# SAMPLING WITH LIGHT TRAPS AND HUMAN BAIT IN EPIDEMIC FOCI FOR EASTERN EQUINE ENCEPHALOMYELITIS VIRUS IN SOUTHEASTERN MASSACHUSETTS

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ABSTRACT. To estimate human exposure to potential vectors of eastern equine encephalomyelitis (EEE) virus, we compared collections of putative EEE virus vectors from human biting collections with collections from CDC, AB (American Biophysics<sup>®</sup>), and New Jersey light traps and resting boxes in enzootic/epidemic foci of EEE virus in southeastern Massachusetts. Human biting collections caught significantly more host-seeking females than resting boxes or unbaited light traps (P < 0.05). Regression analysis of human biting collections to AB traps supplemented with CO<sub>2</sub> could predict 60-70% of the actual human biting risk by *Aedes* and *Coquillettidia perturbans*. The AB traps supplemented with CO<sub>2</sub> and octenol could predict 65% of Anopheles biting risk. No single sampling method was accurate for predicting human biting risk by *Culex salinarius*, and no method could predict human biting risk by all potential vectors surveyed.

## **INTRODUCTION**

Human biting collections directly measure the biting rate of a vector population responsible for disease transmission; however, biting collections are time intensive, inconvenient, costly, difficult to standardize, and pose health risks to the collector. Thus, light traps generally are used as surrogates to sample host-seeking females. Light trap collections can be compared with biting collections to evaluate how accurately they reflect actual human biting risk.

Previous comparisons between light traps and human biting risk have yielded inconsistent results. Slaff et al. (1983) found that a CDC trap with  $CO_2$ best reflected human biting risk, whereas a New Jersey (NJ) trap underestimated species diversity and biting risk. Acuff (1976) collected more mosquitoes with NJ light traps and  $CO_2$ -baited CDC traps than with human biting collections.

Odetoyinbo (1969) first studied unbaited CDC light traps for sampling malaria vectors in Africa. Working in Tanzania, Davis et al. (1995) collected 1.23 times the number of *Anopheles* with indoor CDC traps vs. human bait collections, and Lines et al. (1991) found 3 indoor light traps collected as many *Anopheles* and *Culex* as 2 human volunteers. Light traps underestimated actual biting risk when *Anopheles* abundance was either high or low (Mbogo et al. 1993).

Aedes canadensis (Theobald), Aedes vexans (Meigen), Coquillettidia perturbans (Walker), Anopheles quadrimaculatus Say, Anopheles punctipennis (Say), and Culex salinarius Coquillett are potential vectors of eastern equine encephalomyelitis (EEE) virus from its avian forest cycle to horses and humans in mid- to late summer in southeastern Massachusetts (Edman et al. 1993, Vaidyanathan et al. 1997). *Culiseta melanura* (Coquillett), a bird-feeding mosquito, transmits EEE virus to passerine birds in permanent, forested swamps in summer, thereby maintaining the enzootic foci. Its narrow host range precludes it from transmitting EEE virus to humans and horses.

The CDC light traps, either unbaited or supplemented with  $CO_2$ , have collected *Ae. canadensis*, *Ae. vexans, Cq. perturbans*, and *Cx. salinarius* (Schreck et al. 1972, Howard et al. 1988, Buckley et al. 1994). Artificial dark shelters have been better for collecting *Anopheles* spp. (Morris 1981). We compared CDC and NJ light traps, resting boxes, and a new light trap structurally similar to the CDC trap manufactured by American Biophysics<sup>®</sup> (Jamestown, RI). These traps were compared with human biting collections to determine which surrogate method could best assess risk of EEE virus transmission to humans in Massachusetts.

### MATERIALS AND METHODS

Test sites: Bristol and Plymouth counties in southeastern Massachusetts account for 30,000 acres of the Hockomock Swamp, a red maplewhite cedar freshwater swamp in the Taunton River basin enzootic for EEE virus and Cs. melanura. Four sites were selected in towns along the Hockomock margin (Raynham, Taunton, Easton, and East Bridgewater) with histories of light trap collections of potential vector species and isolations of EEE virus (A. Decastro, Bristol County Mosquito Control, personal communication). Selected sites were near vernal pools, forested swamps, and cattail marshes, which provided breeding habitats for most potential epidemic vectors of EEE virus. Each site was also within 500 m of homes or horse farms. Forested swamps contained white cedar (Chamaecyparis thyoides), swamp oak (Quercus bicolor), maples (Acer spp.), alders (Alnus spp.), willows (Salix spp.), and poison ivy (Rhus radicans).

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	Biting	CO <sub>2</sub>	CO <sub>2</sub> +octenol	CDC	Octenol	Flicker	Steady	NJ	Rest
All spp.	125	74	55	24	15	7	4	4	3
Aedes	92	45	35	10	11	4	1	1	0
Ca. pert	26	21	8	6	1	1	1	1	0
Cx. sali	5	5	8	5	2	1	1	2	0
Anophis	3	3	5	1	1	0	0	0	6

Table 1. Nightly means for 9 sampling methods for mosquitoes in southeastern Massachusetts, 1994 and 1995.1

<sup>1</sup> Aedes = Aedes canadensis, Ae. vexans, Ae. stimulans s.l., Ae. abserratus, Ae. triseriatus, Ae. trivitattus, Ae. aurifer; Cq. pert = Coquillettidia perturbans; Cx. sali = Culex salinarius; Anophls = Anopheles punctipennis, An. quadrimaculatus, An. walkeri.

Total numbers for Ae. canadensis and Ae. vexans were low. Because Ae. canadensis breeds in vernal woodland pools and Ae. vexans breeds in poorly drained alluvial floodplains, we included other univoltine vernal pool Aedes, such as Ae. stimulans (Walker) s.l., and floodplain species, such as Aedes trivittatus (Coquillett), in the analysis.

Experimental design: Trapping methods included CDC miniature light traps (Sudia and Chamberlain 1962), NJ light traps (Headlee 1932), resting boxes (Edman et al. 1968, Nasci and Edman 1981), and American Biophysics<sup>®</sup> (AB) light traps. The AB traps can be set with either a steady or flickering light source and are designed to be easily supplemented with CO<sub>2</sub> or octenol or both.

Along the margin of each site, the following 7 light traps were suspended 1.5 m above the ground at sunset and emptied after sunrise: CDC light trap, NJ light trap, AB steady light, AB flicker light, AB flicker+CO<sub>2</sub>, AB flicker+octenol, AB flicker+CO<sub>2</sub>+octenol. At each site, traps were operated at 8 intrasite locations for about 2 wk per site, June–September, 1994 and 1995. Traps were placed 5 m from one another in a Latin-square design; positions were changed nightly. Traps were suspended along uniform edge vegetation in an attempt to maximize site similarity. Sites were selected so the prevailing wind direction was perpendicular to the trap line.

Octenol was emitted at 0.5 mg/h by octenol packets suspended on the light trap. Octenol was contained in a glass vial within a permeable membrane; crushing the vial released octenol. Carbon dioxide was either delivered as 5–10 lb dry ice (1994) or emitted at 400–500 ml/min from a  $CO_2$  tank (1995), an emission rate equivalent to that of a resting adult human.

Human biting collections were made on one individual (R.V.) stationed at least 20 m from the nearest light trap. Biting collections were made from sunset to 2 h after sunset, June–September, 1994 and 1995. Mosquitoes landing on the host were collected with a hand-held aspirator or with an aerial net if they avoided the aspirator.

During August only, 3 resting boxes were placed within the forest facing the forest margin. Resting boxes were emptied daily by 10 a.m. using a backpack battery-powered aspirator (Nasci and Edman 1981).

Data analysis: Mosquitoes were returned to the laboratory and killed at -20°C; females were identified to species. Because sampling methods violated the assumption of random distribution, they were compared with one another using a Friedman's 2-way nonparametric ANOVA ( $\alpha = 0.05$ ) to determine if there was any difference among methods (NH Analytical Software 1985). If difference was detected, methods were compared pairwise using the Wilcoxon signed rank test to identify which method, if any, was significantly better. If results from the Wilcoxon test found that the biting collection and another method were not significantly different, the nightly catches (x) were transformed to y = log(x + 1) and compared using an unweighted least squares linear regression to calculate the probability of the 2nd method to estimate biting risk. If any trap experienced mechanical failure, corresponding data for all traps for that night were excluded from ANOVA and rank test analysis. Data from stormy nights were also excluded.

#### RESULTS

All species combined: In 1994 and 1995, a total of 9,938 females were collected with 9 sampling methods over 38 nights. Human biting collections had the greatest nightly mean (125), which accounted for 45% of the total catch (P < 0.05; Table 1). The number of mosquitoes taken in the AB flicker+CO<sub>2</sub> light trap did not differ significantly from that in the biting collection (P = 0.20); all other traps took significantly less than the biting collection (Table 2). A linear regression analysis between biting collection and flicker+CO<sub>2</sub> resulted in a coefficient of determination,  $r^2 = 0.612$ , which indicates that this method cannot predict human biting risk; only 61.2% of total variation in biting risk is explained by the regression equation. There was a 78.2% correlation between flicker+CO<sub>2</sub> and human biting risk (Table 3); therefore, although flicker+ $CO_2$  cannot predict human biting risk, it can provide a fair estimation (Fig. 1).

*Trap specificity:* A nightly mean of 92 female *Aedes* was collected from human bait (Table 1). There was no significant difference between biting collection and flicker+CO<sub>2</sub> ( $\bar{x} = 45$ , P = 0.45) or flicker+CO<sub>2</sub>+octenol ( $\bar{x} = 35$ , P = 0.59). Biting collections were significantly better than CDC ( $\bar{x} = 45$ )

Table 2. Pairwise comparisons using the Wilcoxon signed rank test between sampling methods for all species.

Comparison of methods	<i>P</i> -value
Significant difference present (method > trap)	
Biting collection vs. resting box	0.01
Biting collection vs. CDC	0.00
Biting collection vs. flicker+ $CO_2$ +octenol	0.01
No significant difference	
Flicker+CO <sub>2</sub> vs. biting collection	0.20

10, P < 0.05), flicker+octenol ( $\bar{x} = 11$ , P < 0.05), and flicker ( $\bar{x} = 4$ , P < 0.05). Table 3 lists the values of r and r<sup>2</sup> for biting collection, flicker+CO<sub>2</sub>, and flicker+CO<sub>2</sub>+octenol for female Aedes. Plotting log-transformed biting collection and flicker+CO<sub>2</sub> data against each other (Fig. 2) shows a high correlation between the two sampling methods (r = 0.782).

A nightly mean of 26 female Cq. perturbans were collected from human bait (Table 1). Biting collections correlated best with flicker+CO<sub>2</sub> ( $\bar{x} =$ 21, P = 0.35). The number of Cq. perturbans from biting collections was not significantly higher than the number from flicker+CO<sub>2</sub>+octenol (P = 0.06) or CDC (P = 0.05) collections but was significantly higher than the number from flicker (P < 0.05) and flicker+octenol (P < 0.05) collections. Table 3 gives the r and r<sup>2</sup> values for Cq. perturbans biting collection against flicker+CO<sub>2</sub> alone. Plotting logTable 3. Sample correlation coefficient, r, measures the strength of the linear relationship between trap catch (Y

= log[catch + 1]) and human biting collection (X = log[catch + 1]). Sample coefficient of determination,  $r^2$ , measures the closeness of fit of the calculated values of trap catch to observed values of biting collection.

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Trap vs. biting collection	r	r <sup>2</sup>
All species combined	<u></u>	
Flicker+CO <sub>2</sub>	0.782	0.612
Aedes spp.		
$Flicker + CO_2$	0.833	0.694
Flicker+CO <sub>2</sub> +octenol	0.785	0.616
Coquillettidia perturbans		
Flicker+CO <sub>2</sub>	0.795	0.632
Culex salinarius		
Flicker+CO <sub>2</sub>	0.403	0.163
CDC	0.427	0.182
Anopheles spp.		
$Flicker+CO_2+octenol$	0.808	0.653

transformed biting collection and flicker+CO<sub>2</sub> data against each other (Fig. 3) shows a high correlation between the two sampling methods (r = 0.795).

Flicker+CO<sub>2</sub>+octenol traps collected an average of 8 female *Cx. salinarius* per night, the highest for 9 sampling methods (P < 0.05; Table 1). Biting collections were not significantly different from CDC ( $\bar{x} = 5$ , P = 0.55), flicker+CO<sub>2</sub> ( $\bar{x} = 5$ , P =0.78), or flicker+CO<sub>2</sub>+octenol ( $\bar{x} = 8$ , P = 0.44)



Fig. 1. Log[total catch for all species for flicker+CO<sub>2</sub> + 1] plotted against log[human biting collection + 1]. The diagonal line shows the predicted relationship between the 2 sampling methods. The sample coefficient of determination,  $r^2 = 0.612$ , measures the closeness of fit of the sample regression equation of the observed nightly trap catch to human biting collection.



Fig. 2. Log[Aedes catch for flicker+CO<sub>2</sub> + 1] plotted against log[Aedes human biting collection + 1]. The diagonal line shows the predicted relationship between the 2 sampling methods. The sample coefficient of determination,  $r^2 = 0.694$ , measures the closeness of fit of the sample regression equation of the observed nightly trap catch to human biting collection.

collections. Biting collection was significantly different from NJ, resting box, and AB steady light trap collections (P < 0.05). Table 3 lists r and  $r^2$ values for Cx. salinarius biting, CDC, and flicker+CO<sub>2</sub> collections. Plotting log-transformed biting collection and CDC light trap data against each other (Fig. 4) shows a low correlation between these two sampling methods (r = 0.427).

An average of 6 female Anopheles were collected nightly from resting boxes, the highest for 9



Fig. 3. Log[Coquillettidia perturbans catch for flicker+CO<sub>2</sub> + 1] plotted against log[Coquillettidia perturbans human biting collection + 1]. The diagonal line shows the predicted relationship between the 2 sampling methods. The sample coefficient of determination,  $r^2 = 0.632$ , measures the closeness of fit of the sample regression equation of the observed nightly trap catch to human biting collection.



Fig. 4. Log[*Culex salinarius* catch for CDC light trap + 1] plotted against log[*Culex salinarius* human biting collection + 1]. The diagonal line shows the predicted relationship between the 2 sampling methods. The sample coefficient of determination,  $r^2 = 0.183$ , measures the closeness of fit of the sample regression equation of the observed nightly trap catch to human biting collection.

sampling methods (Table 1). Resting boxes were significantly better than biting collection (P < 0.05). Flicker+CO<sub>2</sub> ( $\bar{x} = 3$ , P = 0.14) and flicker+CO<sub>2</sub>+octenol ( $\bar{x} = 5$ , P = 0.23) collections were not significantly different from human biting collection ( $\bar{x} = 3$ ). Table 3 lists the r and r<sup>2</sup> values for biting collection against flicker+CO<sub>2</sub>+octenol; Fig. 5 plots log-transformed biting collection and flicker+CO<sub>2</sub>+octenol data against each other, showing a high correlation between these two sampling methods (r = 0.808).

### DISCUSSION

Linear regression equations were calculated for all traps, but Altman and Bland (1983) gave three reasons why predictions from a linear regression may be inaccurate. First, biting collections and light trap catches are prone to sampling error, which is contrary to the assumptions of the regression calculation. Second, light traps may be more or less efficient at high mosquito densities, and a linear regression is inappropriate considering the logarithmic scale. Third, the confidence limits of the regression coefficient are accurate for the range of densities for which data are available; equations cannot be used to extrapolate biting collections with different habitats and different collectors. Therefore, we present and discuss the sample correlation coefficient, r, which measures the strength of the linear relationship between trap catch and human biting collection, and the sample coefficient of determination,  $r^2$ , which measures the closeness of fit of the calculated values of trap catch to observed values of biting collection.

Traps were operated ca. 2 wk at each site; some variation was caused by trap failure and bad weather. Trap locations within each site were changed every night to equalize all treatments and minimize possible trap interference resulting from CO<sub>2</sub> currents. The level of CO<sub>2</sub> used was equivalent to that of a resting human, insufficient to attract most mammalophagic species beyond 5-10 m (Mogi and Yamamura 1981). In addition, each site was adjacent to a large body of water (lake or marsh), so prevailing winds during the night were generally cross winds, further minimizing trap interference resulting from CO<sub>2</sub>. Traps were compared with each other at 4 sites, and rankings remained the same despite different locales, weather changes, and changes in species composition from site to site.

Biting collections sampled the greatest mean number of host-seeking females, but they are impractical for long-term sampling because of time, cost, inconvenience, and risk of disease transmission to the collector. Temperature and light, geography, microhabitat, time of year, time of evening, and mosquito abundance all influence how well mosquito traps reflect actual human biting risk. The AB light traps supplemented with CO<sub>2</sub> collected an average of 74 females per night (Table 1). This overall mean was the highest for 8 traps and closest to actual human biting numbers (P = 0.20,  $r^2 =$ 0.612). We supplemented only AB light traps with CO<sub>2</sub> or octenol because human biting collections previously have been compared with CDC traps





Fig. 5. Log[Anopheles catch for flicker+CO<sub>2</sub>+octenol + 1] plotted against log[Anopheles human biting collection + 1]. The diagonal line shows the predicted relationship between the 2 sampling methods. The sample coefficient of determination,  $r^2 = 0.654$ , measures the closeness of fit of the sample regression equation of the observed nightly trap catch to human biting collection.

with  $CO_2$  (Acuff 1976, Slaff et al. 1983). In addition, AB traps are relatively new and have not been compared with human biting collections.

In previous studies, NJ light traps were found to underestimate (Slaff et al. 1983) or overestimate (Acuff 1976) biting risk, depending on the species. We found NJ traps poorly correlated with biting risk (P < 0.05). Unbaited AB flicker, AB steady, and flicker+octenol traps also were poor indicators of human biting risk (P < 0.05).

Biting risk from Aedes spp. was predicted best by the addition of  $CO_2$  to AB light traps. The individual regression model for Aedes spp. ( $r^2$  = 0.694) cannot be used to predict risk of human EEE infection because it includes several species not implicated in EEE virus transmission. Although one cannot predict absolute biting risk from trap catch, the high r-value (0.833) suggests that an AB flicker light trap supplemented with  $CO_2$  samples 83.3% of the same population as biting collection. The Aedes spp. analysis included univoltine vernal pool species, which emerge in May and persist until midsummer, and multivoltine floodwater species, which emerge when heavy summer rains reflood their alluvial larval habitats. This analysis primarily assessed the nuisance mosquito problem (Ae. stimulans s.l., Aedes aurifer (Coquillett), Aedes cinereus Meigen, and Aedes abserratus (Felt & Young) are persistent, painful biters in southeastern Massachusetts); in addition, Aedes triseriatus and Ae. trivittatus are important vectors of other arboviruses.

Because we suspected that the value of the re-

gression model for all Aedes spp. ( $r^2 = 0.694$ ) had been decreased because of the extra species added in the analysis, we ran an independent analysis for Ae. canadensis (but not for Ae. vexans, which had record low numbers in 1994 and 1995). Our results agree with previous studies (Howard et al. 1988, Buckley et al. 1994) where light traps supplemented with CO<sub>2</sub> were excellent for sampling Ae. canadensis. There was no significant difference between biting collection and flicker+CO<sub>2</sub> (P = 0.22) or flicker+CO<sub>2</sub>+octenol (P = 0.50). However, neither flicker+CO<sub>2</sub> ( $r^2 = 0.298$ ) nor flicker+CO<sub>2</sub>+octenol  $(r^2 = 0.331)$  traps could predict reliably human biting risk from Ae. canadensis any better than the overall model. Aedes canadensis tends to feed on smaller mammalian hosts along forest margins. Flicker+CO<sub>2</sub> and flicker+CO<sub>2</sub>+octenol traps placed along the forest margin collected more host-seeking Ae. canadensis than a 2-h biting collection in the same locale. This bias in sampling methods contradicts the assumptions of normalcy inherent in linear regression models and may explain why biting risk from this species is difficult to predict from light trap collections.

Biting risk from Cq. perturbans was predicted best by additions of  $CO_2$  to AB light traps. The linear regression model for predicting overall biting risk from flicker+CO<sub>2</sub> traps yielded an  $r^2 = 0.612$ , meaning that only 61.2% of total variation in biting risk (y) is explained by the regression equation (Fig. 3). The individual regression model for Cq. perturbans ( $r^2 = 0.632$ ) collected from flicker+CO<sub>2</sub> traps may estimate risk of human EEE infection better than the overall model or the *Aedes* model. The sample correlation coefficient (r = 0.795) suggests a strong linear relationship between the populations sampled by flicker+CO<sub>2</sub> and by biting.

Coquillettidia perturbans, a documented vector of EEE virus (Boromisa et al. 1987, Vaidyanathan et al. 1997), is abundant in EEE virus enzootic foci throughout July and feeds readily on humans and horses. Light traps may underestimate Cq. perturbans abundance in July when it is at its peak. Because human EEE cases in Massachusetts generally do not appear before the end of August, when Cq.perturbans populations are quite low, our model may be useful for predicting risk of human EEE infection by Cq. perturbans populations that persist into late summer.

Although biting risk from Cx. salinarius was predicted best by an unbaited CDC light trap ( $r^2$  = 0.182), the low  $r^2$  value suggests that the regression equation is a poor indication of human biting risk (Fig. 4). This sampling error leads to an underestimation of the slope of the regression line and overestimation of the y-intercept (Altman and Bland 1983). Light traps often overrepresented human biting risk by Cx. salinarius, suggesting a curvilinear relationship between light trap efficiency and high mosquito density. The application of a linear regression model to light trap data during high Cx. salinarius population density would overestimate biting risk. Regression analyses of CDC light trap (P = 0.55) and flicker+CO<sub>2</sub> trap (P = 0.78) suggests they are poor indicators of Cx. salinarius biting. However, yields from these 2 traps were not significantly different from those of human biting collection. Although nightly trap data among the various traps were highly variable and poor indicators of human biting risk, the CDC and flicker+CO<sub>2</sub> light traps tend to sample host-seeking females and still may be useful for detecting peak periods of Cx. salinarius biting activity. Culex salinarius feeds principally on domestic mammals, but it is opportunistic and may feed equally on birds and mammals (Edman 1974). Females feeding well after sunset are more likely to encounter light traps operated all night than to be sampled in a 2-h, postsunset biting collection.

Resting boxes best sampled Anopheles spp., primarily blood-engorged or parous females. Because these individuals have already fed and are not actively host seeking, the regression model based on resting box collections may give an inaccurate profile of host-seeking intensity and human biting risk. Therefore, estimations of host-seeking Anopheles collected from flicker+CO<sub>2</sub>+octenol traps ( $r^2$  = 0.653) are superior to resting box collections for estimating biting risk. There was no significant difference in Anopheles spp. catch between flicker+CO<sub>2</sub> alone and flicker+CO<sub>2</sub>+octenol (P >0.05). Either method is effective when populations are sampled during peak activity; otherwise, they underestimate biting risk.

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