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LETTER TO THE EDITOR

ABSTRACT. This letter questions the appropriateness of methodology used in a study by Howard and Oliver (*J. Am. Mosq. Control Assoc.* 13:315–325; 1988). Two independent data sets, collected for different purposes by 2 different groups, were subjected to statistical analysis to determine if the data sets differed. The experimental "design," as described by the authors, is an example of pseudoreplication, which arises when replicates are collected at a scale finer than the one for which conclusions of statistical testing are intended to be drawn. All of the components of a properly designed field experiment (control, replication, randomization, and interspersion) are missing from this study. The authors proceed to draw a series of conclusions from the data presented. Few, if any, of the conclusions can be supported by the evidence presented. The assertions put forward in this paper could have a severe negative impact on efforts to prevent transmission of arboviruses or other pathogens to humans and domestic animals.

KEY WORDS Vector control, naled, eastern equine encephalomyelitis, *Culiseta melanura,* experimental design, pseudoreplication

Because I am identified as having "reviewed a previous draft," of the paper by Howard and Oliver (1998), I wish to make several comments about that work. My comments relate to the appropriateness of the methodology and experimental design and the relation between conclusions drawn and the data presented.

1. Methods were not appropriate to the question being asked: In this report, 2 independent data sets, collected for different purposes by different groups, were subjected to statistical analysis to determine if the data sets differed. I pointed out several of the problems in my review of the earlier version of the manuscript, and I do not see that those problems have been corrected. The experimental "design," as described by the authors, is an example of pseudoreplication (Hurlbert 1984). Pseudoreplication arises when replicates are collected at a scale "finer than the one for which conclusions of statistical testing are intended to be drawn" (Dutilleul 1993). All of the components of a properly designed field experiment (control, replication, randomization, and interspersion) are missing.

There were no control sites (i.e., sites that were never treated; Howard and Oliver 1998, p. 317). A 3-year period in which Toad Harbor was not treated is taken to be an indication of "nontreatment population trends of the species of interest. . . ." This can hardly be called a proper control.

There was no replication. Each site basically consisted of a single replicate, sampled multiple times at several locations (pseudoreplicates) within the replicate. A properly replicated study would have followed trends in at least 4–6 swamp areas, with the areas being randomly allocated to either treatment or untreated control status (hence, the randomization quality is also missing). It is important to point out that such an experiment is seldom possible because of the ethical dilemma of not treating an area in the event of an arbovirus outbreak. Finally, there is no interspersion of treated and untreated areas, which would help to overcome the natural variability between swamps. The authors state (p. 321) that "the 2 swamps are remarkably similar." Although this may be true at some spatial scales, it is quite untrue at others. From the air in midsummer, the 2 areas are visibly different, suggesting some underlying ecological differences that might impact the eastern equine encephalomyelitis (EEE) system.

Although the general linear model was probably the best choice for the statistical analysis, I doubt seriously that even the most robust analysis can overcome the basic flaws in the structure of this study.

Additional basic problems exist in the data used in the study. For example, the authors attempted to study the duration of impact of naled application on EEE vectors. Unfortunately, at least at Cicero Swamp, there seems to have been a cessation of trapping on the nights immediately following spraying. In all of the sequences I have examined, there is a gap of several days with no data (apparently because trap collections were too low to make it worthwhile). Although it is possible to confirm that a reduction occurred and that the reductions lasted no more than 1–2 wk, little else can be said (but, see my comments below [2.a.]).

2. The conclusions do not follow from the information presented. The authors offer the following conclusions from their analysis:

a. Application of naled achieved short-term reductions of the 4 vector species and seasonal reductions in the 3 univoltine species, but no long-term impact was observed.

b. The 15-fold increase in *Culiseta melanura* and 83-fold decrease in *Culiseta morsitans* are attributed to multiple long-term impacts of naled.

c. And, finally, "The possibility that applications of naled contributed to increased populations of *Cs. melanura* discredits the rationale that preventive applications of naled reduce the risk of EEE."

I offer the following observations and comments on the conclusions:

a. Naled clearly achieved short-term reductions of the 4 vector species. I am not sure that all of the

seasonal reductions (and the 1 multiyear reduction) are due to naled, but it is certainly a possibility. However, weather factors should have been taken into account as well. It is not clear to me why the authors expected to see a long-term (multiyear) impact from the use of such a short-acting pesticide. Their comments seem to indicate confusion between a philosophy of disease prevention and one of disease eradication.

Theoretically, if the basic reproductive rate of a disease (R_0, the average number of secondary infections attributable to a single infectious case introduced into a fully susceptible population; Fine et al. 1982) is driven below 1.0, transmission will cease. Although the value of R_0 for EEE is not known, it is probably fairly low because of the small number of cases per year in humans and domestic animals. If this is the case, then it seems likely that even an interruption of 1 wk might be sufficient to prevent widespread transmission. If only the "bridge vectors" are of concern (i.e., we do not worry about what happens to the enzootic cycle), the interruption seems to be significantly longer on the basis of the data in this paper. This may be a risky strategy if *Cs. melanura* plays a significant role in dispersing EEE virus outside the swamp habitats (see below).

On the basis of a small study conducted by one of my students, the rapid return of *Cs. melanura* at Cicero Swamp appears to be because of immigration from surrounding areas (T. Welch et al., unpublished research). In this study, female *Cs. melanura* from collections before and after the application of naled in Cicero Swamp were dissected, and the ovaries were examined to determine parity. Our hypothesis was that the returning population would be largely composed of newly emerged nullipars because ultra low volume (ULV) has no effect on immatures. An alternate hypothesis is that the population is replaced through immigration. No significant difference in parity occurred between pre- and postspray populations. Thus, apparently migration is an important factor in the rapid return of *Cs. melanura* following spraying. In fact, Howard et al. (1996) documented the importance of dispersal in moving "from swamp to upland areas and between swamp complexes." Thus, focusing on a larger area than just Cicero and Toad Harbor swamps may be important if an EEE prevention program is to be effective. Knowing whether or not the EEE enzootic cycle is being maintained in other areas besides Toad Harbor and Cicero swamps is important. Are there outlying foci that can re-seed the primary foci following control? The data presented by Howard et al. (1996) suggest that either multiple foci exist or *Cs. melanura* (and/or bridge vector) females fly very long distances.

b. A basic tenet of science is that correlation does not prove causation. There is absolutely no reason, on the basis of the data presented, to conclude that the observed changes in the 2 species resulted from the application of naled. The authors mention, but quickly discard, several possible alternative hypotheses (the original review panel raised several of these hypotheses). Only conducting experiments in such a way that they can be falsified can eliminate the alternate hypotheses. The authors suggest, for example, that beavers are unlikely to have had an impact in Cicero Swamp, but beavers can have a massive impact on freshwater habitats (see, e.g., Naiman et al. 1988, Langston 1998). The authors state that, if rising water levels (from beaver activity) were a factor, "one would expect that the same factor would influence population levels of *Cq. perturbans*. . . ." Unless their fig. 1 is mislabeled, this is exactly what is shown by the data. It seems to me that there is an opportunity for a very interesting study that is being missed.

Similarly, the "exponential increase in *Cs. melanura*" and concurrent "significant reduction" in *Cs. morsitans* are, at this point, observations of change in the numbers of 2 species. These changes may have resulted from interspecific interactions, or they may not. In fact, much of the authors' case rests on only a single year, 1993, which severely skews the longer term trend.

At this point, one would be hard pressed to decide if this was a pattern or simply random variation. Another possibility that the authors do not mention is the impact of interannual/decadal climate patterns. These patterns are known to have a large impact on vectors and vector borne disease.

c. Conclusions in science should be based on data gathered with an appropriate experimental design and interpreted after an appropriate statistical analysis. Presentation of an unfounded "possibility" as a basis for a conclusion (other than that additional study is indicated) is not warranted. In this article, the authors attempt to discredit the use of a public health tool (ULV adulticiding with naled) by extrapolating a "possibility" from inappropriate data and a poor study design.

The authors imply that there is sufficient evidence to conclude that the mosquito *Cs. melanura* is becoming more predominant because of the spraying. Because of the deficiencies outlined above, concluding that such a relationship exists is impossible. Although a relation between spraying with naled and the apparent increase in *Cs. melanura* cannot be proved on the basis of the data presented, that possibility also cannot be excluded by using these data.

One could ask if it is worthwhile (or even possible) to design and conduct an experiment that would actually answer the question posed by Howard and Oliver. I think it would be worthwhile, but there are a number of hurdles to overcome. A properly designed study, one that would overcome the deficiencies of the present study, would be expensive to design and carry out. In addition, there may be ethical questions if some areas are to be left as

untreated controls. (What happens if EEE virus is detected in an untreated control swamp?) Also, a basic difference exists in the way vector surveillance would be conducted in an experimental setting as opposed to the disease surveillance setting. In the latter, we want to have the greatest possible chance of collecting infected females at the earliest possible date—that is an intentional bias that may be undesirable in an experimental setting.

On the other hand, it may be possible to design smaller studies to answer specific questions, such as the issue of beaver impacts on habitats and densities of the major vector species. Similarly, some fairly simple, but labor-intensive, studies might shed light on the issue of competition/displacement between *Cs. melanura* and *Cs. morsitans.*

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