FACTORS INFLUENCING THE INGESTION OF *ONCHOCERCA CERVICALIS* MICROFILARIAE BY *CULICOIDES VARIIPENNIS*[1] (DIPTERA: CERATOPOGONIDAE)

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ABSTRACT. *Culicoides variipennis* were fed under controlled conditions on two ponies that exhibited seasonal changes in *Onchocerca cervicalis* microfilarial (mf) skin density and skin distribution. The seasonal changes did not radically affect mf ingestion. Flies were fed on the umbilicus of infected ponies by two methods: individual feeding in consecutive order at the same site, or by mass feeding. Linear regression analysis indicated that ingestion of microfilaria was independent of feeding time and engorged weight. In the individual feeding data, there was a trend toward an increased ingestion of mf as the feeding time increased. Mass feeding trials suggest early feedings by *C. variipennis* may influence mf ingestion rates of flies that feed on subsequent days. Of the 1,104 flies feeding on both ponies over a 2-year period, 220 (20%) ingested mf and 99 (9% of all engorged flies) ingested only one mf.

INTRODUCTION

Onchocerca cervicalis Railliet and Henry (Filariodea) is a parasite of horses that occurs worldwide. *Culicoides variipennis sonorensis* Wirth and Jones is an established laboratory vector for *O. cervicalis* (Collins and Jones 1978). In Louisiana, Foil et al. (1984) identified *C. variipennis* as a natural vector of *O. cervicalis.*

Seasonal variations in vector and parasite populations have been reported (Foil et al. 1987); changes in *O. cervicalis* microfilaria (mf) density were shown to correspond to changes in local population levels of *C. variipennis.* Similar associations have been observed in other members of the genus *Onchocerca*, e.g., *O. gutterosa* (Neumann) and the vector *Simulium ornatum* (Meigen) (Eichler 1973) and *O. volvulus* and the vectors *S. ochraceum, S. metallicum,* and *S. callidum* (Hashiguchi et al. 1981).

Studies concerning *O. cervicalis* mf ingestion by vectors were conducted in Great Britain (Mellor 1975). *Culicoides nubeculosus* Meigen were fed on the abdomen of a horse, and a positive correlation was found between the length of feeding time and the number of mf ingested. However, there was no correlation between "engorged weight" and feeding time or "engorged weight" and the number of mf ingested. Comparable results have been reported for *S. ornatum* feeding on the umbilicus of a cow (Eichler 1973). The most *O. gutterosa* mf were found in flies that fed the longest, and there was no correlation between "engorged weight" and feeding time.

Previous studies examining the influence of feeding time and engorged weight of vectors upon the ingestion of mf of *Onchocerca* species of livestock have involved feeding flies on one animal or over a short period of time. Furthermore, the effects of seasonal fluctuations of mf populations on the ingestion of mf by vectors have not been adequately investigated. This paper describes the results of feeding *C. variipennis* under controlled conditions on two ponies at different times of the year.

MATERIALS AND METHODS

Eggs of *Culicoides variipennis sonorensis*, furnished by the USDA Arthropod-Borne Animal Diseases Research Laboratory (ABADRL) in Laramie, Wyoming and the Delta Primate Center in Covington, Louisiana, were used to establish a laboratory colony at LSU. Eggs were placed in 26 x 20 cm Nalgene® plastic laboratory animal pans containing moist cotton and finely ground Purina® fly food. The colony was maintained at 21 ± 1°C and a 14L:10D photoperiod. Adults were collected daily upon emergence and maintained in 1 pint ice cream containers with a screen top; a piece of wet cotton and a cube of sugar were provided ad libitum. Anesthetized guinea pigs were the blood source and eggs were collected on a 5.5 cm oval of moistened Whatman® filter paper.

The ponies were maintained on pasture at the St. Gabriel Research Experiment Station, St. Gabriel, Louisiana. Biopsy, microfilarial quantification, and histology were done according to procedures described in Foil et al. (1987). Microfilariae observed in histology sections were assigned a location based on the division of the section into three layers: the outermost epidermis (RI), the adnexal structures (RII) and the region from the adnexal structures to the collagenous tissue layer (RIII) (Foil et al. 1987).

[1] Approved for publication by the Director of the Louisiana Agricultural Station as manuscript number 87-17-1504.

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Nulliparous flies were fed on the shaved umbilicus region of the ponies. An elevated stanchion, which incorporated a platform 77 cm above floor level with a lateral open area giving access to the ventral region of the ponies, was constructed. A feeding belt was fabricated out of a transparent sheet of plastic 60.9 x 20.3 cm, with elastic belts bolted to each end. Holes were melted into the plastic to accommodate sections of 9 mm diameter Tygon® tubing exposing a skin surface area of 63.6 mm^2. The belt was strapped onto the pony with the plastic portion lying tightly against the ventral surface of the pony. *Culicoides variipennis* were individually placed into the tubing and the open end was covered with a piece of moist cellucotton. Before feeding, the cellucotton was removed, and the feeding container was rapidly inserted into the hole in the plastic belt with the top of the container abutted against the skin. Intramuscular injections of a 3 cc mixture of Rompun® (xylazine hydrochloride) and acepromazine were often given to sedate the animals.

Fourteen assays were conducted in which flies were fed individually in sequence until 10 flies had obtained a blood meal. Initiation of feeding was considered to have occurred when the fly stopped probing and remained motionless for one minute, and cessation of feeding was when the mouthparts were withdrawn. After engorgement, the flies were knocked down by placing them in a −20°C freezer for 5 minutes and subsequently were weighed on a Mettler® H20 balance to the nearest 0.01 mg. This weight will be referred to as engorged weight in the remainder of the text. The engorged weight for *C. variipennis* fed on ponies is approximately 1.4 times greater than the unfed weight; this ratio varies with body size (unpublished data). Flies were then transferred to a glass slide, placed in a drop of saline, and the head, thorax, and abdomen were separated and teased apart. The slide was then examined at 100× magnification for mf which were usually alive and readily detectable.

For the mass feeding assays, a feeding cage was made by sewing six elastic straps to the top elastic band of a women's half slip and sewing a CDC light trap collection bag onto the bottom of the slip. The top of the slip was placed underneath the ventral aspect of the pony, and the straps were tied along the dorsum. A hole was made in the lower portion of the CDC light trap bag through which the flies could be introduced and removed. Flies were aspirated from the cage upon completion of the blood meal and dissected. Mass feedings were completed from August to October 1986 and in March 1987.

Statistical analyses of the data were performed using simple linear regression and analysis of variance (ANOVA) as described in Steele and Torrie (1980). For linear regression, r values smaller than the given r value = 0.632 for significance at the $P > 0.05$ level, df = 8, were not considered as being positively correlated (Table A.13, Steele and Torrie 1980). The large number of zero entries in the mass feeding database necessitated the square root transformation $(y + \frac{1}{2})^{\frac{1}{2}}$ of all values prior to ANOVA.

RESULTS

The distribution of mf for each of the three designated tissue layers (RI, RII, and RIII) was determined for ponies 507 and 504 over a period of 15 months, from September 1985 to December 1986. Generally, mf were concentrated in the RII and RIII tissue layers in the colder months and in the RI and RII layers during the warmer months. RII mf were present year-round in pony 507. No mf were observed in the August 1986 sections for both ponies. The mf density was greatest for both ponies in July 1986 and lowest in the winter, i.e., December 1986 for 507 and January 1987 for 504 (Table 1).

Although there was variance in the seasonal spatial distribution and density among the mf populations of the two ponies, mf ingestion was not radically different (Table 1). As the spring mf density increased in 504, the percent of flies ingesting mf increased; however, the percent of flies ingesting more than one mf did not increase. As the mf density decreased in 507 from August 1986 to March 1987, the percent of flies ingesting mf did not decrease, and the percent of flies ingesting 1 mf remained relatively constant (Table 1).

The distribution of the number of mf recovered from 758 flies from individual and mass feedings on the two ponies from March 1985 to March 1987, as well as from 346 flies fed during initial testing of the mass feeding device, is presented in Fig. 1. The distribution of number of mf recovered from flies is highly positive skewed.

There were 14 assays (5 in 1985 and 9 in 1986) in which 10 flies fed in consecutive order at the same site. A trend towards more mf ingested by the flies feeding later in the sequence was observed. The total numbers of mf ingested by the 14 flies fed in each temporal position were: no. 1, 4 mf; no. 2, 8 mf; no. 3, 5 mf; no. 4, 0 mf; no. 5, 4 mf; no. 6, 6 mf; no. 7, 10 mf; no. 8, 7 mf; no. 9, 7 mf; no. 10, 10 mf. No significant differences were observed for total numbers of mf among these 10 positions by ANOVA ($P > 0.05$).

Linear regression analysis was conducted for each of the 14 assays to examine dependence for feeding time on the number of mf ingested, engorged weight on number of mf ingested and

feeding time on the engorged weight. No correlation was observed by regression analysis for any of the 14 assays. Computed r values were smaller than the given r value of 0.632, df = 8, at the $P > 0.05$ level of significance (Steele and Torrie 1980).

The distribution of engorged weights for mass fed flies, and engorged weights and feeding times of flies ingesting mf in the individual feeding assays were compared. There was a trend toward an increased mean mf ingested as the feeding time increased (Table 2). However, no significant differences between feeding time or blood meal weight distributions for mean mf ingested could be detected by ANOVA ($P > 0.05$).

The number of *C. variipennis* engorging, number of flies ingesting mf, and mean number of mf ingested during mass feeding assays using 507 are presented in Table 3. In the initial eight feedings conducted from August 1986 to September 1986 on 507, the number of *C. variipennis* ingesting mf were greatest in the evening time periods. Of the 11 dates that flies were fed in 1986, September 9, 1986 was significantly different by ANOVA. The number of flies ingesting mf on March 7, 1987 was significantly higher than the other feeding periods in that month.

DISCUSSION

Seasonal studies. The seasonal changes observed in mf density and distribution of the two experimental ponies are consistent with a previous report that involved 15 Louisiana ponies (Foil et al. 1987). However, there was no indication that these seasonal changes affected mf ingestion (Table 1). Microfilariae were ingested by *C. variipennis* during individual and mass feedings for every month from March through October (Tables 1 and 3). Similarly, Eichler (1971) observed that *S. ornatum* feeding on a cow infected with *O. gutterosa* were able to ingest mf in all but the winter months of January, February, and March when no mf were detected in superficial skin layers. Unfortunately, our attempts to feed flies from December through February were not successful. Previous reports on *C. variipennis* in Louisiana indicated that wild populations are depressed from December through February (Foil et al. 1987).

Seasonal mf changes have been compared to

Table 1. Changes in *Onchocerca cervicalis* mf density in two ponies (507 and 504) from April 1986 to March 1987 and the number of *Culicoides variipennis* ingesting microfilariae (mf).

Date	mf/mg 507/504	# flies fed 507/504	# ingesting mf 507/504	# ingesting 1 mf 507/504
Apr 1986	NB[a]	10/11	0/1	0/1
May 1986	NB/3.04	−/10[b]	−/2	−/2
June 1986	1.83/10.27	9/10	1/1	0/0
July 1986	164.60/63.11	−/81[c]	−/28	−/6
Aug 1986	8.05/1.66	10/−	1/−	1/−
Sept 1986	3.88/2.20	320[c]/−	75[c]/−	34/−
Oct 1986	2.00/1.18	71[c]/−	15/−	10/−
Dec 1986	0.29/2.00	−/11	−/0	−/0
Jan 1987	2.85/0.38	−/−	−/−	−/−
Feb 1987	2.00/2.25	−/−	−/−	−/−
Mar 1987	2.20/0.83	215[c]/−	37[c]/−	24/−
Total		635/123	129/32	69/9

[a] N.B. = no biopsies taken.
[b] − = no attempt made to feed flies.
[c] Includes results from mass feedings.

Table 2. Results of individual and mass feeding assays: feeding time, engorged weight, and mean microfilaria (mf) ingested distributions of *Culicoides variipennis* ingesting *Onchocerca cervicalis* mf.

Time (in min)	# of flies ingesting mf	X ± SD mf ingested	Weight (in mg)	# of flies ingesting mf	X ± SD mf ingested
<3:00	0	0			
3:00–6:00	6	1.50 ± 0.8	0.20–0.29	2	1.50 ± 0.7
6:00–9:00	6	1.67 ± 0.8	0.30–0.39	14	1.86 ± 1.4
9:00–12:00	12	1.75 ± 1.2	0.40–0.49	13	2.46 ± 1.8
12:00–15:00	15	2.13 ± 2.1	0.50–0.59	22	2.40 ± 2.9
15:00–18:00	7	2.43 ± 1.8	0.60–0.69	14	2.86 ± 5.0
>18:00	8	3.25 ± 3.5	0.70–0.79	5	1.40 ± 0.5
			>0.80	3	1.33 ± 0.6

vector incidence which, in turn, has led to various hypotheses regarding a potential adaptive mechanism between parasites and vectors to enhance transmission (Hashiguchi et al. 1981, Foil et al. 1987). While earlier studies indicated a correlation between mf densities and vector populations (Hashiguchi et al. 1981, Foil et al. 1987), the effects of the seasonal changes upon mf ingestion by vectors were not investigated. The seasonal coincidence of mf and vector populations may be an adaptation to provide enhanced transmission during those times of the year with optimum environmental conditions. Alternatively, availability of mf for ingestion throughout most of the year could provide plasticity to adapt to different climatic conditions and vectors, allowing *O. cervicalis* access to new vector and equine populations.

Individual fly feedings. The lack of correlation between feeding time and engorged weight observed in this study is similar to reports from other studies on telmophagous flies (Mellor 1975, Eichler 1971). Similarly, lack of correlation between blood meal size and feeding time was reported by Jordan and Goatly (1962) working with the solenophagous *Culex pipiens fatigans (quinquefasciatus* Say).

While there was no statistical correlation between feeding time and numbers of mf ingested observed for *C. variipennis* feeding in succession at the same site in this study, data from individual feeding assays revealed that mean numbers of mf ingested increased with feeding time (Table 2). Similar results were reported by Mellor (1975) and Eichler (1971). Mellor hypothesized that tissue damage caused by the mouthparts of feeding flies could result in the passive movement of mf from surrounding tissues to the wound by fluid mechanics. Alternatively, a chemotactic factor released from the mouthparts could attract mf to actively migrate to the feeding site. However, flies that feed longer could ingest more interstitial fluids and consequently more mf before blood is released into the feeding pool from injured vasculature.

Data provided by the mass feeding assays indicates that previous *C. variipennis* feedings appear to influence the numbers of mf ingested in subsequent days of feeding (Table 3). The results of the August and September 1986 assays indicated that mf were more accessible to ingestion by *C. variipennis* during the evening. However, the experimental design failed to block for one variable, i.e., the effect of previous feedings upon subsequent *C. variipennis* feedings and mf ingestion. The large numbers of mf ingested by flies feeding in the evenings of September 9 and 10, 1986 may have been influenced by the previous six feedings (Table 3). Flies fed during the evening period in the October assays did not ingest more mf. In the March 1987 feedings, there was a statistically significant increase in the number of flies ingesting mf on the fourth day, suggesting that previous feedings could affect mf ingestion in subsequent feedings.

A similar phenomenon was noted in the individual feeding assays in a single day, in which flies feeding in positions 1–5 ingested a total of 21 mf, while flies in positions 6–10 ingested 40. Further mass feeding assays at different times of the year will be required to determine if previous feedings and/or periodicity influence

Table 3. Results of mass feedings of *Culicoides variipennis* on pony 507 from August 1986 to March 1987. The # flies engorging, # flies ingesting *Onchocerca cervicalis* microfilaria (mf), and mean number mf ingested for each assay are given.

Date	Time	# Flies engorging	# Flies ingesting mf	mf/fly ± SD
Aug 27, 1986	1340–1440	43	10	1.70 ± 5.77
Sept 3, 1986	0730–0830	41	6	0.46 ± 1.42
Sept 4, 1986	2250–2350	51	8	0.41 ± 1.50
Sept 6, 1986	0730–0830	39	5	0.15 ± 0.43
Sept 7, 1986	2250–2350	31	4	0.23 ± 0.67
Sept 8, 1986	1340–1440	35	7	2.17 ± 7.17
Sept 9, 1986	1910–2010	43	21[a]	2.28 ± 1.54
Sept 10, 1986	1910–2010	37	14	1.54 ± 3.30
Oct 1, 1986	1310–1410	21	5	0.57 ± 1.57
Oct 2, 1986	1910–2010	22	2	0.36 ± 1.50
Oct 5, 1986	2250–2350	22	8	0.54 ± 1.10
Mar 4, 1987	1430–1630	61	6	0.11 ± 0.37
Mar 5, 1987	1430–1630	31	5	0.32 ± 0.91
Mar 6, 1987	1430–1630	46	5	0.13 ± 0.40
Mar 7, 1987	1430–1630	71	21[b]	3.23 ± 4.67

[a] Statistically significant at the 0.05 level from the other dates in August and September 1986.
[b] Statistically significant at the 0.05 level from the other dates in March 1987.

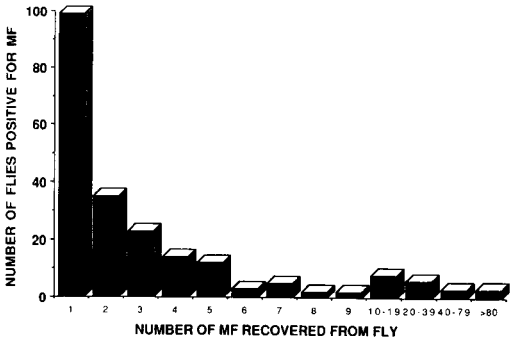

Fig. 1. Distribution of the number of *Onchocerca cervicalis* microfilariae (mf) recovered from *Culicoides variipennis* fed on ponies 507 and 504 from March 1985 to March 1987.

mf ingestion. If the trend of previous feeding contributing to increased ingestion of mf is reproduced in future studies, a mechanism that contributes to the seasonal correlation between vector populations and mf density measured by biopsy techniques could be described. However, the influence of vector feeding on the occurrence of mf at the feeding site would likely be only part of the seasonal phenomena since periodic reproduction of *O. volvulus* and *Wehrdikmansia cervipedis* have been described (Schulz-Key and Karam 1986, Weinmann et al. 1973).

Some conclusions regarding the transmission of *O. cervicalis* by *C. variipennis* can be drawn from mf distribution data for all flies used in both individual and mass feeding studies. The distribution of mf recovered from engorged flies is positively skewed, with the majority of flies (99, 45%) ingesting one mf (Fig. 1). The ingestion of large numbers of mf has been shown to contribute significantly to increased mortality in *C. nubeculosus* and *C. variipennis* (Mellor 1975), *Simulium ochraceum* (Collins et al. 1977), and *Culex quinquefasciatus* (Jordan and Goatly 1962). Therefore, flies ingesting small numbers of mf are more likely to survive and support development of mf to the infective L3 stage. In this study, those flies ingesting one mf constituted 9% of the total of 1100 flies that blood fed on the two ponies. For all flies feeding on pony 507, 10% ingested one mf and for all flies feeding on pony 504, 7% ingested one mf. Interestingly, the percentage of *C. variipennis* harboring infective L3 stage larvae has been reported as 7% for wild populations in Louisiana (Foil et al. 1984), and 7.3% for flies fed on an infected horse (Mellor 1975).

Culicoides variipennis has been described as a competent natural vector of *O. cervicalis* (Foil et al. 1984). There are factors that appear to contribute to this vector status. *Culicoides variipennis* is a large fly capable of surviving the development of this filarid. Also, it is a telmophagous species that feeds on the ventral midline of equids where the extravascular mf density is greatest (Rabalais and Votava 1974). In Louisiana, *C. variipennis* population density is maximum when mf density is greatest in equids (Foil et al. 1987). This study provides additional information that a high percentage of the flies that ingest mf ingest a low number of mf and that feeding flies may influence the occurrence of mf available for ingestion.

ACKNOWLEDGMENTS

The authors would like to thank Greg Hunt and Dr. Fred Holbrook of the USDA-ARS in Laramie, Wyoming, and Dr. Robert Lowrie of the Delta Primate Center in Covington, Louisiana for providing both *Culicoides* eggs and assistance in establishing the colony. W. V. Adams, Jr., Melanie Chapman, Dr. Fred Enright, Louis Fitzmorris, Dr. Denny French, Dr. James Fuxa, Laurie Issel, Martin Klass, Dr. Daniel Leprince, and William Roper also made technical contributions to the study.

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