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

LANE FOIL, DAVE STAGE and THOMAS R. KLEI

**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 *Onchoerca 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**

*Onchoerca cervicalis* is a filarial parasite of horses reported throughout the United States. Reported prevalence rates are 61% in the East (Lloyd and Soubby 1978), 57% in the Midwest (Kabalus 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a prevalence rate of 79% was reported from 135 ponies and horses examined in Louisiana (T. R. Klei and L. Foil, unpublished data).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 ophthalmia (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 Lidocaine®. 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 1.85 m × 1.85 m × 1.83 m wooden frame and covering it with 52 × 52 mesh saran cloth. 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 1.33 m from the ground which allowed the entrance 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.
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 filarial larvae was C. variipennis with a 7% infection rate. Eleven filarial larvae were observed in 8 C. variipennis. One larva was distinctly in the third stage (L3) as judged by its size and morphology. This L3 was observed in a fly maintained for 14 days post-feeding. The remainder of larvae were in the L2 stage and measured 135–210 µm long and 20–25 µm wide with mean length of 157.1 ± 23.9 µm, and width of 20.7 ± 5.4 µm. These 10 larvae were recovered from the thoracic muscles of flies maintained for 10 to 11 days post-feeding. One of these L3 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. 1989). 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 L2 stage at 10–11 days postinfectious blood meal, and of a size considered by Mellor (1975) to be infected.

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 filarial larval development and the laboratory maintenance of different Culicoides spp. would aid future indigenous vector filariasis studies.

ACKNOWLEDGEMENTS

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References Cited


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

<table>
<thead>
<tr>
<th>Culicoides spp.</th>
<th>No. collected (Blood engorged)</th>
<th>No. dissected (10–14 days)</th>
<th>Percent mortality</th>
<th>No. with developing larvae</th>
</tr>
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<tbody>
<tr>
<td>Culicoides gigittatus (Coquillett)</td>
<td>5557</td>
<td>65</td>
<td>98.8</td>
<td>0</td>
</tr>
<tr>
<td>Culicoides dehliopilus (Lutz)</td>
<td>993</td>
<td>28</td>
<td>97.2</td>
<td>0</td>
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<tr>
<td>Culicoides variipennis (Coquillett)</td>
<td>354</td>
<td>114</td>
<td>67.8</td>
<td>8</td>
</tr>
<tr>
<td>Culicoides parvus (Coquillett)</td>
<td>38</td>
<td>1</td>
<td>98.3</td>
<td>0</td>
</tr>
<tr>
<td>Culicoides arboricola (Root and Hoffman)</td>
<td>152</td>
<td>3</td>
<td>98.0</td>
<td>0</td>
</tr>
<tr>
<td>Culicoides guittipennis (Coquillett)</td>
<td>11</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
</tr>
</tbody>
</table>
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