ASSESSMENT OF WILD-CAUGHT *CULICOIDES* (CERATOPOGONIDAE) SPECIES AS NATURAL VECTORS OF *ONCHOCERCA CERVICALIS* IN LOUISIANA

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ABSTRACT. A total of 7,125 blood engorged *Culicoides* representing 6 species were collected over a 2-year period from a stable trap baited with *Onchocerca cervicalis*-infected ponies. Specimens that survived 10–14 days postfeeding were dissected and examined for advanced stages of parasite development. Only *C. variipennis* supported growth of *O. cervicalis* with the natural infection rate being 7%.

INTRODUCTION

Onchocerca cervicalis is a filarid parasite of horses reported throughout the United States. Reported prevalence rates are 61% in the East (Lloyd and Soulsby 1978), 57% in the Midwest (Rabalais et al. 1974), 52% in Kentucky (Lyons et al. 1981), and 48% in the West (Stannard and Cello 1975). Recently, a prevalence rate of 79% was reported from 135 ponies and horses examined in Louisiana (T. R. Klei and L. Foil, unpublished data). Infections by *O. cervicalis* have been frequently associated with dermatitis (McMullan 1972) and less commonly with periodic opthalmia (Cello 1971).

Culicoides spp. have been incriminated as a vector of *O. cervicalis* abroad (Steward 1935, Mellor 1975). However, in the United States, only laboratory studies have documented the vector role of *Culicoides* (Collins and Jones 1978). There are no reports of the development of *O. cervicalis* in indigenous insects after feeding upon infected horses in the U.S. under natural conditions. This report summarizes the natural infection rate of 6 *Culicoides* spp. indigenous to Louisiana collected over a two-year period.

MATERIALS AND METHODS

Skin biopsies for microfilarial examinations of the bait ponies were obtained with an 8 mm keyhole biopsy punch taken from the umbilicus region. Prior to biopsy, the area was blocked with a 1 ml subcutaneous inoculation of Lidocane®. Biopsies were weighed to the nearest mg, finely minced in RPMI-1640 tissue culture medium buffered with 0.1 M Hepes (pH 7.2–7.4) and incubated overnight in 3–5 ml of medium at 37°C. The tissue debris was subsequently removed and the microfilariae concentrated by centrifugation. The pelleted microfilariae were fixed with 4% neutral buffered formalin, stained with one drop of New Methylene Blue, pooled, and stored for later measurement with an ocular micrometer. Microfilariae concentrations of the two bait ponies ranged from 0.27 to 0.52 microfilaria/mg of biopsied tissue.

Blood engorged *Culicoides* spp. were obtained over a 2-year period from a stable trap constructed in a pasture in Brusly, Louisiana, where *O. cervicalis* transmission among ponies had been validated by the biopsy of ponies that had never left the farm. The stable trap was constructed by erecting a 1.83 m × 1.83 m × 1.83 m wooden frame and covering it with 52 × 52 mesh saran cloth.[4] A zipper was installed in both the front and rear panels of the trap for entry and exit of the pony and researchers. Plexiglas® baffles were constructed along the side panels of the trap at 0.31 m, 0.92 m and 1.53 m from the ground which allowed the entry but not the exit of insects. The pony was secured inside the trap in a stanchion constructed of tubular steel with a wooden floor. The animal was placed in the trap in the late afternoon and left until the following morning.

After removal of the pony, the blood engorged *Culicoides* spp. were collected with a mouth aspirator, placed in 500 ml cardboard containers, and held in the laboratory at 21±1°C for 10–14 days with 10% sucrose *ad libitum* as described in detail by Lowry et al. (1978). The flies were then dissected on glass microscope slides in 0.85% saline using 30 gauge needles, and examined microscopically for developing filarial larvae. The larvae observed were identified as to stage of development and measured alive with an ocular micrometer. Representative specimens of the *Culicoides* spp. were collected in CDC light traps

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in the same pasture and mounted on slides for identification.

RESULTS

A total of 7,125 engorged flies representing 6 *Culicoides* spp. were collected over a 2-year period. However, only 114 *C. variipennis* and 97 other *Culicoides* spp. survived until the time of dissection (Table 1). The only *Culicoides* sp. shown to be infected with filarid larvae was *C. variipennis* with a 7% infection rate. Eleven filarid larvae were observed in 8 *C. variipennis*. One larva was distinctly in the third stage (L_3) as judged by its size and morphology. This L_3 was observed in a fly maintained for 14 days postfeeding. The remainder of larvae were in the L_2 stage and measured 135–210 μm long and 20–25 μm wide with mean length of 157.1 $\pm$ 23.9 μm, and width of 20.7 $\pm$ 3.4 μm. These 10 larvae were recovered from the thoracic muscles of flies maintained for 10 to 11 days postfeeding. One of these L_2 was partially melanized. Three flies contained 2 second stage larvae each.

DISCUSSION

The high mortality among *Culicoides* spp. other than *C. variipennis* held under laboratory conditions (Table 1) has also been observed in other laboratories (Jones et al. 1983). The conditions under which they were held may have not been conducive to the survival of these species or the midges could have been relatively aged upon collection. However, these midges are more probably short-lived or susceptible to mortality induced by developing filarial larvae. Either of these two factors would reduce the possibility of them being potential *O. cervicalis* vectors. Due to the relatively good survival of *C. variipennis* under laboratory conditions, developmental studies such as this one are biased toward this species.

The *C. variipennis* infection rate of 7% in this study was similar to the 7.3% infection rate of a laboratory strain of *C. variipennis* fed upon a horse with 7 microfilariae/mg skin reported by Mellor (1975). Since *C. variipennis* is the known vector of bluetongue virus in cattle, many studies of the distribution and prevalence of this insect have been conducted (Foster et al. 1963). This midge species has been found to be widely distributed and reaches high population densities in many areas of the U.S. (Jones 1961). Therefore, with infection rates even lower than 7%, dense midge populations could account for the high prevalence of *O. cervicalis* reported in horses. The majority of the larvae recovered from *C. variipennis* were of the early L_2 stage at 10–11 days postinfectious blood meal, and of a size considered by Mellor (1975) to be retarded. This may indicate that wild caught *C. variipennis* held under laboratory conditions are not the optimum host for *O. cervicalis*, or that environmental conditions necessary for development are significantly different from those in other studies.

This report of the natural infection rate of midges obtained from one pasture in southern Louisiana confirms that *O. cervicalis* develops to some degree in indigenous *C. variipennis*. Further studies will be necessary to determine whether other indigenous *Culicoides* spp. can serve as vectors. Studies concerning the specific environmental conditions necessary for filarid larval development and the laboratory maintenance of different *Culicoides* spp. would aid future indigenous vector filariasis studies.

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Table 1. *Culicoides* spp. which were collected in a stable trap after feeding upon a pony infected with *Onchocerca cervicalis*.

	No. collected (Blood engorged)	No. dissected (10–14 days)	Percent mortality	No. with developing larvae
Culicoides biguttatus (Coquillett)	5557	65	98.8	0
Culicoides debilipalpis (Lutz)	993	28	97.2	0
Culicoides variipennis (Coquillett)	354	114	67.8	8
Culicoides paraensis (Goeldi)	58	1	98.3	0
Culicoides arboricola (Root and Hoffman)	152	3	98.0	0
Culicoides guttipennis (Coquillett)	11	0	100.0	0

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