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Fundamental issues in mosquito surveillance for arboviral transmission

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Summary

Marked spatiotemporal variabilities in mosquito infection of arboviruses, exemplified by the transmission of West Nile virus (WNV) in America, require adaptive strategies for mosquito sampling, pool screening and data analyses. Currently there is a lack of reliable and consistent measures of risk exposure, which may compromise comparison of surveillance data. Based on quantitative reasoning, we critically examined fundamental issues regarding mosquito sampling design and estimation of transmission intensity. Two surveillance strategies were proposed, each with a distinct focus, i.e. targeted surveillance for detection of low rates of mosquito infection and extensive surveillance for evaluation of risk exposure with high levels of mosquito infection. We strongly recommend the use of indicators embodying both mosquito abundance and infection rates as measures of risk exposure. Aggregation of surveillance data over long periods of time and across broad areas obscures patterns of focal arboviral transmission. We believe that these quantitative issues, once addressed by mosquito surveillance programs, can improve the epidemiological intelligence of arbovirus transmission.

Keywords

West Nile virus; Mosquitoes; Maximum likelihood estimate; Minimum infection rate; Epidemiological monitoring; Disease transmission

1. Introduction

The importance of mosquito surveillance was underscored by Reeves in his statement “... each epidemic... that was evolved in recent years could have been prevented or abated early in the course of its development by means of surveillance and vector abatement” (Reeves, 1980). Mosquito-based surveillance consists of the systematic collection of mosquito samples and testing of mosquito pools for arboviruses in order to assess the status of transmission and allow informed decision-making. Mosquito surveillance, as a tool for monitoring arboviral

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transmission, has been largely conducted by local public health agencies and mosquito control districts. For example, the recurrent West Nile virus (WNV) epidemics in the United States have resulted in enhanced mosquito surveillance at all levels across the nation. This has greatly accelerated the accumulation of critical data for estimation of risk exposure, so that appropriate preventive and intervention measures can be put in place.

Arboviral transmission, in general, is maintained at low levels in vector mosquitoes and reservoir hosts, with transient, sporadic outbreaks among humans. When environmental variables are favourable, arboviral circulation may exhibit explosive dynamics with high prevalence of infection in vector mosquitoes and avian hosts, as manifested by WNV transmission in North America (Hayes et al., 2005; Lampman et al., 2006). Substantial fluctuations in mosquito abundance and arboviral infections pose a challenge for mosquito-based surveillance programs because different surveillance strategies should be applied depending on the level of arboviral transmission. Public health workers are not always fully aware of relevant methodologies that can improve effectiveness and efficiency of surveillance. This can result in the choice of inefficient indicators of risk exposure, e.g. numbers of positive pools and minimal infection rates (MIR), thus compromising comparison and interpretation of the surveillance data (Gu et al., 2003). On the other hand, estimation of mosquito infection rates based on pool screening might be compromised by the use of a constant pool size, especially when mosquito infection rates are variable (Gu et al., 2004).

Given the complexity of arboviral transmission, it should be realized that there are no ubiquitous solutions and sampling programmes must be designed with the best knowledge available regarding the ecology of vector species and arboviral transmission. In this paper, we attempt to develop a comprehensive framework that tailors mosquito surveillance to variable arboviral transmission. Based on quantitative reasoning, we aim to derive general principles governing mosquito-based surveillance for estimation of risk exposure. We propose two different sampling strategies corresponding to different objectives of mosquito surveillance and identify issues associated with estimation of risk exposure. We highlight the role of targeted surveillance in the detection of low transmission and review critical issues regarding the estimation of prevalence of infected mosquitoes, especially during periods of rapid change in infection rates. We propose a composite index, i.e. the density of infected mosquitoes, as a better indicator of transmission intensity and raise caveats to the aggregation of sample data.

2. Targeted sampling design in low-level mosquito infections

In most elementary statistical textbooks, the principle of random sampling is emphasized to obtain representative samples for inferring quantities of interest in the population. Random sampling has many advantages for statistical inference, e.g. generalizability of sampling results to unsampled areas and flexibility to choose a pre-specified level of confidence. However, there are cases when random sampling fails to address the issues of interest. For example, random sampling makes no use of auxiliary information that could make the collection of samples more efficient, resulting in low power of detection when events are rare. Therefore, overemphasis of random sampling may lead to a shift from reliance upon important epidemiological considerations to more statistical ones.

Mosquito workers whose jurisdiction covers a large area tend to sample as many sites as resources allow. The emphasis on spatial coverage in surveillance programs results in less sampling effort at each of the sampling sites. This is particularly problematic in the case of early detection, when transmission foci are sporadic and mosquito infection rates low. Under these situations, the design of mosquito surveillance programs should aim at enhanced detection by increased sampling effort at a few 'hotspots' rather than extensive coverage. Early detection of arboviral activity provides useful information for projection of the potential course

of transmission later in the season and for unravelling the environmental factors associated with the maintenance and establishment of enzootic cycles. These are among some of the least understood aspects of arboviral transmission (DeFoliart et al., 1987; Komar, 2003; Nasci et al., 2002). Early detection is also critical because the timing of virus activity is important for understanding the intricacies of viral amplification, as well as extending the window for intervention. In temperate areas, the initiation of arboviral transmission cycles is often associated with low vector densities and low levels of transmission. Collection of sufficient mosquito samples for obtaining sensible detection power can be difficult when mosquito abundances are low. As one would expect, detection of mosquito infection when there is low transmission requires collection of large samples of mosquitoes. For example, 700 mosquitoes are needed for a modest detection probability of 0.5 when the natural infection rate is 0.1% (Gu and Novak, 2004). As a result, non-detection is common in areas of low transmission. The low sensitivity of conventional mosquito surveillance early in the season has led to the proposal of dead-bird surveillance for the early detection of WNV activity (Tesh et al., 2004; Watson et al., 2004). However, the sensitivity of dead-bird surveillance may decline due to the loss of wild sentinels, like crows, and the development of herd immunity (Buckley et al., 2003; Hochachka et al., 2004; Yaremych et al., 2004).

We recommend targeted surveillance to enhance the efficiency of early detection. This term was first used in the surveillance of bovine spongiform encephalopathy (BSE), in which sampling for screening tests was concentrated on sub-populations of cattle that were expected to have a higher prevalence of the disease (Doherr et al., 2001). Here, we define targeted surveillance as increased mosquito sampling at sites where interactions between vector mosquitoes and reservoir hosts are favourable for a greater likelihood of arboviral transmission. Identification of target sites however, raises uncertainties which can be overcome in part by the availability of epidemiological intelligence of arboviruses. Assuming that the epidemiological intelligence about the hotspots is correct, targeted surveillance yields higher sensitivity and efficiency than random surveillance for a fixed surveillance investment (Stark et al., 2006). For illustrative purposes, a hypothetical scenario was produced for comparison of two sampling strategies. Assume that a public health worker had jurisdiction over a total of 20 sites of interest and resources for maintaining a total of 20 trap-nights weekly. One sampling plan was to cover all 20 sites, resulting in only one trap-night at each site. Assume that a daily capture (50 mosquitoes per trap) was constant over these sites. If 5 of 20 sites were transmission foci with mosquito infection of 0.1%, the detection probability was 0.22 (i.e. $1 - (1 - 0.001)^{1 \times 5 \times 50}$) (Gu and Novak, 2004). On the other hand, if the epidemiological intelligence suggested five sites were more likely to be foci than others, there would be four trap-nights per site. If the epidemiological intelligence was largely correct, with three of those targeted sites being transmission foci, the resulting detection probability of this surveillance would be 0.45 (i.e. $1 - (1 - 0.001)^{4 \times 3 \times 50}$), double that of the first sampling plan. With increased accuracy of epidemiological intelligence, targeted mosquito surveillance can be a sensitive tool for early warning of arboviral activity.

3. Estimation of mosquito infection rates in periods of high transmission

As illustrated above, mosquito surveillance programs in the early season or in areas of low transmission are concerned mainly with enhancement of the detection power of sampling schemes. Later in the season, however, arboviral transmission activity may increase to the extent that detection of mosquito infection becomes frequent. Circulation of arboviruses exhibits marked fluctuations, particularly when meteorological conditions become favourable for virus replication and mosquito activity (Epstein, 2001; Hubalek, 2000; Reisen et al., 2004). For example, mosquito infection rates with WNV can change dramatically from week to week during the amplification phase (Gu et al., 2004; Lampman et al., 2006). As a result, the focus of surveillance programs should shift from detection of mosquito infection to

estimation of the transmission intensity, meanwhile expanding the number of sampling sites to evaluate the range of arboviral transmission.

The common procedure for estimating the level of mosquito infection is to group mosquito samples into pools for viral testing using immunoassays or DNA-based screening. The goal of pool screening is to estimate the proportion of infected mosquitoes more efficiently by reducing the number of tests required compared to individual screening. Pool screening is highly efficient when infection rates are low. For simplicity, we assume the assay systems have perfect detection with no false negative or false positive results (i.e. 100% sensitivity and specificity). These assumptions can be relaxed by more complicated analyses when the sensitivity and specificity of the assay systems are not perfect (Venette et al., 2002). It should be noted that there are several distinct quantities derived from viral testing of various parts of mosquitoes: infection rates (the whole mosquito body), dissemination rates (legs) and transmission rates (salivary glands or heads) (DeFoliart et al., 1987). Most screening systems test samples of the whole mosquito; infection rates thus derived measure the potential but not the actual status of the mosquitoes as disease-transmitting vectors. Here, we use infection rates as surrogates of transmission rates by assuming that these two quantities are correlated. However, this may not serve to identify vector mosquitoes because there may be a series of barriers preventing or reducing further dissemination and transmission of the infected mosquitoes (Hardy et al., 1983).

The most commonly used indicator for estimating levels of mosquito infection is MIR (an equivalent variant is minimal field infection rate MFIR). MIR, calculated as the ratio of the number of positive pools to the total number of tested mosquitoes, assumes that there is only one infected individual in each positive pool. Rather than estimating actual infection rates, MIR measures the lower bound. Additionally, application of MIR is restricted to situations of low transmission (Gu et al., 2003). The popularity of MIR is largely due to the fact that arboviral transmission is generally low, usually less than 0.1%. In fact, an infection rate of 0.1% was initially adopted as an indicator of potential outbreak for arboviruses in the United States (Bernard and Kramer, 2001; Day, 2001; Nasci et al., 1993; Reisen et al., 2002). However, mosquito infection rates can be much higher than 0.1% (Gu et al., 2004; Hayes et al., 2005). Use of MIR may greatly underestimate mosquito infections in situations when levels of mosquito infection are high and/or pool size is large. In order to define the domains in which the use of MIR is justified, the conditional probability ($p_{>1}$) of more than one infected mosquito, given the pool is positive, can be specified based on a binomial model:

$$P_{>1} = 1 - \frac{mf(1-f)^{m-1}}{1 - (1-f)^m}$$

where m and f are pool size and proportions of infected mosquitoes, respectively. The second term on the right-hand side of the equation is the probability of there being only one infected mosquito in positive pools. Using the cut-off value of $\alpha = 0.05$, invalidation of MIR occurs when the infection rate is $>0.2\%$ and the pool size equals 50. MIR is also not valid when infection rates are $>0.35\%$ for a smaller pool size of 30 mosquitoes (Figure 1).

Given the limitations of MIR in coping with variable arboviral transmission, we highlight the advantages of another indicator, maximal likelihood estimation (MLE). MLE has been in existence for decades (Chiang and Reeves, 1962; Walter et al., 1980). Instead of estimating the lower bound of mosquito infection, as MIR is intended for, MLE directly estimates the proportion of infected mosquitoes in the sample. Furthermore, calculation of MLE requires no more data than are required for the MIR calculation, i.e. the number of mosquitoes per pool and the test result for each pool. Although these two estimators are very similar under low transmission conditions, the discrepancy is marked when levels of mosquito infection are high.

For example, during the 2002 epidemic outbreak in Chicago, the MLE could be three times greater than the MIR (Gu et al., 2004).

It should be noted that the application of MLE alone cannot guarantee the accurate estimation of proportions of infected mosquitoes. Another important consideration of pool screening is choice of pool size. Besides the physical limitations of the testing system, e.g. sensitivity and specificity, estimation of infection rates is substantially affected by pool size as our previous simulation studies indicated (Gu et al., 2004). Inappropriate choice of pool size leads to the vast majority of or even all pools being positive and thus inaccurate estimation of infection rates. Choice of pool size, e.g. 50 mosquitoes per pool, is often automatically made in the field by mosquito collectors who are not fully aware of the potential consequences. For example, 80 to 100% of the mosquito pools provided by local mosquito control agencies were positive at several sites in Chicago during extensive enzootic and epidemic transmission of WNV (R.J. Novak, unpublished data), thus making reliable estimation of infection rates impossible.

Given the undesirable consequences of inappropriate pooling, several authors recommended the use of an optimal pool size based on a prior guessed natural infection rate (Gu, 1995; Swallow, 1985; Thompson, 1962). However, this approach may not be appropriate when mosquito infection rates are variable and educated guesses not possible. To shoot the 'moving target', we have suggested a solution that adopts variable size pooling in the face of uncertainties about infection rates (Gu et al., 2004). Variable size pooling groups mosquitoes into a gradient of pool size, e.g. 5, 10, 20, 30, 40 and 50, for viral testing to ensure that some pools remain negative. For a wide range (0–10%) of infection rates, our simulation studies showed that variable size pooling, coupled with MLE, provided better estimation (Gu et al., 2004).

4. Indicators of risk exposure

Mosquito surveillance programs are mostly concerned with estimation of risk exposure to arboviral infections. Assuming that contact between humans and mosquitoes is random, the probability (P) of exposure can be estimated by

$$P = 1 - e^{-mr}$$

where m is the mosquito biting rate per person and r is the mosquito infection rate (Li and Rossignol, 1998). Clearly, it is the product of m and r that measures the frequency of risk exposure. If one uses mosquito samples captured by the sampling device as an estimate of m , then the density of infected mosquitoes (DIM), calculated as the product of the abundance of mosquito samples and the proportion of infected mosquitoes, serves as the indicator of risk exposure (Ezenwa et al., 2006; Gu et al., 2006). Similar indicators, e.g. the entomological inoculation rate (EIR) and the annual transmission potential (ATP), are commonly used in mosquito surveillance for malaria and onchocerciasis (Basanez et al., 2002; Beier et al., 1999). However, this is not the case with mosquito surveillance for arboviruses. DIM estimates the frequency of contact between humans and infected mosquitoes during a period of time (day, week, month or year); use of mosquito infection rates alone is not sufficient and can be misleading. For example, suppose that mosquito density and the proportion of mosquito infection at one of two sites was 500/week and 0.1%, respectively, while the corresponding numbers at the other site were 100/week and 0.5%, respectively. Calculated DIMs were the same at the two sites (50 infected mosquitoes/week) although the mosquito infection rates were substantially different. The advantage of DIM becomes particularly noticeable when sample data are aggregated over sites. For instance, the average DIM of WNV in *Culex* mosquitoes in October 2004 at sites close to the Des Plaines River (< ca. two miles) was much lower than that at sites outside of the river area (0.063 vs. 0.244), which was opposite to the pattern of mosquito infection rates (0.65% vs. 0.54%) (Gu et al., 2006). The phenomenon is due to the

fact that the average of the product of the two variables (density and proportion of mosquito infection) might differ from the product of the averages of the two variables, because of heterogeneity in these variables; for example some sites where high levels of mosquito infection were associated with low mosquito densities. Furthermore, estimation of DIM can be readily made by the same data required for calculation of mosquito infection rates.

Some investigators have combined DIM with other parameters such as vector competency and blood-feeding preference (Bell et al., 2005; Kilpatrick et al., 2005; Kilpatrick et al., 2006) in order to refine the measure of risk exposure. However, variabilities in vector competency and feeding patterns have been frequently reported (Apperson et al., 2004; Hardy et al., 1983; Lardeux et al., 2007; Reisen et al., 2005; Vaidyanathan and Scott, 2007). It is not always sensible to compute a general estimate of exposure risk to be applied in the whole distribution range of the vector species. We advocate the use of DIM for most surveillance programs because it is a quantity measuring the frequency of local exposure to arboviral transmission. We believe that the thresholds of risk exposure can be estimated by accumulating DIM data for various vector species in relation to human clinical cases.

5. Spatiotemporal analysis for identifying transmission patterns

Transmission of arboviral diseases depends on intimate interactions between viruses, mosquitoes and hosts. These interactions are affected by a range of abiotic (e.g. temperature, rainfall, and humidity) and biotic factors (e.g. abundance of vertebrate hosts and vector mosquitoes). Variabilities in these factors across a landscape can result in enormous heterogeneity in transmission (Komar, 2001). In arboviral transmission systems, the transmission foci themselves should be the basic unit for data analysis and reporting because they are crucial for revealing influencing factors and local transmission intensity. Aggregation of estimates of transmission indices across numerous sites and over a long time period tends to obscure the signature of focal transmission.

Given the enormous effort being undertaken to sample mosquito populations at numerous sites by local health officials, it is necessary to report the data in a manner that allows spatiotemporal tracking of transmission dynamics. The lack of suitable spatiotemporal resolution in reports and publications compromises the development of understanding of focal arboviral transmission. For example, consider a situation where a total of 50 pools of a certain *Culex* mosquito were found positive. If most of the positive pools occurred at only a few sites during a short period of time this would strongly suggest a major role for the species in local transmission. By contrast, if these positive pools appeared sporadically through the whole season and across numerous sites it would indicate only a minor role for the species. Therefore, the key information for implicating vector mosquitoes and characterizing patterns of transmission would be lost by data aggregation. Although it is tempting and convenient to use summaries, it should be realized that the key information for elucidation of arboviral transmission is at the local level. We suggest that mosquito surveillance reports should provide sufficient details of sampling schemes (the number of sampling sites and frequency of sampling) so that local transmission intensity can be estimated. Spatiotemporal data are essential for investigation of the interrelationship between human cases and environmental variables (Brownstein et al., 2004; Ezenwa et al., 2007; Ward, 2005) and social and demographic variables (Ruiz et al., 2004). With the development of geographical information systems and the wide availability of geo-referenced environmental data, researchers and public health workers are increasingly able to overlay multiple sources of spatial-temporal data to track the evolution of arboviruses. For example, we were able to identify the area with higher potential transmission intensities adjacent to the Des Plaines River, where there existed a landmark corridor harbouring disproportionately large areas of natural woodlands and

wetlands, by mapping indicators of transmission intensities collected over 39 sites in the Chicago area (Gu et al., 2006).

6. Conclusions

As noted by Fine (1981), more precise terminology is needed for characterizing arboviral transmission. Remarkable variation in arboviral transmission calls for adaptive strategies with distinct objectives in correspondence with changing arboviral transmission. We recommend the following guidelines: (1) targeted surveillance can significantly improve the overall efficiency of sampling programs for early detection in low transmission areas; (2) estimation of mosquito infection rates in high transmission areas should consider variable size pooling coupled with maximal likelihood estimation; (3) estimation of risk exposure should be made using the composite index incorporating mosquito abundances and infection rates; and (4) heterogeneity in arboviral transmission requires data analyses and reports to be carried out at the focal level. We believe that our understanding of the epidemiology of arboviruses would be greatly advanced if these guidelines were applied in mosquito surveillance programs.

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Figure 1.
The conditional probability of there being more than one infected mosquito in positive pools as a function of infection rates.