually spend most of the time along the west edge of the pasture or inside the stable on the north side of the pasture. Other animals on the property are a cat, a goat, 2 nesting pairs of barn swallows and 3 dogs. The pasture is mostly flat ground with a sparse growth of vegetation and scrub; it is bordered on the west by the owner's house, a storage shed, and a garden

Table 1. Culicoides collected from horses within 1/4 mile of site A.*

Species	Site A	Fisher's Farm	Subtotal
C. obsoletus	55	35	90
C. stellifer	189	759	948
C. v. variipennis	0	3	3
Total	244	797	1041

* Collected from July 17 to October 12 (1984).

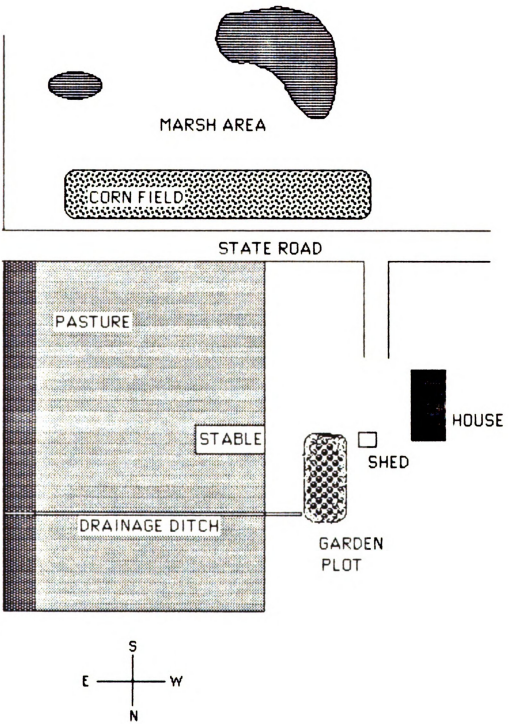

Figure 3. Study site B.

plot. A drainage ditch extends from the garden plot along the entire length of the north side of the pasture. The pasture is bordered on the north by an open field, on the east by a field covered with a dense growth of vegetation and scrub, and the south by a dirt road. Across the dirt road is a corn field with a marshy area at the far side.

MATERIALS AND METHODS

Equine Onchocerca Infections

A horse from site A had been diagnosed positive for onchocerciasis on August 30, 1977 by the veterinary medicine clinic at Michigan State University. However, the owner had no record of which horse was examined that day. She assumed it may have been one of the older horses. According to the owners at site B, their ponies had never been examined for onchocerciasis. The equines from both sites were examined at the beginning of this study by a veterinarian, Dr. E. J. Rosser from Michigan State University. According to Dr. Rosser, clinical signs of onchocerciasis were noted in 2 of 3 horses at site A and 3 of 5 ponies at site B. He suggested that skin biopsies should be taken from the skin lesions to confirm the presence of Onchocerca microfilaria in the tissue.

Two methods were used to diagnose Onchocerca infections in the equines: skin biopsies and the presence of Onchocerca microfilariae in blood smears from engorged Culicoides that recently had fed on one of the horses or ponies. At site A the owner would not permit a skin biopsy to be taken from the horse called Lacey, one of the animals Dr. Rosser thought was

infected. It had been observed from previous collections of engorged biting flies from this horse that an unidentified black fly species ingested large numbers of microfilariae while feeding (Figures 4 and 5) on this horse, so it was assumed to be infected with Onchocerca. It was a 20 year old mare, that

Figure 4. Microfilariae found in the midgut of an unknown black fly species which had fed on the infected horse at Site A (x 125).

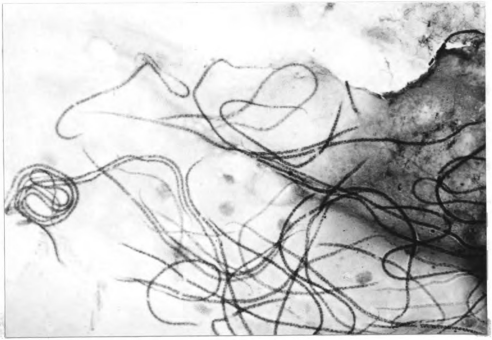

Figure 5. Microfilariae from the above black fly seen at a higher magnification (x 313).

was born and raised in Michigan. Culicoides feeding on it were collected from the ventral abdomen of the horse with an aspirator beginning 1-2 hours before and until 1 hour after sunset. Initially, a battery operated aspirator was used to collect the midges, but had to be abandoned because it frightened the horse. A tube aspirator described by Endris et al. (1982) was tried, but was discontinued because the collector became allergic to inhaled foreign particles from either the horse or the midges. An aspirator described by Hill (1947) with slight modifications was used. The glass tubes inserted in the cork were replaced with plastic tubes and 10 dram clear plastic collection vials were filled to a depth of 1 inch with plaster of paris and allowed to dry. Before field use, 2 inch by 2 inch pieces of saran cloth were placed on top of the vials, then covered with plastic lids with the center portions removed. The construction of these vials is similar to the cage used by Endris et al. (1982) for rearing sand flies (Diptera: Psychodidae) in the laboratory. The vials were placed inside a 2 qt square polystyrene food container, the bottom of the container was lined with damp paper towels and the plastic lid was placed over the container. The collection vials were transported to and from the field in this manner.

In the laboratory, the midges were anesthetized with CO_2 from dry ice or chilled in the freezer compartment of a refigerator for 45-60 seconds. They then were sorted in a glass petri dish into engorged and unengorged groups using a dissection microscope (magnification 40x). The unengorged flies were killed with an alcohol saturated camel hair brush and stored in 70% ethanol. The engorged flies were placed in 3 ml of 0.9% saline in a 5 ml blood tube. The blood tube was sealed with a rubber stopper and the solution was shaken vigorously

until the flies settled to the bottom of the tube. They were left in the saline solution for 20-30 minutes, then dissected in a drop of saline solution on a microscope slide under a dissection microscope. Minuten insect pins embedded in wooden applicator sticks were used to separate the abdomens from the thorax and head segments (the latter two segments were placed in 70% ethanol in 5 ml blood tubes until their species identification was confirmed). The abdomens on the slides were gently teased apart to remove the blood mass, additional saline was added to completely dissolve the blood mass and then the preparations were allowed to dry overnight. The dried blood was stained either with Giemsa, using a method described by Belding (1942), or Mayer's Acid Hematoxylin (1 part stock stain/ 9 parts distilled water by volume) using a method similar to Menon (1960) except the slides were maintained in the stain for 10 minutes and rinsed in distilled water (ph 7.0) for 1 minute.

The second method routinely used to diagnose Onchocerca infection in equines was conducted at site B. Two ponies were biopsied, one was a 2 year old mare named Pepper, the other a 14 year old stallion named Comanche. A single biopsy sample was obtained from the abdominal midline of Comanche and from an abdominal lesion of Pepper. Each pony was administered 3 ml of Xylzin (Rompun) intravenously and the biopsy sites were scrubbed with a 2 inch by 2 inch sterile gauze saturated with a betadine/soap solution. The hair was removed from the biopsy site with a disposable razor, then the area was scrubbed again with betadine and allowed to dry. The site was infiltrated with 3 ml of lidocaine and 6 mm Baker's biopsy punches were used to remove the skin samples. Each sample was cut in half with scissors. One piece was mounted on a

wooden tongue depressor and placed in 100 ml of 10% buffered formalin in a plastic virology container and submitted to the Animal Health Diagnostic Laboratory at Michigan State University for histopathological examination. The other piece was placed in 100 ml of 20% Tyrode/Horse Serum solution (THS) in a plastic virology container. Slight bleeding was observed after removing the biopsy specimen so a 2 inch by 2 inch sterile gauze pad with digital pressure was used to control the bleeding. A tetanus antitoxin was administered subcutaneously to both ponies. The skin samples were transported back to the laboratory approximately 2 hours after removal.

In the laboratory, the skin samples were removed from the THS solution with sterile forceps, rinsed in fresh THS solution, cut into small strips, using sterile scissors, and placed in a 6 ml plastic culture tube. Two ml of THS, 200 ug of streptomycin, 200 units of penicillin, and 100 units of nystatin were added to both culture tubes. The two culture tubes were held in an incubator at 37^{o}C for 18 hours to allow microfilariae to leave the tissue. The supernatant from each culture tube was poured into a clean culture tube, the incubation tube was rinsed twice with 2 ml of Tyrode solution, then the solution was poured into the clean tube. Both tubes were centrifuged at 1500 rpm for 15 minutes, the supernatant decanted, then 1 ml of 2% neutral buffered formalin (2 ml buffered formalin/ 98 ml Tyrode solution) was added to the residue in each tube. The tubes were centrifuged again at 1500 rpm for 15 minutes, the supernatant decanted, then the residue was removed with a pasteur pipet and smeared on a microscope slide and allowed to dry over night. The slide smears were immersed in 1% glacial acetic acid, then 1% sodium bicarbonate, rinsed in distilled water,

then 4% aluminum ammonium sulfate, rinsed in distilled water, then placed in Mayer's Acid Hematoxylin (1 part stock stain/9 parts distilled water) for 5 minutes in each solution, then rinsed in distilled water. After staining, the smears were dehydrated in 35%, 50%, 70%, 80%, and 95% ethanol (1 minute in each solution), then immersed in Euparal essence for the same amount of time. The smears were mounted in euparal vert under a No. 2 cover glass and held in an incubator at 55^{o}C for 3-4 days.

Natural Infection Rate

Wild adult Culicoides were collected by aspirator, as described above for the engorged flies, as they landed on the ventral abdomen of the infected equines. Collections were made one evening a week at site A from May 3 until October 25 (1985) and twice a week at site B from August 20 until October 4, except on windy or rainy evenings. Flies were fixed in Carnoy solution for 16-18 hours at room temperature, then rinsed in 70% ethanol for the same time period, and stored in 5% glycerine alcohol until staining.

The midges were stained in groups of 20 to 300 in 5 ml blood tubes using a method described by Nelson (1958), except that they were rinsed in 70%, 50%, and 35% ethanol (30 minutes in each alcohol dilution), then were placed in Mayer's Acid Hematoxylin (1 part stock stain/1 part 4% glacial acetic acid) for approximately 3 hours. They were rinsed in distilled water or 1% glacial acetic acid for about 1 minute to remove excess stain, then dehydrated in 35% and 50% ethanol (30 minutes in each solution), then held in 70% ethanol for 24 hours. This method stained the filarial larvae inside the midges and allowed the parasites to be seen during dissection.

The weekly collection of Culicoides from each site were pooled together. Eighty per cent of the midges collected each month were dissected and the number of infected midges recorded. Each was dissected in a drop of glycerol on a microscope slide under a dissection microscope (45x). The head, thorax, and abdomen were separated and examined for filarial larvae. The larvae were mounted in glycerol under a coverslip, ringed with euparal, and measured with a micrometer on a phase contrast microscope.

Light Trap Collection

A light trap similar to the Monks Wood light trap described by Service (1976) was used to collect Culicoides from May 24 until September 4, 1985. An Ellisco^{R} light trap with a 15 watt blacklight tube provided the light source, a CDC minature light trap (without a bulb) with an attached collection bag was secured to the body of each trap by a metal wire (Figure 6). The collection bag consisted of a 15 inch long saran cloth bag with an elastic garter sewn at one end. The other end was sewn to the threaded lid of a 4 oz urine specimen cup. A 1 3/4 inch hole was cut in the center of the lid which was screwed on to the specimen cup.

Two of these light traps were operated one evening a week during the collection period in 1985, one at the Veterinary Research Farm on Michigan State University (Figure 7) and the other was alternated between two horse stables in Clinton County: one located at a farm on DeWitt road, about 11 miles north of site B, and the other at site A (Figure 8).

The midges were collected in 40 ml of Carnoy solution placed in the urine specimen cups, held in the solution for approximately 16-18 hours, then rinsed

Figure 6. Blacklight trap used to collect Culicoides.

Figure 7. Blacklight trap in operation at the Veterinary Research Farm.

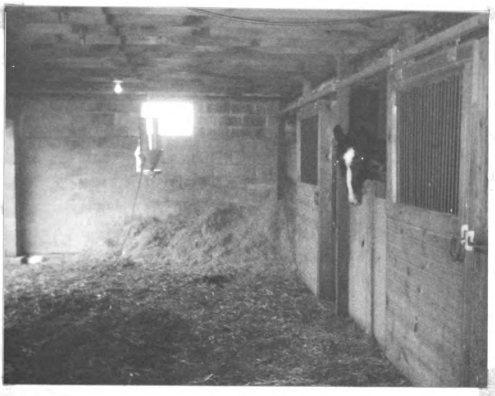

Figure 8. Blacklight trap in operation near the west entrance of the stable at site A.

in 40 ml of 70% ethanol overnight. The Culicoides spp. were separated from the other specimens under a dissection microscope and stored in 5% glycerin alcohol. Microscope slide mounts were prepared for identification for a representive sample of each species of Culicoides using the method of Wirth and Marston (1968). The morphological characters listed by Blanton and Wirth (1979) were used to identify most of the Culicoides and the characters described by Jamnback and Wirth (1963) and Jamnback (1965) were used to separate the obsoletus group. Identified Culicoides were submitted to Dr. W. W. Wirth, Systematic Entomology Laboratory, Gainesville, Florida for confirmation of their identification.

Attempts to locate the breeding site of C. obsoletus

Two methods were used to locate the breeding site of C. obsoletus: emergence traps and a flotation technique. The emergence traps were 6 1/2 inch planting pots with 6 dram glass vials secured in the pot drain holes. The vials interiors were coated with a mixture of lubricant grease and 10W40 motor oil. Twenty of these traps were used in the west pasture at site A (Figure 9). Eight were placed on manure piles and 12 on horse manure droppings in the pasture and woodlot.

In the second obsoletus collecting method, suspected breeding materials were collected from within a one mile radius of the equine baited and light trap collection sites. A six inch garden spade was used to collect soil along the base of manure piles, soil contaminated with animal excrement, and damp soil along the corners of horse stalls. Wet soil from the margins of drains, ponds, and streams, and damp leaves from tree holes and wooded areas were also collected.

Figure 9. Emergence traps on the manure pile in the west pasture at site A.

Soil samples were collected from the first one inch of top soil and placed in 12 oz insulated drinking cups, 2-3 scoops in each container. The foliage samples were placed in 10 inch by 13 1/2 inch plastic ziplock bags. Since such a large number of habitats were surveyed, no attempt was made to standardize the number of samples collected. In addition, some pupae were collected with an eye dropper from tree holes and horse water troughs. In the laboratory, the samples were placed in white enamel pans and flooded with distilled water. An eye dropper was used to collect the Culicoides pupae that rose to the surface of the water. Pupae were placed individually in 2 dram glass vials on wet cotton pads, the vials were plugged with cotton, then maintained at 69.8-71.6^{o}F in a dark incubator. After the adults emerged, they were killed with an alcohol saturated camel hair brush; the adults and pupal skins stored in 5% glycerin alcohol overnight, then mounted on microscope slides, identified, and forwarded to Dr. W. W. Wirth for confirmation.

Experimental Infections

Two studies were conducted to determine if O. cervicalis develops to the infective stage in C. obsoletus and C. v. variipennis after feeding on an infected horse in the field. In study 1, engorged wild C. obsoletus and C. v. variipennis were collected (as previously described) from the ventral abdomen of the infected horse at site A. Culicoides v. variipennis was easily recognized by its larger size and its feeding mainly on or near the umbilicus. The engorged midges were transported back to the laboratory and released into a 6 3/4 inch long by 6 1/2 inch wide by 6 inch high plexiglass cage. Except for C. v. variipennis, no attempt was made to separate the midges by species. The individual midges

were aspirated into 10 dram plastic vials with plaster of paris bottoms (as described above). Cotton pads soaked in 10% dextrose solution were placed on the saran cloth as a nutrient source. The vials were then placed in 2 qt plastic food containers, lined with damp paper towel to maintain high humidity and the lids were placed over the containers. The midges were maintained in a dark incubator at 62.6 - 66.2^{o}F. Each day the sugar pads were changed and dead flies were removed, identified, then fixed in Carnoy solution overnight. The following morning, they were rinsed in 70% ethanol for 18 hours, then stored in 5% glycerin alcohol.

In study 2, C. v. variipennis pupae were collected from the wet soil near the drain pipe outlet in the south pasture at site A (Figure 10) and about 8 to 10 ft from the base of a compost pile on the Michigan State University tree nursery (Figure 11), then placed in 4 oz urine specimen containers on wet cotton. A piece of saran cloth was placed on top of each container, then capped with the lid (a hole in the center), sugar pads were placed on the saran cloth and the cups were placed in a plastic food container. The pupae were held in a dark incubator at 62.6-66.2^{o}F until the adults emerged. Adults were maintained under these conditions for 3-5 days before attempting to feed them on the infected equines. In the feeding experiments the midges from the laboratory were released inside a small plexiglass cage and removed from the cage with a tube aspirator. The mouth of the aspirator was plugged with cotton and the midges were immobilized by chilling in the freezer compartment of a refigerator for 45-60 seconds. Then they were gently blown into a nylon feeding bag and the neck of the bag was closed with a metal hair clip. Approximately 25 midges were placed

Figure 10. Breeding site of C. v. variipennis near the drain pipe outlet in the south pasture at site A.

Figure 11. Breeding site of C. v. variipennis near the base of a compost pile on the tree nursey at Michigan State University.

in the bag.

The bag (Figure 12) consisted of 3 inch long pieces of nylon, cut from the toe portion of a pair of Hanes^{R} nylon stockings. The nylon material was sewn to a 2.5 inch by 2.5 inch piece of foam sponge (packing material from VWR Scientific, Disposable Pasteur Pipets) and a 1 inch by 1 inch square was cut from the center of the foam.

The midges were transported to and from the sites in a plastic food container, lined with damp paper towels. To allow feeding, the nylon bag was placed slightly anterior to the umbilicus or over the abdominal lesions of each equine (Figures 13 and 14). The site was rubbed by hand to dislodge any blood feeding flies, then visually checked before the bag was attached to the animal with Dermiclear^{R} (Johnson and Johnson) transparent tape. The metal clip was removed and the midges were allowed to feed for 20-30 minutes. After this time period the metal clip was replaced and the bag was slowly removed; midges still feeding were collected with the aspirator. The replete midges were returned to the laboratory and maintained in 10 dram vials as described above for the engorged flies. The dead flies were removed, fixed in carnoy solution, and stored in glycerin alcohol over a 23 days period. Flies still alive on the 23rd day were immobilized by chilling in the refrigerator, then dissected in warm horse serum (98.6^{o}F).

The engorged midges in both infection studies were removed from the 5% glycerin alcohol, then hydrated through descending dilutions of ethanol to 35% ethanol and stained in Mayer's Acid Hematoxylin (as described above). The midges were dissected under a dissection microscope and the filarial larvae

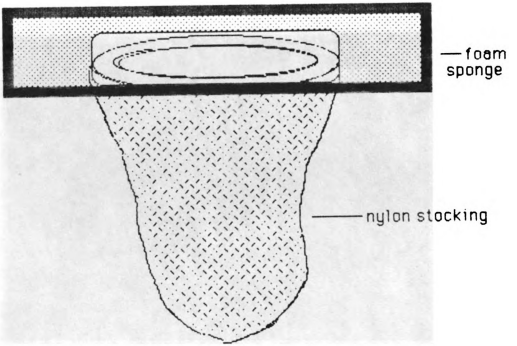

Figure 12 Nylon feeding bag.

Figure 13. Nylon feeding bag attached to the ventral abdomen of an infected pony at site B.

Figure 14. Nylon feeding bag attached near the umbilicus of the pony.

mounted in glycerol under a coverslip.

RESULTS

Equine Onchocerca Infections

During the period July 14 to October 12 (1984), a total of 36 engorged wild Culicoides spp. were collected at site A from the ventral abdomen of Lacey during 4 collection evenings. Three species of Culicoides were collected: C. obsoletus, C. stellifer, and C. venustus (Hoffman). It was found that 2 of 22 C. obsoletus and 2 of 7 C. stellifer had ingested microfilariae during feeding (Figures 15 and 16), but none of the 7 engorged C. venustus were found to be infected. In addition, a total of 9 engorged wild C. v. variipennis were collected from Lacey on September 7 and 18 (1985) and 4 had ingested microfilariae (Figure 17).

The results of the skin biopsy confirmed the presence of microfilariae in the skin of both ponies at site B. Microfilariae were extracted from the pieces of skin soaked in THS solution (Figure 18). A random sample of 14 microfilariae were measured and they had an mean length of 224.38 +/- 7.15 u and width of 2.73 +/- 0.32 u. Several morphological structures were frequently seen in the microfilariae: the cephalic space, nerve ring, rectal cell, and terminal nucleus. The distance of these structures from the anterior end of the microfilariae were measured and expressed as the mean distance and percentage of the total body length (Table 2). Other structures such as the excretory pore, excretory cell, and anal pore were observed in only a few microfilariae.They were 66.22

Figure 15. Microfilaria ingested by C. obsoletus after feeding on an Onchocerca infected horse (x 500).

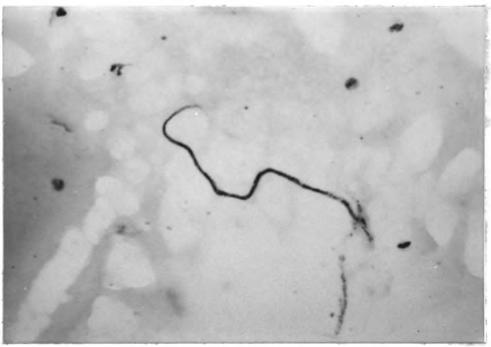

Figure 16. Microfilaria ingested by C. stellifer after feeding on an infected horse (x 500).

Figure 17. Microfilaria ingested by C. v. variipennis after feeding on the infected horse (x 400).

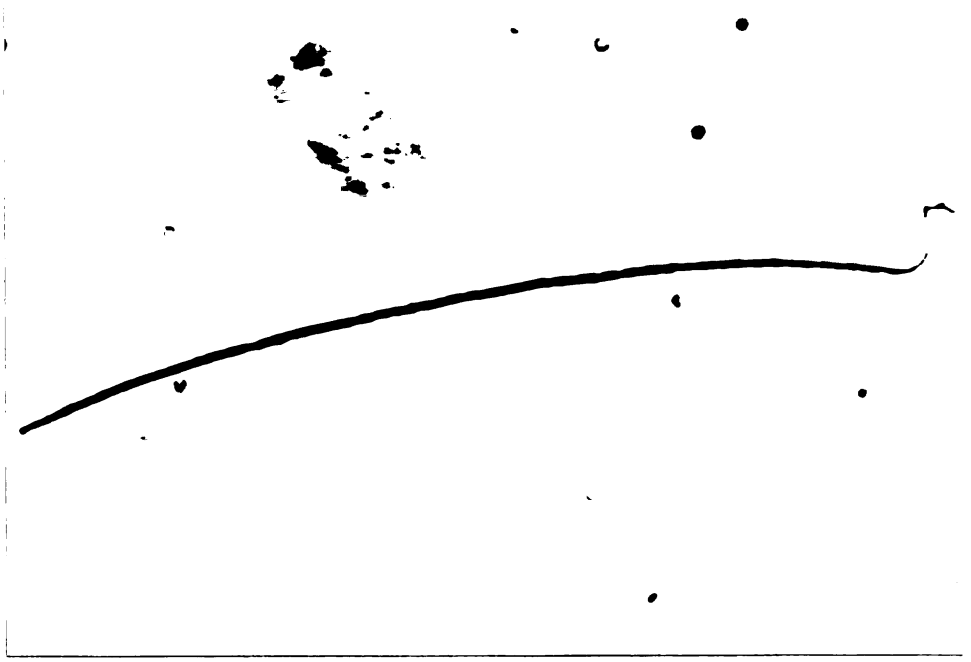

Figure 18. Microfilaria from the skin of an infected pony (x 500).

Table 2. Morphological dimensions of microfilariae from skin biopsy.*

Structures	Distance from ant. end	% distance from ant. end
Cephalic space	3.65+/-0.78**	1.62+/-0.34
Ant. edge of nerve ring	53.09+/-3.14	23.65+/-0.83
Rectal cell	154.77+/-5.54	68.98+/-1.14
Terminal nucleus	216.81+/-6.96	96.63+/-0.70

*Samples were collected from two infected ponies at site B.
**All measurements are in um and expressed as Mean +/- S.D.

+/- 1.31 u (30.68 +/- 0.45%), n=2; 72.69 +/- 3.06 u (33.52 +/- 1.37%), n=4; and 178.64 +/- 6.66 u (81.69 +/- 1.44%), n=3 respectively, from the anterior end of the microfilariae. These measurements for the microfilariae are similar to the results obtained by Collins (1973) and Mellor (1974a) for Onchocerca in equines.

Microfilariae were also found in the two pieces of skin submitted for histopathological examintion (Figure 19). The microfilariae were identified as an Onchocerca sp., probably O. cervicalis.

Natural Infection Rate

A total of 1,868 wild Culicoides comprising 8 species were collected with a mouth aspirator from the ventral abdomen of the horse at site A and the ponies at site B during the 1985 collection period (Tables 3 and 4). However, four species were commonly found on the horse at site A: C. obsoletus, C. v. variipennis, C. biguttatus (Coquillett), and C. stellifer. Culicoides obsoletus was the most abundant species on the horse at site A in May and at both sites in August, September, and October. At site A C. biguttatus was the most common species occurring on the horse in June and C. stellifer in July. Culicoides variipennis variipennis was most evident on the horse at site A in early May and again from August to September. The other four species C. sanguisuga, C. venustus, C. spinosus (Root and Hoffman), and C. guttipennis (Coquillett) were collected in small numbers during the collection period.

Filarial larvae were found in C. obsoletus, C. v. variipennis, C. stellifer, and C. biguttatus at site A (Table 5). However, larvae similar to Onchocerca were found in only the first three species (Figures 20-23). All larvae were in the thoracic muscles of the Culicoides; none were present in the head or

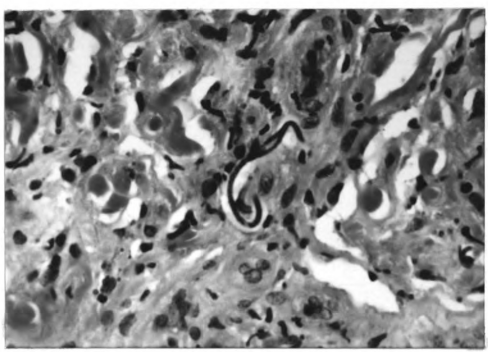

Figure 19. Microfilaria in the skin of an infected pony (x 500).

Table 3. Species of *Culicoides* collected from an infected horse at site A in 1985.

Species	Collection dates									
	5/3	5/10	5/17	6/17	6/24	7/1	7/11	7/16	7/23	8/1
C. obsoletus	75	4	0	34	9	7	2	1	8	4
C. v. variipennis	3	0	2	0	0	0	0	0	0	0
C. biguttatus	0	0	1	384	24	18	15	15	16	2
C. stellifer	0	0	0	70	4	17	12	153	101	22
C. sanguisuga	0	0	0	1	0	0	0	2	0	0
C. venustus	0	0	0	1	0	0	0	2	0	0
C. spinosus	0	0	0	2	0	0	0	0	0	0
C. guttipennis	0	0	0	0	0	0	0	0	0	0

Table 3 (cont'd.)

Species	Collection dates									
	8/8	8/19	8/28	9/4	9/17	9/25	10/2	10/9	10/17	10/25
C. obsoletus	0	89	5	21	1	43	33	33	49	8
C. v. variipennis	2	2	0	7	10	9	4	1	1	0
C. biguttatus	2	0	0	1	0	0	0	0	0	0
C. stellifer	10	5	4	1	0	0	0	0	0	0
C. sanguisuga	0	5	0	3	0	0	0	0	0	0
C. venustus	1	1	2	0	0	0	0	1	0	0
C. spinosus	0	0	0	0	0	0	0	0	0	0
C. guttipennis	0	0	1	0	0	0	0	0	0	0

Table 4. Species of *Culicoides* collected from infected ponies at site B in 1985.

Species	Collection dates							
	8/20	8/22	8/27	8/29	9/7	9/11	9/24	10/4
C. obsoletus	4	54	22	0	33	65	9	44
C. v. variipennis	0	0	0	0	0	5	0	1
C. biguttatus	0	0	0	0	0	3	0	0
C. stellifer	1	1	0	0	0	0	0	0
C. sanguisuga	1	3	0	0	0	0	0	0
C. venustus	0	0	0	0	0	0	0	0
C. spinosus	0	0	0	0	0	0	0	0
C. guttipennis	0	0	0	0	0	0	0	0

Table 5. Natural filarial infections of Culicoides. *

Species	Month					
	May	June	July	Aug.	Sept.	Oct.
C. obsoletus	0/63**	0/34	0/14	1/81	1/54	3/98
C. v. variipennis	0/2	0	0	0/4	1/26	0/5
C. biguttatus	0	0/326	2/51	0/3	0	0
C. stellifer	0	1/59	8/226	1/35	0/2	0
C. sanguisuga	0	0	0	0/3	0/2	0
C. venustus	0	0	0	0/4	0	0
C. guttipennis	0	0	0	0	0	0

* Collected from the horse at site A; filarial nematode infections were found only in the thoracic muscles.
** No. of infected flies/No. dissected.

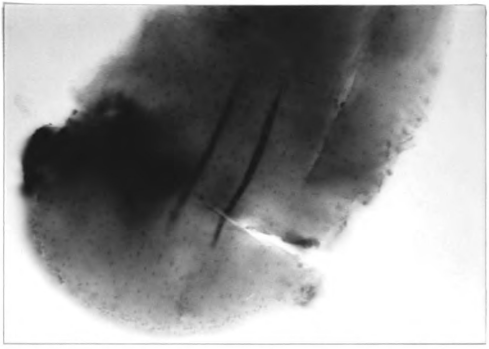

Figure 20. Onchocerca type larvae in the thoracic muscles of wild caught C. obsoletus (x 313).

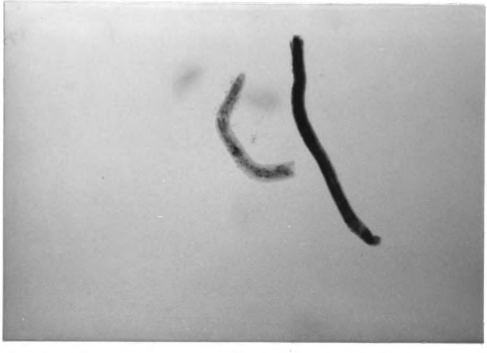

Figure 21. Onchocerca type larva (broken specimen) dissected from the thoracic muscles of wild caught C. stellifer (x 200).

Figure 22. Onchocerca type larva dissected from the thoracic muscles of wild caught C. v. variipennis (x 125).

Figure 23. Onchocerca type larva in the thoracic muscles of the above C. v. variipennis midge (x 125).

abdomen. Several non-filarial type nematodes were found in the thoracic muscles of C. obsoletus, C. stellifer, and C. biguttatus (Figures 24-26). No filarial infections were observed in the Culicoides collected from site B.

Light Trap Collection

A total of 2,134 Culicoides and 3 other genera, Forcipomyia, Dasyhelea, and Bezzia were collected in the blacklight traps at three collection sites: the two horse stables in Clinton county (Tables 6 and 7) and the veterinary research farm in Ingham county (Table 8). Five Culicoides spp. were commonly collected: C. obsoletus, C. v. variipennis, C. biguttatus, C. stellifer and C. crepuscularis (Malloch). Culicoides obsoletus was frequently collected at the two horse stables thoughout the collection period. At site A this species was most abundant in early June and from late July to early August. They were found in small numbers at the veterinary research farm. Culicoides variipennis variipennis was collected only at site A and the veterinary research farm; it was present in small numbers during each month of collection, except for May at site A. Culicoides biguttatus and C. stellifer were found at all three blacklight trap sites. Culicoides crepuscularis was most abundant at the veterinary research farm.

Attempts to locate the breeding sites of C. obsoletus

The emergence trap method for collecting C. obsoletus was discontinued after a month of operation at site A because only 2 obsoletus group spp. were collected during this time period. The two adult midges were collected along the base of the manure pile. Large numbers of Forcipomyia bipunctata (Linnaeus) (Diptera: Ceratopogonidae) were found breeding in the manure pile.

Figure 24. Non-Onchocerca type larva (arrow) in the thoracic muscles of wild caught C. obsoletus (x 125).

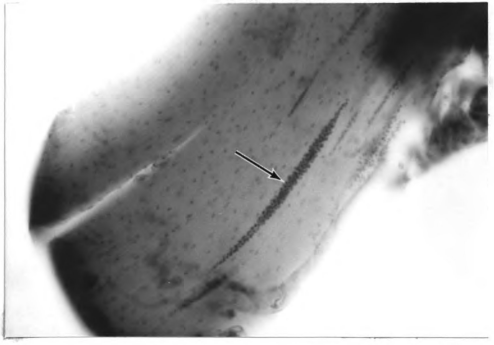

Figure 25. Non-Onchocerca type larva (arrow) in the thoracic muscles of wild caught C. stellifer (x 313).

Figure 26. Non-Onchocerca type larva (arrow) in the thoracic muscles of wild caught C. biguttatus (x 160).

Table 6. Blacklight trap collection of Culicoides and other Ceratopogonids at site A in 1985.

Species	Collection dates									
	5/24	6/7	6/13	7/1	7/11	7/23	8/1	8/8	8/21	9/4
C. obsoletus	60	124	176	31	15	233	232	1	60	38
C. biguttatus	7	16	61	74	20	71	7	0	1	8
C. v. variipennis	0	16	3	0	5	7	9	0	0	3
C. crepuscularis	0	1	0	0	0	1	0	0	0	3
C. stellifer	0	1	2	36	13	95	41	0	26	0
C. venustus	0	0	1	0	0	1	1	0	0	0
C. spinosus	0	0	1	0	0	0	1	0	0	0
C. haematopotus	0	0	1	0	0	0	1	0	0	0
C. chiopterus	0	0	0	0	0	0	0	1	3	1
C. sanguisuga	0	0	0	0	0	0	0	1	0	0
Forcipomyia bipunctata	286	131	0	161	3	173	90	0	51	25
F. brevipennis	0	0	0	0	0	0	1	0	1	1
Dasyhelea sp.	0	0	0	0	3	0	0	0	0	1
Bezzia nobilis	0	0	0	0	0	0	1	0	0	1

Table 7. Blacklight trap collection of Culicoides and other Ceratopogonids at the DeWitt stable in 1985.

Species	Collection dates						
	6/28	7/5	7/12	7/19	8/2	8/9	8/23
C. obsoletus	0	57	52	48	130	46	8
C. biguttatus	0	21	1	1	0	0	0
C. v. variipennis	0	0	0	0	0	0	0
C. crepuscularis	0	0	0	0	0	1	0
C. stellifer	0	7	4	2	12	1	0
C. venustus	0	0	0	0	0	0	0
C. spinosus	0	0	0	0	0	0	0
C. haematopotus	0	0	0	0	0	0	0
C. chiopterus	0	0	0	0	2	0	0
C. sanguisuga	0	0	0	0	0	0	0
Forcipomyia bipunctata	4	135	783	900	466	461	62
F. brevipennis	0	0	0	0	1	10	0

Table 8. Blacklight trap collection of *Culicoides* and other Ceratopogonids at the MSU Veterinary Research Farm in 1985.

Species	Collection dates					
	6/25	7/3	7/9	7/16	7/27	8/31
C. obsoletus	0	2	0	1	17	2
C. biguttatus	5	3	3	7	2	0
C. v. variipennis	0	0	0	0	7	21
C. crepuscularis	0	1	47	156	113	1
C. stellifer	0	1	0	1	1	0
C. venustus	0	0	0	0	0	0
C. spinosus	0	0	0	0	0	0
C. haematopotus	0	0	0	0	0	2
C. chiopterus	0	0	0	0	0	0
C. sanguisuga	0	0	0	0	0	0
Forcipomyia bipunctata	5	3	3	7	2	0
F. brevipennis	0	0	0	1	0	2
Dasyhelea sp.	0	0	53	121	45	0
Bezzia nobilis	0	0	5	6	0	0

In method 2, a six inch garden spade was used to collect samples of suspected C. obsoletus breeding materials within a 1 mile radius of site A and B, the DeWitt farm, and the veterinary research farm. In the laboratory, the samples were placed in white enamel pans, flooded with distilled water. The pupae that rose to the surface were collected and held in 2 dram glass vials on wet cotten until the adults emerged. This was an attempt to locate this species breeding site and to use as a source of clean flies or adults reared from the pupal stage in experimental Onchocerca infection studies. No C. obsoletus breeding sites were found, but several other Culicoides spp. habitats were located. At site A, C. v. variipennis, C. crepuscularis, C. wisconsensis (Jones), and C. piliferus group pupae were collected from the wet soil in the south pasture (Figure 10); Culicoides guttipennis was found breeding in several beechwood tree holes in the woodlot; C. haematopotus (Malloch) and C. crepuscularis pupae were collected along the margin of a duck pond, 1/4 mile south of the site. Culicoides haemotopotus, C. crepuscularis, C. piliferus group, and C. venustus were found breeding along the margins of Summers Drain, 4/5 mile north of site A. A single Culicoides guttipennis pupa was collected from a horse water trough at the DeWitt farm about 11 miles from site B. Culicoides variipennis variipennis and C. crepuscularis pupae were collected from wet soil at the base of a compost heap adjacent to the Veterinary Research Farm site (Figure 11).

Experimental Infections

Study 1 was to determine if O. cervicalis develops to the infective (L_3) larvae in C. obsoletus and C. v. variipennis after feeding on an infected horse in

the field. Engorged wild C. obsoletus and C. v. variipennis were collected by aspirator from the ventral abdomen of the horse at site A and maintained at 62.6-66.2°F for 25 days post feeding.

Upon dissection, both species were found to be infected with filarial larvae. Second stage larvae were seen in 2 of 115 (1.7 %) C. obsoletus, 2 and 11 days, respectively, after feeding (Figures 27 and 28). Two of 5 larvae found on day 2 measured 77.2 and 61.5 u long by 15.7 u wide. The single larva observed on day 11 measured 143 u long and 15.7 u wide. Both second and third stage larvae were present in 9 of 54 (16.7%) C. v. variipennis (Figures 29-40). Infective larvae (L_3) were observed in the head of C. v. variipennis 6 and 25 days, respectively, after feeding (Table 9). The individual measurements of the four L_3 larvae in the head were: 627, 627, 627, and 684 u long by 18.5, 18.0, 19.1, and 17.9 u wide, respectively. These measurements are within the range for Onchocerca infective stage larvae (Steward, 1933; Moignoux, 1952).

In study 2, three replicates of 25 laboratory reared C. v. variipennis were fed on Lacey and two replicates on a 15 year old pony named Ginger at site B (Table 10). Ginger was one of the three ponies examined by Dr. Rosser which had clinical signs of onchocerciasis.

The first feeding was to determine if the midges would ingest microfilariae from the area of skin near the umbilicus. It was found that 3 of 7 engorged midges did ingest microfilariae from Lacey and 2 of 2 from Ginger. In the second replicate,10 C. v. variipennis fed on Lacey and one on Ginger. Of the midges that fed on Lacey, developmental stages were observed in 4 specimens

Figure 27. Onchocerca type larva (arrow) in the thoracic muscles of C. obsoletus 2 days after feeding on an infected horse (x 160).

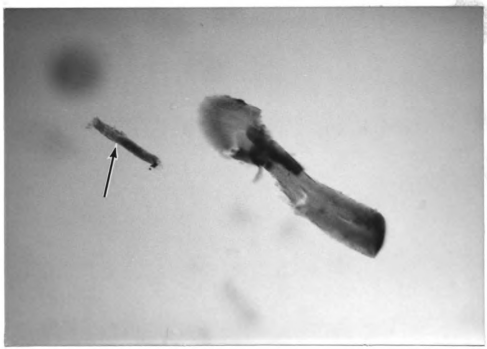

Figure 28. Onchocerca type larva (arrow) dissected from the thoracic muscles of C. obsoletus 11 days after feeding (x 125).

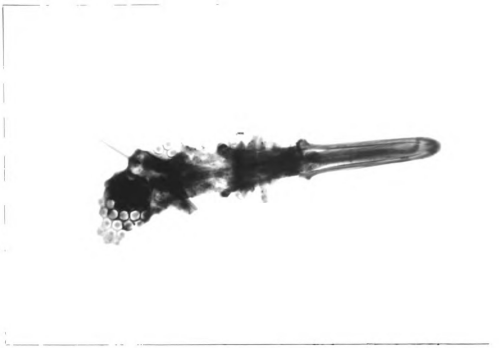

Figure 29. Onchocerca type infective (L_3) larva in the head of C. v. variipennis 6 days after feeding on the infected horse (x 125).

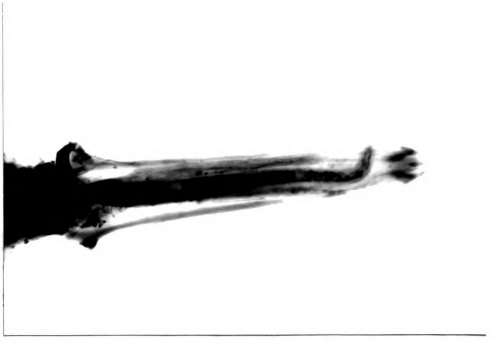

Figure 30. Same infective (L_3) larva in the proboscis of C. v. variipennis (x 313).

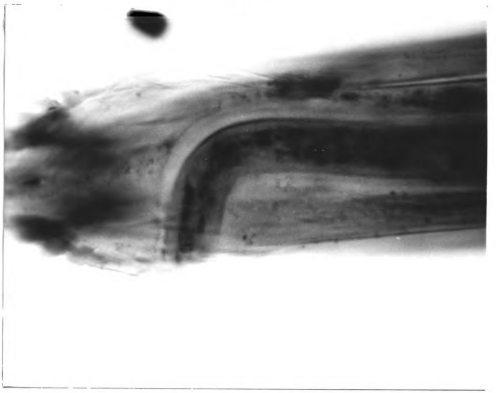

Figure 31. The infective (L_3) larva emerging from the proboscis (x 400).

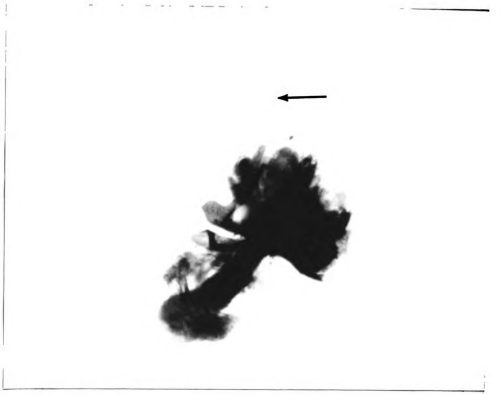

Figure 32. A second third stage infective (L_3) larva (arrow) found in the head of the same C. v. variipennis (x 80).

Figure 33. The infective stage (L_3) larva dissected from the head of C. v. variipennis (x 125).

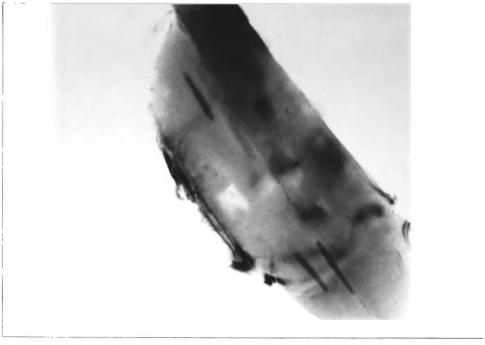

Figure 34. Second stage filarial larvae in the thoracic muscles of C. v. variipennis 7 days after feeding (x 160).

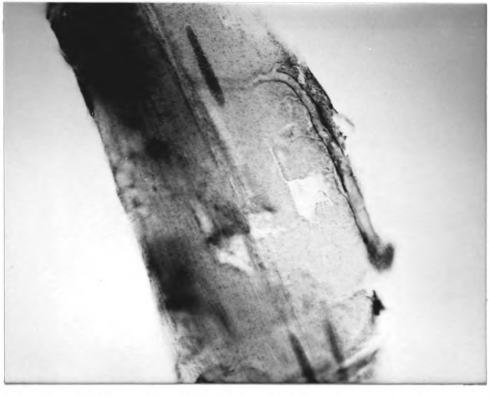

Figure 35. Second stage filarial larvae in the thoracic muscles of C. v. variipennis 8 days after feeding (x 313).

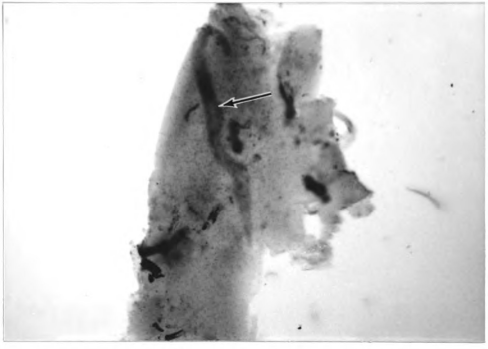

Figure 36. Second stage filarial larva (arrow) in the thoracic muscles of C. v. variipennis 10 days after feeding (x 125).

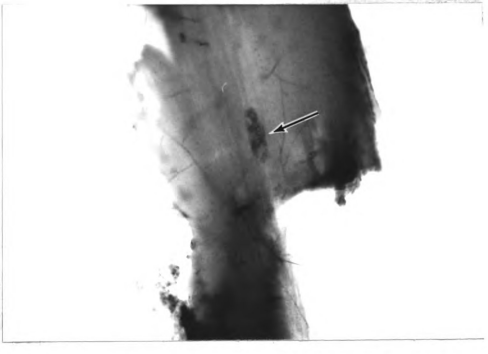

Figure 37. Second stage filarial larva (arrow) in the thoracic muscles of C. v. variipennis 11 days after feeding (x 125).

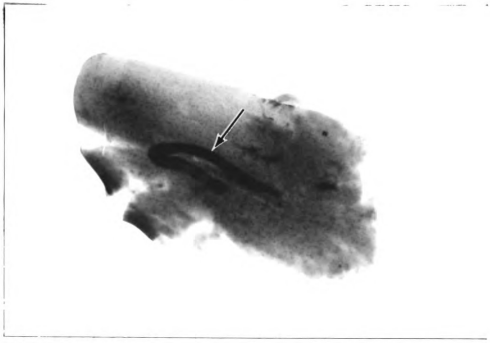

Figure 38. Second stage filarial larva (arrow) in the thoracic muscles of C. v. variipennis 18 days after feeding (x 125).

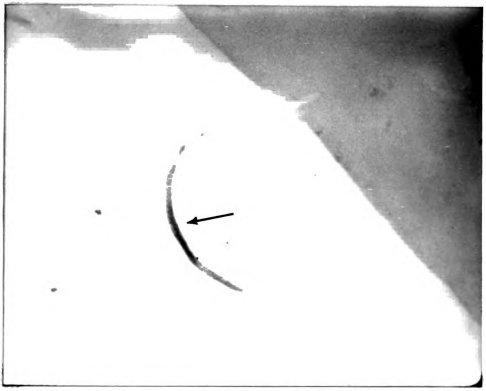

Figure 39. Second stage filarial larva (arrow) emerged from the thoracic of C. v. variipennis 20 days after feeding (x 44).

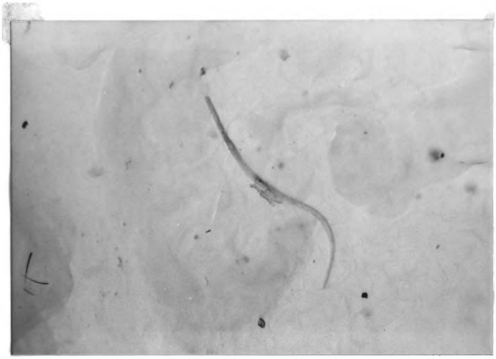

Figure 40. Infective (L_3) filarial larva emerged from the head of C. v. variipennis 25 days after feeding (x 80).

Table 9. Development of Onchocerca in wild caught C. v. variipennis.

Days post feeding	# Dissected	# Infected	# Larvae/Stage	Site	Dimension*
1	1	0	0	-	-
2	1	0	0	-	-
3	0	0	0	-	-
4	0	0	0	-	-
5	0	0	0	-	-
6	3	2	$3/L_2$	T	535.8 x 22.9
			$2/L_3$	H	627.0 x 18.5
7	2	1	$9/L_2$	T	105.0 x 14.6
8	3	1	$3/L_2$	T	-**
9	3	0	0	-	-
10	8	1	$1/L_2$	T	465.8 x 28.8
11	6	1	$1/L_2$	T	81.7 x 23.0
12	6	0	0	-	-
13	4	0	0	-	-
14	8	0	0	-	-
15	1	0	0	-	-
16	1	0	0	-	-
17	1	0	0	-	-
18	1	1	$1/L_2$	T	425.5 x 20.7
19	0	0	0	-	-
20	1	1	$1/L_2$	T	499.0 x 19.6
21	0	0	0	-	-
22	1	0	0	-	-
23	1	0	0	-	-
24	1	0	0	-	-
25	1	1	$2/L_3$	H	655.5 x 17.5

* Average length and width in um, ** specimen lost, T= thoracic muscles, H= head
L_2= second stage larvae, and L_3= infective stage larvae.

Table 10. Experimental infection of laboratory reared C. v. variipennis.

	Lacey			Ginger		
Replicate	# Fed	# Infected	Stage	# Fed	# Infected	Stage
1	7	3	MF	2	2	MF
2	10	4	L_2,L_3	1	1	L_2
3	2	1	L_2	0	0	0

MF= microfilariae

dissected 4, 6, 14, and 23 days post feeding (Figures 41-46). The single infective stage larva was found in the head of the specimen dissected on the 23rd day (Figures 44-46). This larva was 627 u long by 19.1 u wide. The single midge that fed on Ginger died 7 days after feeding and 15 second stage larvae were present in the thoracic muscles (Figure 47). In replicate 3, only 2 midges fed on Lacey and 6 second stage larvae were found in one midge 12 days after feeding (Figure 48). Table 11 shows the development of the Onchocerca microfilariae ingested from the equines at site A and B by C. v. variipennis in replicates 2 and 3.

Figure 41. Second stage filarial larva (arrow) in the thoracic muscles of clean C. v. variipennis 4 days after feeding (x 125).

Figure 42. Second stage filarial larva (arrow) in the thoracic muscles of C. v. variipennis 6 days after feeding (x 125).

Figure 43. Second stage filarial larva (arrow) in the thoracic muscles of C. v. variipennis 14 days after feeding (x 160).

Figure 44. An infective (L_3) larva in the head of C. v. variipennis 23 days after feeding (x 50).

Figure 45. Infective (L_3) larva emerging from the proboscis of C. v. variipennis (x 200).

Figure 46. Infective (L_3) larva emerging from the mandible of C. v. variipennis (x 160).

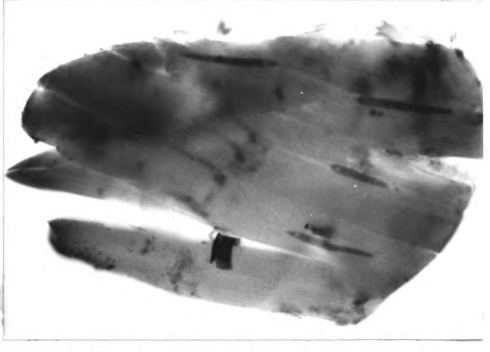

Figure 47. Second stage larvae in the thoracic muscles of C. v. variipennis 7 days after feeding (x 160).

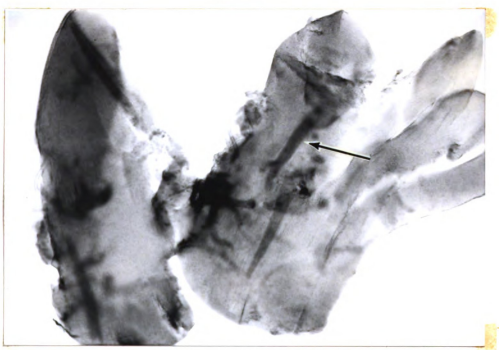

Figure 48. Second stage larvae (arrow) in the thoracic muscles of C. v. variipennis 12 days after feeding (x 160).

Table 11. Development of Onchocerca in laboratory reared C. v. variipennis.

Days post feeding	# Dissected	# Infected	# Larvae/Stage	Site	Dimension
1	0	0	0	-	-
2	0	0	0	-	-
3	0	0	0	-	-
4	1	1	$1/L_2$	T	86.3 x 17.3
5	0	0	0	-	-
6	1	1	$3/L_2$	T	155.0 x 20.1
*7	1	1	$15/L_2$	T	124.9 x 14.5
8	0	0	0	-	-
9	0	0	0	-	-
10	0	0	0	-	-
11	0	0	0	-	-
12	1	1	$6/L_2$	T	209.2 x 22.6
13	0	0	0	-	-
14	1	1	$1/L_2$	T	356.5 x 24.2
15	0	0	0	-	-
16	0	0	0	-	-
17	0	0	0	-	-
18	0	0	0	-	-
19	0	0	0	-	-
20	0	0	0	-	-
21	0	0	0	-	-
22	0	0	0	-	-
23	2	1	$3/L_2$	T	483.3 x 17.0
			$1/L_3$	H	627.0 x 19.1

* Fly fed on Ginger at site B.

DISCUSSION

The original purpose of this study was to evalute the role of C. obsoletus as a potential vector of Onchocerca in equines, but C. v. variipennis was later included in the study for two reasons: 1) It was observed during the second year of the study that wild caught C. v. variipennis midges were repeatedly found infected with microfilariae after feeding on the horse at site A; 2) two breeding sites of C. v. variipennis were found and moderate numbers of larvae and pupae were collected from these sites, so it was an ideal opportunity to determine if Onchocerca species would develop to the infective stage in this subspecies of C. variipennis after feeding on an infected equine in the field.

According to Barnett (1960), four criteria must be demonstrated to incriminate an arthropod as a vector:

1. Demonstrate that the suspected arthropod feeds on or makes effective contact with the host under natural conditions.
2. Demonstrate a biological association in time and/or space between the suspected arthropod vector and the occurrence of clinical or subclinical infection in the host.
3. Repeatedly demonstrate that the suspected arthropod vector harbors the identified infectious agent in the infective stage under natural conditions.
4. Demonstrate the transmission of the infective stage to a susceptible

host under controlled conditions.

Previous authors have reported C. obsoletus feeding on equines in the field. Steward (1933) collected engorged C. obsoletus from horses in England and Shemanchuk (1972) reported that this species bites horses in Canada. Schmidtmann et al. (1980) collected them from ponies in New York. According to Blanton and Wirth (1979), Malloch in 1915 reported C variipennis biting horses in Illinois. This northeastern subspecies of C. variipennis is considered to be C. v. variipennis. In this study, both C. v. variipennis and C. obsoletus were collected by mouth aspirator from the infected equines at site A and B (Tables 3 and 4). This demonstrates Barnett's first criterion for incrimination of a vector.

The second criterion requires proof of biological association between the suspected vector and occurrence of clinical infection in the host. According to McMullen (1972), clinical signs of equine onchocerciasis are common from spring to late autumn. These signs include loss of hair and the presence of scaly skin lesions on the body of the equines, particularly on the head, face, chest, neck, and ventral abdominal midline; there may be mild to severe itching associated with the infection. These conditions usually improve during the winter, but reappear in the spring. The duration of Onchocerca infections in equines is unknown, however, Mellor (1973a) recorded O. cervicalis microfilariae in the skin of horses from June to February in England. McMullen (1972) reported that adult O. volvulus lives for 10-15 years in man. Clinical signs of onchocerciasis in equines are not evident in all infected equines so a skin biopsy or other means of demonstrating the presence of microfilariae is necessary for confirmation of infection.

The horse at site A was used for breeding and appeared to be in relatively good health. Clinical signs of onchocerciasis in that horse were first observed on July 14, 1984 and during the month of June in 1985. These signs included thinning of the hair along the midline of the ventral abdomen, extending from the xiphoid process to the umbilicus and a single skin lesion about 1 inch in diameter lying midway between the forelegs. This horse was not biopsied, but engorged wild C. obsoletus and C. v. variipennis were collected from the ventral abdomen of the horse and later dissections demonstrated several apparently different types of microfilariae in their blood smears. The blood smear prepared from C. obsoletus was stained with Giemsa and three microfilariae were observed in the blood smear. Two of the microfilariae were about the same size,195 u long by 2.5 u wide. They were within the size range of O. cervicalis microfilariae stained with Giemsa by Mellor (1974a). The other nematode was broken during the preparation of the blood smear; it was smaller about 139.8 u long by 3.1 u wide and lacked a cephalic space at the anterior end. This nematode was not within the size range for Onchocerca. Two types of microfilariae were also observed in a hematoxylin stained blood smear from an engorged wild C. v. variipennis that had fed on this horse. These microfilariae were fixed and stained using the method described by Collins (1973). The measurements of the two microfilariae were about the same size, 214.4 u long by 2.5 u wide and 218.7 u long by 3.1 u. The cuticular striations in the latter microfilaria were more evident under oil immersion than the former microfilaria. It is assumed that Onchocerca species are host specific and the species occurring in equines in the United States is O. cervicalis. Both microfilariae in the blood smear from

C. v. variipennis were considered to be Onchocerca, since their measurements were within the size range of O. cervicalis reported by Collins (1973). At site B abdominal lesions were observed in 3 of 5 ponies. Biopsy samples taken from the lesion and umbilicus area of the two ponies contained Onchocerca microfilariae.

The results of the blacklight trap and horse baited collections showed that both C. obsoletus and C. v. variipennis were found on or near the equines at sites A and B throughout the 1985 study period, except that C. v. variipennis was not collected during the month of August at site B. In addition, C. v. variipennis was found breeding in the south pasture and limited breeding of a C. obsoletus group species was found in the west pasture at site A. Several potential breeding sites for C. obsoletus were located near the site a pond, the Watson and Summers drain, and a large marshy area located 400 ft from the western edge of the site. Wet leaflitter and soil samples were collected from the several sites along the margin of the marsh and the Watson and Summers drain, but no C. obsoletus were found. Time limitations in the study did not allow a more detailed survey of these areas for C. obsoletus. No breeding sites for C. obsoletus or C. v. variipennis were found at site B, but potential breeding habitats such as a corn field and marshy area, drainage ditch, and manure pile were located nearby. The results of the biting and light trap collections and the origin of these species in the vicinity of the collection sites satisfy Barnett's second criterion for vector incrimination.

The third criterion states that the suspected vector must repeatedly be found to harbor the identified infective stage under natural conditions. Unlike O.

volvulus, there have been few detailed descriptions of equine Onchocerca larvae in the arthropod host. The identity of equine Onchocerca larvae has been based on their size, site of development of the larvae and the assumption that the species occurring in the skin of equines are O. cervicalis and O. reticulata. Steward (1933) followed the development of O. cervicalis to the infective (L_3) stage in engorged field collected C. nubeculosus in England; they measured 600-700 u long by 18-21 u wide. Foil et al. (1984) reported finding a third stage O. cervicalis larva in a wild C. variipennis collected in a pony baited stable trap in Louisiana, but they did not indicate it's measurements. Bain and Petit (1978) redescribed the infective (L_3) larvae from laboratory infected C. nubeculosus in more detail and reported that the infective (L_3) larvae ranged in length from 680 to 870 u, the longest measured 870 u long by 18 u wide. They also listed measurements for several internal structures: buccal capsule, nerve ring, excretory pore, oesophagus, intestine, rectum, and tail. Wild caught C. obsoletus and C. v. variipennis at site A were found infected with filarial larvae in the thoracic muscles. Several different types and stages of larvae were observed in the thoracic muscles of C. obsoletus, but no Onchocerca infective (L_3) larvae were observed. The size and shape of most of the larvae in C. obsoletus did not resemble the developmental stages of O. cervicalis described by Steward (1933) and Mellor (1975). However, three larvae were found in the thoracic muscles of C. obsoletus which did resemble first stage Onchocerca larvae in appearance and body width, but their average size, 112.97 u long by 5.72 u wide (n=3) was below the size range for Onchocerca. Two second stage Onchocerca type larvae were

found in 1 of 37 (2.7%) wild caught C. v. variipennis. A late second stage Onchocerca type larva was found in 1 of 322 (0.3%) C. stellifer. This species was the predominant species occurring on the infected horse in July during 1984 and 1985. In addition, non- Onchocerca larvae were found in the thoracic muscles of 2 C. biguttatus specimens dissected. It is possible that these non-Onchocerca type larvae found in the thoracic muscles of C. obsoletus, C. stellifer, and C. biguttatus may be from a non-equine host. Lichtenfels (1975) listed 6 non-equine filarial larvae found in the horse. According to Linley (1985), ceratopogonids are vectors of 17 filarial nematodes of which 8 are bird filariae. They may also be the larvae of a parasite of Culicoides. Mermithid parasites have been reported from adult C. obsoletus (Service, 1974). According to Buckley (1938), mermithid infections in the thoracic muscles of Culicoides resemble filarial "sausage" stages.

In experimental infection studies, filarial nematodes were present in engorged wild caught C. obsoletus and C. v. variipennis after they had fed on the infected horse at site A. Second stage larvae were found in 2 of 115 (1.7%) C. obsoletus. These larvae were in the thoracic muscles 2 and 11 days post feeding. Although the larval stages were similar in shape, their size was below the range for Onchocerca. Because of a lack of experimentally infected flies for comparison, it was not possible to determine if these larvae were ingested from the skin of the horse or had been acquired from a previous blood meal. Infective (L_3) larvae were present in the heads of 2 engorged wild caught C. v. variipennis 6 and 25 days after feeding on the horse at site A. The lengths of the infective

(L_3) larvae measured 627, 627, 627, and 684 u, their widths were 18.5, 18.0, 19.1, and 17.9 u, respectively. The length of these infective (L_3) larvae were within the size range of Onchocerca (Steward, 1933). The presence of third stage larvae in the head of Culicoides indicate that they are infective and able to transmit the infective (L_3) larvae to the equine host during feeding.

The infective (L_3) stage of Onchocerca also developed in laboratory reared C. v. variipennis allowed to feed on an infected horse at site A (Tables 10 and 11). The horse was exposed to a total of 75 C. v. variipennis and 19 fed. A single infective (L_3) larva was found in the proboscis (lying between the mandibles) of this midge 23 days post feeding and the larva measured 627 u long by 19.1 u wide. Since this larva was similar in appearance and within the size range of O. cervicalis reported by Steward (1933) in English horses , it was assumed to be that species of Onchocerca. The finding of infective (L_3) larvae of Onchocerca sp. in wild caught and wild and experimentally infected C. v. variipennis at site A satisfy Barnett's third criterion. Three out of 50 of these flies fed on the pony at site B; they were dissected 0 and 7 days post feeding, but no infective (L_3) larvae were found.

The fourth criterion, demonstration of transmission of the infective stage to a susceptible host under controlled conditions was not attempted in this study. The lack of a susceptible host other than equines and the cost of utilizing these animals in controlled studies prevented the complete incrimination of Culicoides as a vector of Onchocerca in this study. Collins and Jones (1978)

allowed infective C. variipennis to feed on horse serum using a membrane feeder and observed transmission of O. cervicalis infective larvae into the horse serum solution. They also fed the infective C. variipennis on jirds (Meriones unguiculatus), and dissected samples of midges before and after feeding on the jirds to determine the number of infective (L_3) larvae in the flies and how many were lost during the feeding process. In addition, the skin surface of the jirds were examined and no larvae were seen. From the results of their study they concluded that 62.5% of 369 midges shed all their infective larvae while feeding on the jird. No mention was made of whether the infective larvae continued their development in the jirds.

Based on the findings in this and other studies on the transmission of Onchocerca by Culicoides, it is concluded that C. v. variipennis is a potential vector of equine onchocerciasis in Michigan and that Culicoides stellifer may be a potential secondary vector. The role of C. obsoletus as a potential vector is still unresolved, since no Onchocerca infective stage larvae were found in several hundred dissections of wild caught midges. This is not uncommon since the natural infection rate of Onchocerca in Culicoides species is quite low. In Malaysia, Buckley (1938) found that only 13 of 3,734 (0.35%) C. pungens (de Meij) were naturally infected with O. gibsoni (Cleland and Johnston), a bovine parasite. In France, Moignoux (1952) reported that 2.1% of wild caught C. nubeculosus were naturally infected with O. reticulata. In this study, natural infection rates of 2.7% and 0.3% for C. v. variipennis and C. stellifer, respectively were found.

SUMMARY AND CONCLUSIONS

1. Onchocerca infected equines were diagnosed by demonstrating the presence of microfilaria in blood taken from engorged Culicoides and in skin biopsies from suspected equines.
2. Wild caught C. obsoletus and C. v. variipennis were collected from infected equines at both study sites.
3. Natural infections of Onchocerca were found in 1 of 37 (2.7%) wild caught C. v. variipennis and in 1 of 322 (0.3%) C. stellifer; however, no infections were observed in wild caught C. obsoletus.
4. Onchocerca infective stage larvae were found in the head of both wild and experimentally infected C. v. variipennis midges.
5. Using the four criteria described by Barnett (1960), it appears that C. v. variipennis is a potential vector of equine onchocerciasis in Michigan and that Culicoides stellifer may be a secondary potential vector. The role of C. obsoletus as a potential vector is still unresolved, since no Onchocerca infective stage larvae were found in several hundred dissections of wild caught flies of this species.

LIST OF REFERENCES

LIST OF REFERENCES

Bain, O. 1975. Redescription de cinq especes d' onchocerques. Annales de Parasitologie Humaine et Comparee. 50: 763-788.

Bain, O., R. L. Muller, Y. Khamis, J. Guilhon, and T. Schillhorn Van Veen. 1976. Onchocerca raillieti n. sp. (Filarioidea) chez l' ane domestique en Afrique. J. Helminth. 50: 287-293.

Bain, O., and G. Petit. 1978. Redescription du stade infestant d' Onchocerca cervicalis R. and H., 1910. Ann. De Parasitologie Humaine et Compareacutee. 53: 315-318.

Barnett, H. C. 1960. The incrimination of arthropods as vectors of disease. Proc. 11th Inter. Congr. Entomol. 2: 341-345.

Belding, D. C. 1942. Textbook of clinical parasitology. Appleton-Century-Crofts Publishing Co., N. Y. and London. 781-782.

Bennett, G. F. 1960. On some ornithophilic blood-sucking diptera in Algonquin Park, Canada. Can. J. Zool. 38: 377-389.

Birley, M. H., Y. Braverman, and K. Frish. 1984. Survival and blood-feeding rate of some Culicoides species (Diptera: Ceratopogonidae) in Israel. Environ. Entomol. 13: 424-429.

Blanton, F. S., and W. W. Wirth. 1979. Arthropods of Florida and neighboring land areas. Vol. 10. The sand flies (Culicoides) of Florida (Diptera: Ceratopogonidae). Florida Dept. of Agric. and Consumer Services.

Buckley, J. J. C. 1938. On Culicoides as a vector of Onchocerca gibsoni (Cleland and Johnston 1910). J. Helminth. 16: 121-158.

Cello, R. M. 1971. Ocular onchocerciasis in the horse. Equine Vet. J. 3: 149-154.

Collins, R. C. 1973. Onchocerciasis of horses in southeastern Louisiana. J. Parasitol. 59: 1016-1020.

Collins, R. C., and R. H. Jones. 1978. Laboratory transmission of Onchocerca cervicalis with Culicoides variipennis. Amer. J. Trop. Med. Hyg. 27: 46-50.

Cummings, E., and E. R. James. 1985. Prevalence of equine onchocerciasis in southeastern and midwestern United States. J. Amer. Vet. Med. Assoc. 57: 186: 1202-1203.

El Sammani, S. E., and H. S. Hussein. 1983. Onchocerca raillieti: adult location and skin distribution of the microfilariae in Sudanese donkeys. J. Helminth. 57: 355-360.

Endris, R. G., P. V. Perkins, D. G. Young, and R. N. Johnson. 1982. Techniques for laboratory rearing of sand flies (Diptera: Psychodidae). Mosq. News 12: 400-407.

Foil, L., Stage, D., and T. R. Klei. 1984. Assessment of wild-caught Culicoides (Ceratopogonidae) species as natural vectors of Onchocerca cervicalis in Louisiana. Mosq. News. 44: 204-206.

Gibson, C. L. 1952. Comparative morphology of the skin inhabiting microfilariae of man, cattle, and equines in Guatemala. Amer. J. Trop. Med. and Hyg. 1: 250-261.

Hair, J. A., E. C. Turner, Jr., and D. H. Messersmith. 1966. Larval habitats of some Virginia Culicoides (Diptera: Ceratopogonidae). Mosq. News. 26: 195-204.

Hall, M. C. 1923. The relation of parasites to poll evil and fistulous withers. Vet. Medicine. 18: 715-718.

Herd, R. P., and J. C. Donham. 1983. Efficacy of ivermectin against Onchocerca cervicalis microfilarial dermatitis in horses. Amer. J. Vet. Res. 44: 1102-1105.

Hill, M. A. 1947. The life cycle and habits of Culicoides impunctatus Goetghebuer and Culicoides obsoletus Meigen, together with some observations on the life cycle of C. odibilis Austen, C. pallidicornis Kieffer, C. cubitalis Edwards and C. chiopterus Meigen. Ann. Trop. Med. Parasit. 41: 55-115.

Jamnback, H. 1961. Observations on Culicoides obsoletus (Meigen) in the laboratory (Diptera: Ceratopogonidae). Mosq. News. 21: 48-53.

Jamnback, H. 1965. The Culicoides of New York State (Diptera: Ceratopogonidae). N. Y. State Mus. Sci. Serv. Bulletin 399: 1-154.

Jamnback, H., and T. Watthews. 1963. Studies of populations of adult and immature Culicoides sanguisuga (Diptera: Ceratopogonidae). Ann. Entomol. Soc. Amer. 56: 728-732.

Jamnback, H., and W. W. Wirth. 1963. The species of Culicoides related to obsoletus in eastern North America. Ann. Entomol. Soc. Amer. 56: 185-198.

Jones, R. H. 1961. Observation on the larval habitats of some North American species of Culicoides (Diptera: Ceratopogonidae). Ann. Entomol. Soc. Amer. 54: 702-710.

Jones, R. H., R. O. Hayes, H. W. Potter, Jr., and D. B. Francy. 1977. A survey of biting flies attacking equines in three states of the southwestern United States, 1972. J. Med. Entomol. 14: 441-447.

Jorgensen, N. M. 1969. The systematics, occurrence, and host preference of Culicoides (Diptera: Ceratopogonidae) in southeastern Washington. Melanderia. 3: 47 pp.

Kardatzke, J. T., and W. A. Rowley. 1971. Comparison of Culicoides larval habitats and populations in central Iowa. Ann. Entomol. Soc. Amer. 64: 215-218.

Kettle, D. S., and J. W. H. Lawson. 1952. The early stages of british biting midges, Culicoides Latreille (Diptera: Ceratopogonidae) and allied genera. Bull. Entomol. Res. 43: 421-467.

Lawson, J. W. H. 1951. The anatomy and morphology of the early stages of Culicoides nubeculosus Meigen (Diptera: Ceratopogonidae=Heleidae). Trans. Roy. Entomol. Soc. London. 102: 511-570.

Le Roux, P. L. 1950. Onchocerca cervicalis Railliet and Henry, 1910, is a doubtful synonym of O. reticulata Diesing, in Hermann, 1841. Trans. Roy. Soc. Trop. Med. Hyg. 44: 5.

Levine, N. D. 1968. Nematode parasites of domestic animals and of man. Burgess Publishing Co., Minneapolis, 457-485.

Lichtenfels, J. R. 1975. Helminths of domestic equids. Proc. Helminth. Soc. Wash. 42: 82 pp.

Linley, J. R. 1985. Biting midges (Diptera: Ceratopogonidae) as vectors of nonviral animals pathogens. J. Med. Entomol. 22: 589-599.

Lloyd, S., and E. J. L. Soulsby. 1978. Survey for infections with Onchocerca cervicalis in horses in eastern United States. Amer. J. Vet. Res. 39: 1962-1963.

M' Fadyean, J. 1902. The anatomy of the horse. A dissection guide. 2nd. ed. W. and A. K. Johnson, Edinburgh and London, 161-162.

McCullough, C., M. McCullough, and G. Wilhelm. 1977. Onchocerciasis among ungulate animals in Maryland. J. Parasitology. 63: 1065.

McMullan, W. C. 1972. Onchocercal filariasis. Southwestern Veterinarian. 25: 179-191.

Mellor, P. S. 1971. A membrane feeding technique for the infection of Culicoides nubeculosus Mg. and Culicoides variipennis sonorensis Coq. with Onchocerca cervicalis Railliet and Henry. Trans. Roy. Soc. Trop. Med. Hyg. 65: 199-201.

Mellor, P. S. 1973a. Studies on Onchocerca cervicalis Railliet and Henry 1910: 1. Onchocerca cervicalis in British Horses. J. Helminth. 47: 97-110.

Mellor, P. S. 1973b. Studies on Onchocerca cervicalis Railliet and Henry, 1910: 2. Pathology in the horse. J. Helminth. 47: 111-118.

Mellor, P. S. 1974a. Studies on Onchocerca cervicalis Railliet and Henry, 1910: 3. Morphological and taxonomic studies on Onchocerca cervicalis from british horses. J. Helminth. 48: 145-153.

Mellor, P. S. 1974b. Studies on Onchocerca cervicalis Railliet and Henry, 1910: 4. Behavior of the vector Culicoides nubeculosus in relation to the transmission of Onchocerca cervicalis. J. Helminth. 48: 283-288.

Mellor, P. S. 1974c. The probable cause of "sweet itch" in England. Vet. Record. 95: 411-415.

Mellor, P. S. 1975. Studies on Onchocerca cervicalis Railliet and Henry, 1910: 5. The development of Onchocerca cervicalis larvae in the vectors. J. Helminth. 49: 33-42.

Menon, M. A. U. 1960. A rapid method of preparing dry mounts of filarial larvae. Indian J. Malariology. 14: 659-662.

Moignoux, J. B. 1952. Les onchocerques des equides. Acta Tropica. 9: 125-150.

Murray, W. S. 1957. Investigations on the bionomics of Culicoides obsoletus (Meigen) and other biting midges at Mount Solon, Virginia. Mosq. News. 17: 77-82.

Nelson, G. S. 1958. Staining of filarial larvae in insects before dissection. Bull. Wld. Health Org. 19: 204.

Nelson, G. S. 1970. Onchocerciasis. Part 10. Onchocerciasis in domestic animals. Adv. Parasitology. 8: 208-210.

Ottley, M. L., and D. E. Moorhouse. 1978. Equine onchocerciasis. Australian Vet. J. 54: 545.

Ottley, M. L., C. Dallemagne, and D. E. Moorhouse. 1983. Equine onchcerciasis in queenland and the northern territory of Australia. Australian Vet. J. 60: 200-203.

Ottley, M. L., D. E. Moorhouse, and P. A. Holdsworth. 1985. Equine and bovine onchocerciasis. Veterinary Bulletin. 55: 571-588.

Polley, L. 1984. Onchocerca in horses from western Canada and the northwestern United States: An abattoir survey of the prevalence of Infection. Can. Vet. J. 25: 128-129.

Rabalais, F. C., and C. L. Votava. 1974. Cutaneous distribution of microfilariae of Onchocerca cervicalis. Amer. J. Vet. Res. 35: 1369-1370.

Railliet, A., and A. Henry. 1910. Les onchocerques, nematodes parasites du tissu conjonctif. Comp. Rend. Soc. Biol. 68: 248-251.

Roberts, S. R. 1963. Etiology of equine periodic ophthalmia. Amer. J. Opthal. 55: 1049-1055.

Robson, J. 1918. Filariasis of the withers in the horse. Vet. Rec. 30: 348-351.

Sandground, J. H. 1934. Onchocerciasis. Part 2. On the validity of various species of the genus Onchocerca (Diesing). Harvard University Press, Cambridge, Massachusetts, 134-172.

Schmidt, G. M., J. D. Krehbiel, S. C. Coley, and R. W. Leid. 1982. Equine onchocerciasis: Lesions in the nuchal ligament of midwestern U. S. horses. Vet. Pathology. 19: 16-22.

Schmidtmann, E. T., C. J. Jones, and B. Gollands. 1980. Comparative host-seeking activity of Culicoides (Diptera: Ceratopogonidae) attracted to pastured livestock in central New York State, USA. J. Med. Entomol. 17: 221-231.

Service, M. W. 1969. Studies on the biting habits of Culicoides impunctatus Goetghebuer, C. obsoletus (Meigen), and C. punctatus (Meigen) (Diptera: Ceratopogonidae) in southern England. Proc. Roy. Entomol. Soc. London (A). 44: 1-10.

Service, M. W. 1974. Further results of catches of Culicoides (Diptera: Ceratopogonidae) and mosquitoes from suction traps. J. Med. Entomol. 11: 471-479.

Service, M. W. 1976. Mosquito ecology. Halsted press, New York and Toronto, 349-351.

Shemanchuk, J. A. 1972. Observations on the abundance and activity of three species of Ceratopogonidae (Diptera) in northeastern Alberta. Can. Entomol. 104; 445-448.

Stannard, A. A., and R. M. Cello. 1975. Onchocerca cervicalis infection in horses from the western United States. Amer. J. Vet. Res. 36: 1029-1031.

Steward, J. S. 1933. Onchocerca cervicalis (Railliet and Henry 1910) and its development in Culicoides nubeculosus Meigen. 3rd Report of The Cambridge University Institue of Animal Pathology. 272-284.

Steward, J. S. 1937. The occurrence of Onchocerca gutturosa Neumann in cattle in England, with an account of its life history and development in Simulium ornatum Meigen. Parasitology. 29: 212-219.

Supperer, R. 1953. Filariosen der pferde in Osterreich. Wiener Tierarztliche Monatsschrift. 40: 193-220.

Thomas, A. D. 1963. Microfilariasis in the horse. J. South African Vet. Med. Assoc. 34: 17-23.

Twinn, C. R. 1931. Note on the biting midge, Culicoides obsoletus Mgn., in eastern Canada. Can. Entomol. 63: 248.

Van Volkenberg, H. L. 1921. Calcification of the ligamentum nuchae. Cornell Vet. 11: 55-63.

Williams, R. W. 1951. Observation on the bionomics of Culicoides tristriatulus Hoffman with notes on C. alaskensis Wirth and other species at Valdez, Alaska, Summer 1949. (Diptera: Heleidae). Ann. Entomol. Soc. Amer. 44: 173-183.

Williams, R. W. 1955. Observations on the bionomics of some Culicoides of Cheboygan County, Michigan, (Diptera: Heleidae). Bull. Brooklyn Entomol. Soc. 50: 113-120.

Wirth, W. W. 1977. A review of the pathogens and parasites of the biting midges (Diptera: Ceratopogonidae). J. Wash. Acad. Sci. 67: 60-74.

Wirth, W. W., and N. Marston. 1968. A method for mounting small Insects on microscope slides in canada balsam. Ann. Entomol. Soc. Amer. 6: 783-784.

World Hlth Org. 1966. WHO expert committee on onchocerciasis. Tech. Rep. Ser. No. 335. 2nd Report. 5-90.