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Subject: Epi-Aid 2012-069 Final Report: Evaluation of the impact of adult mosquito control during a West Nile virus outbreak in Dallas, Tarrant, Denton, and Collin Counties — Texas, 2012

BACKGROUND

In early summer 2012, the Texas Department of State Health Services (DSHS) noted a substantial increase in West Nile virus (WNV) activity. By August 17, a total of 552 cases of WNV disease had been reported to Texas DSHS, including 21 deaths. In comparison, only six cases of WNV disease had been reported to Texas DSHS by the same time in 2011. Among the 552 cases in 2012, 412 (75%) resided in four counties in north central Texas. In response, local health departments and mosquito control districts increased surveillance and control efforts. Larviciding and ground-based adulticide spraying were performed variably throughout the area and aerial insecticide spraying was implemented in some areas.

On August 18, Texas DSHS requested assistance from the Centers for Disease Control and Prevention (CDC). The objectives of the investigation were to: 1) Compile available epidemiologic and mosquito surveillance data and provide technical advice for prevention and control activities in Collin, Dallas, Denton, and Tarrant Counties in Texas Health Service Region 2/3; 2) Evaluate the impact of adult mosquito control on mosquito abundance, WNV mosquito infection rates, and incident human infections in the four counties; and 3) Assist with the development of public health strategies for future WNV virus prevention and control efforts in Texas. From August 23 to September 4, a CDC team worked at the Texas DSHS Region 2/3 office in Arlington and an interim report was provided to Texas DSHS on September 4. This report contains the final results of the impact evaluation using data gathered through the end of 2012, and programmatic observations.

IMPACT ANALYSIS AND PROGRAM EVALUATION

1. Description of the outbreak

<u>Methods</u>

We performed the evaluation in four counties in north central Texas, including Collin County (0.8 million population; 886 square miles), Dallas County (2.4 million population; 908 square miles), Denton County (0.7 million population, 958 square miles), and Tarrant County (1.8 million population; 898 square miles). We defined a case as a resident of one of the four counties who, in 2012, had a clinically compatible illness and laboratory evidence of WNV disease according to the national surveillance case definition [CDC 2012]. Cases were classified as neuroinvasive (e.g., meningitis, encephalitis, or acute flaccid paralysis) or non-neuroinvasive.

We obtained demographic and epidemiologic data for cases reported to Texas DSHS as of December 31, 2012. Because of the considerable morbidity associated with neuroinvasive disease cases, detection and reporting is assumed to be more consistent and complete than for non-neuroinvasive disease. Therefore, incidence rates were calculated only for neuroinvasive disease cases using U.S. Census Bureau 2010 mid-year population estimates.

<u>Results</u>

As of December 31, 2012, a total of 904 cases of WNV disease were reported to Texas DSHS from the four counties, including 362 (40%) classified as neuroinvasive and 542 (60%) classified as non-neuroinvasive disease (**Table 1**). The outbreak peaked in mid-summer and 88% of cases had illness onset in July or August (**Figure 1**). The median age of cases was 53.5 years (range: 2 months–93 years); 434 (48%) were female. The case-fatality ratio was 9% (32/362) for neuroinvasive and 0.4% (2/542) for non-neuroinvasive disease cases. The incidence of WNV neuroinvasive disease was 6.4 cases per 100,000 population.

2. Impact of aerial spraying on human WNV disease

<u>Methods</u>

Adult mosquito control measures. Dates and locations of aerial or ground-based application of insecticides targeting adult mosquitoes were requested from the municipalities and other jurisdictions in the four counties. In addition, we conducted an internet search for additional maps and ground spray data that were not directly provided. For aerial spray data, insecticide applications that occurred in a pre-defined area on more than 1 day were classified as a single aerial spray event. For ground-based applications, data were aggregated and the total area sprayed each week was mapped. Locations of aerial and ground-based insecticide applications were mapped using ArcMap 10.0 (ESRI, Redlands, CA).

Three aerial spray events were conducted in northeastern Texas during 2012, covering approximately 1,117 (31%) of the 3,659 total square miles in the four counties (**Table 2 and Figure 2**). The first occurred from August 16–20 and covered 559 square miles primarily in northern and central Dallas County (Dallas County Zones 1 and 2); for the purpose of this analysis, this was designated Area 1. The event was interrupted due to weather conditions and spread over five nights but the full area was covered once by August 19. The second spray event occurred from August 22–23 and covered 117 square miles primarily in eastern and southern Dallas County (Dallas County Zone 3); this was designated Area 2 and the full area was covered once on August 22. The final aerial spray event occurred from August 31–September 2 and covered 441 square miles of Denton County; this was designated Area 3 and the full area was covered once by September 1.

Defining exposure periods. We assigned cut-off dates to define time periods before and after the aerial insecticide applications. In areas where aerial spraying was performed, we defined the cut-off as the earliest date by which the entire treated area was covered by aerial spraying, regardless of additional applications (i.e., August 19 for Area 1, August 22 for Area 2, and September 1 for Area 3).

In areas where aerial spraying was not conducted, the cut-off dates were selected from the three sprayed areas. In Denton County, the cut-off date used in the county's sprayed area (September 1) was also assigned to the unsprayed area. For the untreated areas in Dallas, Collin, and Tarrant Counties, no single cut-off date could be assigned from an adjoining area (two spray events occurred in Dallas County and no aerial spray events were focused in Collin or Tarrant Counties). Therefore, for the unsprayed areas in these three counties, the cut-off date yielding the most conservative conclusions about the effectiveness of spraying was chosen for all three. The date was selected using a computer model that simulated possible courses of the outbreak and varied effectiveness of spraying. Nine scenarios were considered incorporating three different patterns of disease rates over time and three different levels of spraying effectiveness; for each scenario, 5000 data sets were generated. With each data set, we compared incidence rates before and after spraying using each of the three possible cut-off dates (i.e., August 19, August 22, or September 1). As a result of this analysis, August 19 was determined to be the most conservative cut-off date for the unsprayed areas of Dallas, Collin and Tarrant Counties.

Categorizing disease cases. WNV neuroinvasive and non-neuroinvasive disease cases were categorized as living within or outside an aerial-sprayed area. Cases without a known home street address were excluded. To account for the incubation period for WNV disease, we used the date of symptom onset minus 7 days (i.e., the average incubation period) to define if the case was exposed before or after an aerial spray event.

Data analysis. To assess consistency in detection and reporting of cases, we calculated the proportion of WNV disease cases that were classified as non-neuroinvasive disease in aerial-sprayed and unsprayed areas before and after the cut-off dates and compared them using the Mantel-Haenszel χ^2 test. To determine if the individual treated areas could be combined, we calculated odds ratios for being a neuroinvasive or non-neuroinvasive disease case before and after spraying in each area and compared the result from each area using Woolf's test for homogeneity; the same calculation was performed for each untreated area in each county.

To evaluate the impact of aerial spraying on human disease, we calculated incidence rates per 100,000 population for WNV disease cases occurring in 2012 in the combined treated and combined untreated areas before and after the cut-off dates. For post-treatment calculations, the denominator was determined by subtracting the number of WNV disease cases that had occurred before the cut-off date from the relevant population figure. Incidence rate ratios (IRR) before and after aerial spraying were calculated for the treated and untreated areas (e.g., Incidence rate in the treated area before spraying/Incidence rate in the treated area after spraying). We then calculated the ratio of IRRs and 95% confidence intervals (CI) in treated versus untreated areas (i.e., IRR in the treated area/IRR in the untreated area). To account for the possible impact of ground-based insecticide applications in the aerially treated and untreated areas, we calculated the square miles and proportion of the total area covered by ground-based spraying approximately 3 weeks before and after the cut-off dates and compared them using the Mantel-Haenszel χ^2 test. For all analyses, a 2-sided $P < 0.05$ was considered statistically significant.

Results

There were 904 WNV disease cases reported to Texas DSHS from the four counties in 2012. Among these, four (0.4%) did not have an available home street address (two homeless patients, one with an unidentifiable street name, and one with a post office box only) and were excluded from the impact analysis. Of the remaining 900 cases, 498 (55%) occurred in areas that underwent aerial spraying and 402 (45%) occurred in areas that were not aerially sprayed. In the aerially treated areas, the proportion of cases classified as non-neuroinvasive disease increased from 59% (277/466) before to 78% (25/32) after the aerial spraying ($P = 0.04$). In the areas where aerial spraying was not performed, the proportion of non-neuroinvasive disease cases was similar before 59% (215/363) and after 64% (25/39) the cut-off dates ($P = 0.6$). Given the possible increase in the diagnosis of non-neuroinvasive disease in the treated areas after aerial spraying, the impact evaluation was limited to neuroinvasive disease cases as they are believed to be a more consistent indicator of WNV infections. For neuroinvasive disease cases, Woolf's test for homogeneity indicated that odds ratios for the three aerially treated areas were similar ($P = 0.3$) and four untreated areas were similar ($P = 0.7$), indicating these areas could be combined and evaluated as one treated and one untreated group.

In aerial sprayed areas, neuroinvasive disease incidence before and after spraying was 7.5 and 0.3 per 100,000 persons, respectively; the IRR was 27.0 (95% CI: 12.7–57.4) (**Table 3**). In untreated areas, the before and after incidence was 4.8 and 0.5 per 100,000 persons, respectively; the IRR was 10.6 (95% CI: 6.1–18.3). The ratio of these IRRs was 2.6 (95% CI: 1.0–6.5), indicating the decrease was an estimated 2.6 times, and between 1.0 and 6.5 times, greater in the aerial sprayed area.

Due to the large numbers, variable size, and poor documentation of ground-based insecticide applications, mapping of these activities was limited to the >400 spray events that occurred in the 3 weeks before and after aerial spraying. In the combined aerial sprayed areas, the proportion covered by ground-based spraying was similar before (10%, 115/1,117 square miles) and after (9%, 99/1,117 square miles) the aerial applications ($P = 0.3$) (**Table 2**). However, in the combined areas where aerial spraying was not performed, the proportion covered by ground-based insecticide applications increased from 5% (129/2,542 square miles) in the 3 weeks prior to the comparable cut-off dates to 11% (285/2,542 square miles) covered after ($P < 0.001$).

3. Impact of aerial spraying on mosquito abundance and WNV mosquito infection rates

Methods

Mosquito surveillance data

We contacted county agencies, mosquito control districts, contractors performing mosquito surveillance and control, and laboratories that performed WNV testing on mosquitoes to obtain mosquito surveillance data. We collected sampling dates and location for each trap site, and mapped location by address or global positioning system (GPS) coordinates to determine if it was in a treated or untreated area. We used only data from gravid traps at stationary sites defined as sites with multiple collection dates in which at least one collection occurred within 3 weeks before and 3 weeks after aerial spraying. *Culex quinquefasciatus*, the southern house mosquito, is the main WNV vector in north Texas and was the focus of this analysis. We collected data on the number of *Cx. quinquefasciatus* mosquitoes and WNV mosquito testing results (number, size, and total WNV positive pools) by trap location and date.

Mosquito surveillance practices varied across communities in the four counties (e.g., some municipalities did no mosquito trapping, some put traps out only after a human case was detected, some trapped routinely but used different locations each week, and some collected mosquitoes for virus testing but did not compile abundance or species identification data). The most complete information was available from Dallas County. Therefore, the evaluation of impact of aerial spraying on mosquito abundance and infection rate was limited to that area.

Adult mosquito control measures. Dates and locations of aerial applications of insecticides targeting adult mosquitoes were collected and mapped as described above. Three aerial spray events were conducted in Dallas County in 2012 (**Figure 3**). The first event covered primarily north central Dallas County (Zone 1); it was interrupted by inclement weather and occurred over a 5 day period from August 16–20. The second spray event occurred on two consecutive nights from August 19-20 and covered large areas of the county (Zone 2). The final event also occurred on two consecutive nights from August 22-23 and covered small areas in the east and south (Zone 3). The remainder of Dallas County was designated as the unsprayed area and each of the three zones was compared to this same untreated area.

Defining the pre- and post-spray periods. For the purposes of this analysis, the pre- and post-spray periods were defined based on the date that aerial spraying was completed in each of the three zones. Using this procedure, the cut-off dates for the "before" and "after" analyses in the three treated zones were assigned as August 20 for Zones 1 and 2, and August 23 for Zone 3. For the untreated area, the data were reanalyzed using the cut-off date for the sprayed area to which it was being compared (i.e., August 20 or August 23). This method was slightly different from the evaluation of impact on human disease which used data from all four counties and defined the pre- and post-spray periods as the earliest date that each treated area was covered once.

Data analysis. Mosquito abundance was defined as the average number of *Cx quinquefasciatus* mosquitoes collected per trap per night from stationary trap sites and was calculated for each of the three treatment zones and the untreated area in the 1 week before and after treatment. A generalized, linear, mixed model assuming a Poisson distribution was used to estimate the rate of change and 95% CI from before to after aerial spraying. Zone, treatment status and their interaction were included in the model as fixed effects. Each trap, based on the date of collection, had its treatment status as either "before spray" or "after spray". Traps in the untreated area were classified as "before spray" or "after spray" based on dates the treated areas were sprayed. A random effect for trap location was included in the model to account for the correlation of repeated measures in the same location. Using model estimates, we then calculated the abundance rate ratio and 95% CI for each treated zone versus the untreated area (i.e., Rate of change in the treated area/Rate of change in the untreated area). WNV infection rates and 95% CI were calculated as the bias corrected, maximum likelihood estimate of the number of infected *Cx quinquefasciatus* mosquitoes per 1000 tested in the 1 week and 3 weeks before and after aerial spraying [Biggerstaff 2009]; we determined the difference and 95% CI from the pre- to post-spray periods in each area. The Vector Index (Average number of *Cx. quinquefasciatus* mosquitoes collected per trap night x proportion of *Cx. quinquefasciatus* mosquitoes infected) was calculated for each treated zone and the untreated area and the difference before and after aerial treatment events was determined (**Appendix 2**) [Gujaral 2007, Jones 2011, Kwan 2012]. The Vector Index combines information about vector species presence, vector species density, and vector species infection rate in a single value; however, there are no established methods to calculate variances or make statistical comparisons.

Results

Mosquito trapping and testing effort. In Dallas County, 42 stationary sites were sampled at least once before and after aerial spraying in the treated and untreated areas. In general, the numbers of trap nights, individual *Cx quinquefasciatus* mosquitoes tested, mosquito pools tested, and WNV-positive pools in each zone before and after aerial spraying were relatively small, making assessments of impact difficult (**Table 4)**.

Mosquito abundance. The numbers and trends in the average number of *Cx. quinquefasciatus* mosquitoes collected per trap night varied by zone (**Table 5**). There were small but statistically significant increases in mosquito abundance in Zone 1 (Modeled rate of change 2.3; 95% CI 1.8, 2.9) and Zone 3 (Modeled rate of change 1.8; 95% CI 1.4, 2.2) in the 1 week before and after spraying. Zone 2 showed a similar small increase in mosquito abundance but the difference was not significant. In the untreated areas, mosquito abundance also showed a small but significant increase (Modeled rate of change 0.7; 95% CI 0.4, 0.9) from the 1 week before to 1 week after August 20; abundance was unchanged when the calculations were repeated using the August 23 cut-off. The rate ratio comparing the abundance rate change between the treated and untreated areas was only significant for Zone 3 versus the untreated area using the August 23 cut-off (Abundance rate ratio 1.9; 95% CI 1.4, 2.6). As shown previously, in areas of Dallas County that did not perform aerial spraying, ground-based spraying increased from 28% of the area covered in the 3 weeks before August 19 to 76% in the 3 weeks after ($P < 0.001$) (**Table 2**).

WNV infection rates. The rates and trends of WNV infections in *Cx. quinquefasciatus* mosquitoes varied across areas (**Table 6**). In Zone 1, the infection rates per 1,000 mosquitoes tested were similar in the 1 week and 3 weeks before and after aerial spraying. However, WNV infection rates decreased significantly in Zone 2 (Difference -37.4; 95% CI -129.9, -12.3) and Zone 3 (Difference -20.8; 95% CI -69.2, -9.8) with no infected mosquitoes identified in the 3 weeks following aerial spraying in either area; the same trends were observed in the 1 week before and after spraying but due to the small numbers of mosquitoes tested these differences were not statistically significant. It is notable that spraying in Zone 1 was interrupted due to inclement weather and extended over 5 nights while Zones 2 and 3 received two consecutive nights of treatment. In the unsprayed area of Dallas County, WNV infection rates were similar in the 1 week before and after the August 20 cut-off. Rates declined in the 1 week after the August 23 cut-off and the 3 weeks after either date; however, these differences were not statistically significant.

Vector Index. Overall, the differences in the Vector Index between areas and following aerial spraying were similar to those for the WNV infection rates (**Table 7**). In spray Zone 1, the Vector Index was relatively unchanged before and after aerial spraying for both the 1 week and 3 week time periods. In Zones 2 and 3, the Vector Index decreased substantially with no WNV-infected mosquitoes detected in either area during the 3 weeks following aerial treatment. In the unsprayed area, changes in the Vector Index varied with the cut-off and timeframe; it was unchanged from 1 week before to 1 week after August 20 but decreased from 3 weeks before to 3 weeks after the August 20 cut-off and for both the shorter and longer periods around the August 23 cut-off date.

4. Mosquito surveillance and control program assessment

Methods

A systematic comprehensive evaluation of the WNV mosquito surveillance and control program was not a pre-defined objective of the Epi-Aid. However, during the course of the investigation, we had the opportunity to observe the programs in Dallas and the surrounding counties and informally assess their procedures and capacities through requests for surveillance and control data and queries regarding routine practices.

Results

We identified >90 entities responsible for mosquito surveillance or control activities in the four-county area. This large number of programs using a variety of surveillance, control, and data management procedures made it difficult to communicate and coordinate activities across the region. We also noted considerable variability in the technical expertise, experience, and capacity among programs.

Our observations suggested the mosquito surveillance activities conducted to monitor WNV activity were limited and often did not provide the data needed to facilitate timely implementation or monitoring of control measures. The information we obtained suggested that mosquito surveillance procedures did not include consistent trapping at regular, fixed sites. In the jurisdictions that had fixed mosquito sentinel sites, sampling was often irregular (e.g., conducted over consecutive days but not repeated at that site until several weeks later). This discontinuity restricted the ability to observe developing virus activity patterns. Some jurisdictions only conducted trapping following the identification of a human case and limited it to the area around the case residence. The mosquito identification data, laboratory test results, and information on larval surveillance activities that were provided did not indicate that a coordinated surveillance program was in place across the jurisdictions. Although many jurisdictions were making efforts to keep accurate records, this lack of central coordination compromised the ability to use the data for monitoring, evaluation, or future planning.

LIMITATIONS

Effect of aerial adulticide applications on human disease

Several factors limited our ability to conduct a comprehensive and accurate evaluation of the impact of adult mosquito control measures on human disease cases:

- Due to apparent changes in the detection of non-neuroinvasive disease cases after the aerial spraying, the analysis was limited to neuroinvasive cases. The higher proportion of non-neuroinvasive disease cases reported in the treated areas after aerial spraying may have resulted from increased awareness or concern and more visits to healthcare providers or laboratory testing for people with milder symptoms.
- In analyzing the human case data, we assumed that WNV infections were acquired at the residential address and that the incubation period was 7 days from exposure to illness onset.
- Since the aerial spraying occurred relatively late in the season, there were relatively few cases identified during the post-spray period in both treated and untreated areas.

- Aerial spray events occurred over several days and at different times in each area. We used conservative cut-off dates to define the before and after periods and applied them to seemingly comparable non-sprayed areas. However, factors other than the aerial insecticide applications may have accounted for the changes in disease incidence before and after spraying and for the greater decline observed in treated versus untreated areas (e.g., differences in disease diagnosis or case classification, weather patterns, other mosquito control efforts, or varying impact of spraying in different ecological areas).
- Data describing ground-based mosquito control activities were often incomplete or difficult to interpret in terms of the date and location of the application. The type and application rate of the insecticide products could not be taken into account in the evaluation.

Effect of aerial adulticide applications on mosquito abundance and WNV infection rate

Multiple factors limited our ability to evaluate impact of adult mosquito control measures on *Cx. quinquefasciatus* mosquito abundance and WNV infection rates:

- The availability, quality, completeness, and accuracy, of the mosquito surveillance and control data varied across the four-county area, and the data provided were often not in a readily usable format. As a result, the mosquito analysis was limited to Dallas County.
- Mosquito surveillance and testing conducted in the area was not designed to monitor the effectiveness of the aerial insecticide treatments in reducing vector abundance or the subsequent calculations of infection rates and Vector Index values.
- Relatively few stationary traps were available that met our criteria for timing and placement, which resulted in small sample sizes available to estimate abundance and infection rate, and large confidence intervals around the indices, indicating substantial uncertainty in the estimates.
- Areas of Dallas County that did not perform aerial spraying received increased ground spraying following the aerial spray events, which introduces a confounding factor in efforts to quantify the effects of the aerial spraying on abundance, infection rate and Vector Index data.

Mosquito surveillance and control program assessment

- A systematic, comprehensive program evaluation was beyond the scope of project objectives and was not conducted. The procedures and capacities of the mosquito surveillance and control programs in the area were observed in the course of interacting with these programs to obtain mosquito surveillance and control data for use in the quantitative analyses.

CONCLUSIONS AND RECOMMENDATIONS

Effect of aerial adulticide applications on human disease

- The aerial spraying measures implemented for WNV control had a measurable impact in preventing WNV neuroinvasive disease. As expected given the timing of the intervention, disease incidence decreased in the after-spray period in both treated and untreated areas, but the relative change was significantly greater in aerial-sprayed areas.
- It was difficult to assess the impact of ground-based insecticide applications in the prevention of human disease during this outbreak. However, based on the available data, the proportion of the aerially-treated area covered by ground-based spraying was similar before and after the aerial applications. In contrast, in the combined areas where aerial spraying was not performed, the proportion covered by ground-based insecticide applications more than

doubled between the 3 weeks before and after the cut-off dates. These data suggest that it is unlikely that ground-based spraying accounted for the significantly greater decline in neuroinvasive disease incidence in aerially-treated versus untreated areas.

- Further studies should assess possible confounding factors (e.g., ground spraying) and evaluate the impact of earlier spraying implementation.

<u>Effect of aerial adulticide applications on mosquito abundance and WNV infection rate</u>

- There was a significant decrease in mosquito infection rates in Zones 2 and 3 from 3 weeks before to 3 weeks after aerial spraying, with no WNV-infected mosquitoes identified in those areas following treatment. During the same time period, infection rates remained unchanged in Zone 1 and the untreated areas. It is notable that Zones 2 and 3 received two consecutive nights of treatment, while aerial spraying in Zone 1 was interrupted due to inclement weather and had to be completed over 5 nights.
- Vector Index calculations supported decreases in the number of infected mosquitoes collected per trap night in Zones 2 and 3 in the 3 weeks following treatment in those areas. This was the only difference that is supported by significant decreases in infection rate in the mosquitoes in those areas. Other changes in Vector Index are likely not significant, as they are based on differences in infection rates that are not statistically significant.
- Mosquito abundance estimates for the 1 week pre and 1 week post spray periods suggested that there was a small increase in abundance in two of the three treatment zones over this time period, but that there was little or no meaningful change in abundance when the treatment zones were compared to the untreated area.
    - The apparent lack of impact on *Cx. quinquefasciatus* abundance following the aerial spray applications can be explained in part by the relatively small number of traps available to accurately monitor vector abundance, the timing of trap placement in respect to the treatment dates, and by use of gravid traps to monitor abundance. Published research suggests that the bloodfed and gravid components of the mosquito population are less susceptible to ULV control, given their behavioral and physiological state [Reiter 1990, Eliason 1990, Moore 1990) and changes in *Cx. quinquefasciatus* abundance following control may not be reflected when sampling this population segment. In addition, *Cx. quinquefasciatus* populations are continually replenished by emergence of new adults [Andis 1987], resulting in rapid replacement of the adult mosquito population. However, the newly emerged, nulliparous adult mosquitoes are not infected with WNV and do not pose the same risk of WNV infection as older populations containing numerous infected adult mosquitoes. The reduction of human disease incidence in the aerially-treated areas supports the contention that the aerial spray applications reduced the abundance of the older, infectious mosquito population, resulting in a reduction of human infection risk, even though decreases in abundance were not documented.
    - The observation that there was little difference in mosquito abundance following the aerial spray applications is different from what was observed in the preliminary report. In retrospect following receipt of a more complete accounting of the mosquito surveillance that was conducted, mosquito abundance was compared using an incomplete data set that did not allow accurate determination of the number, timing, and location of trapping. In the preliminary analysis, total numbers of mosquitoes were used to compare areas, while in the current analysis, abundance was corrected for

trapping effort, providing a more accurate estimate of mosquito population density. As a result, the estimates utilized in the preliminary report were incorrect and inaccurately portrayed the mosquito population size before and after the aerial applications.

Mosquito surveillance and control program assessment

- There are many independent mosquito control districts in the four-county area. These programs use an array of surveillance, control, and data management procedures and vary in their technical expertise, experience, and capacity. These factors made it difficult to communicate and centrally coordinate activities across the region.
- Mosquito control programs should collaborate to implement Integrated Vector Management (IVM) procedures in a coordinated, district-wide vector control program (**Appendix 1**).
    - Assistance from the State (Texas DSHS and Texas Ag Life Cooperative Extension program) in the form of technical expertise can facilitate and support development of a comprehensive IVM program at the county or local level.
    - The Texas Ag Life Cooperative Extension program held workshops and training programs for vector control personnel late in 2012, and has plans to continue these programs into 2013.
    - To our knowledge, there is not currently a medical entomologist or public health entomologist with specific knowledge and practical experience in mosquito surveillance and control, and vector-borne disease transmission ecology, within the Texas state or regional health agencies. Such expertise would be valuable to coordinate planning efforts, assist programs in developing and implementing enhanced surveillance programs, and providing timely consultation and assistance during outbreak responses.
- Assessment of the amount of the affected area receiving ground-based mosquito adulticide applications during the WNV outbreak indicates there is insufficient ground-spraying capacity in the region to quickly and repeatedly treat the large populated areas in the region during an epidemic response. Contingencies for obtaining aerial-spray capacities should be developed for use when surveillance indicates rapid, large scale mosquito control is required to reduce human disease risk.
- Based on the informal observations during this outbreak response, we make the following specific recommendations to improve vector surveillance and control:

    Vector surveillance activities

    - Mosquito sampling sites should be fixed so data gathered is comparable over time.
    - The number of fixed trapping sites and frequency of routine trapping should be increased.
    - Surveillance information gathered from routine trapping sites should be compiled in a standard format that is used or accessible by all of the local programs involved in the district, in order to facilitate data sharing and comparability.
    - Surveillance information should be available to local public health departments to support and guide efforts such as targeted community awareness and education activities.
    - Vector surveillance indices should be standardized, using common units of vector abundance (i.e., number collected per trap night per week) and measures of infection

rate (i.e., maximum likelihood estimate of the infection rate in the vector population each week for each surveillance area).
- Use of the surveillance data should be maximized by evaluating historical data to establish associations of the indices with human risk. Consider incorporating the Vector Index into the surveillance procedures (**Appendix 2**).
- Consider adding dead bird surveillance in areas where human population density would make it a useful tool to complement mosquito-based surveillance.

Vector Control Activities
- Identify mosquito production sites that are conducive to source reduction measures and build lines of communication with local governmental agencies to address these sites (e.g., fix blocked drainages in storm water systems, clean vegetation from retention ponds.)
- Establish surveillance to determine the extent of *Culex quinquefasciatus* production from the storm water management system and implement appropriate control measures should they be found to be important sources of this vector species.
- Develop a program for proactively locating, monitoring, and treating neglected (green) swimming pools which have been identified as important sources of WNV vector mosquitoes in other areas of the country.
- Increase larval source mapping, source reduction, and larval control efforts, particularly early in the WNV transmission season, to suppress mosquito populations to levels insufficient to support WNV amplification.
- Use historical surveillance data and consistent indices to establish thresholds for implementing routine mosquito control procedures.
- Establish response plans that use these thresholds for pre-defined control actions in order to avoid delays in implementation and the additional human infections that would occur. To the extent possible, the response plan should include contingencies for obtaining funds to support emergency, large scale mosquito control efforts.
- Evaluate the efficacy of any control measures (larvicide or adulticide), including monitoring vector mosquito populations for resistance to the pesticides being used.

REFERENCES

1. Andis, MD, Sackett, SR, Carroll, MK, Bordes ES. Strategies for the emergency control of arboviral epipemics in New Orleans. J Am. Mosq. Control Assoc. 1987. 3:125-130.
2. Biggerstaff, Brad J. PooledInfRate, Version 4.0: a Microsoft® Office© Excel Add-In to compute prevalence estimates from pooled samples. Centers for Disease Control and Prevention, Fort Collins, CO U.S.A., 2009. http://www.cdc.gov/ncidod/dvbid/westnile/software.htm
3. CDC. Arboviral neuroinvasive and non-neuroinvasive diseases. 2012 Nationally notifiable diseases and conditions and current case definitions. Centers for Disease Control and Prevention, Atlanta, GA. Available at http://wwwn.cdc.gov/nndss/document/2012_Case%20Definitions.pdf. Accessed January 29, 2013.
4. Eliason, D. A., E. G. Campos, C. G. Moore and P.Reiter. 1990. Apparent influence of the stage of blood meal digestion the efficacy of ground applied ULV aerosols for the control of urban Culex mosquitoes. II. Laboratory evidence. J. Am. Mosq. Control Assoc. 6:371-375.

5. Gujral IB, Zielinski-Gutierrez EC, LeBailly A, Nasci R. Behavioral risks for West Nile virus disease, northern Colorado, 2003. Emerg Infect Dis. 2007;13:419–425.
6. Jones RC, Weaver KN, Smith S, et al. Use of the vector index and geographic information system to prospectively inform West Nile virus interventions. 2011; Am Mosq Control Assoc.; 27(3):315-9.
7. Kwan JL, Park BK, Carpenter TE, Ngo V, Civen R, Reisen WK. Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010.2012; Emerg Infect Dis.18(8):1298-306.
8. Moore, C. G., P. Reiter, D. A. Eliason, R. E. Bailey and E. G. Campos. 1990. Apparent influence of the stage of blood meal digestion on the efficacy of ground applied ULV aerosols for the control of urban Culex mosquitoes. III. Results of computer simulation. J. Am. Mosq. Control Assoc. 6:376-383.
9. Reiter, P., DA Eliason,, DB Francy. et al. 1990. Apparent influence of the stage of blood meal digestion on the efficacy of ground applied ULV aerosols for the control of urban *Culex* mosquitoes. I. Field Evidence. J. Am. Mosq. Control Assoc. 6(3): 366-70.

Table 1. West Nile virus disease cases and neuroinvasive disease incidence (per 100,000 population) in 2012 for four northeast Texas counties

County	2010 population	Non-neuroinvasive disease cases No.	(%)	Neuroinvasive disease cases No.	(%)	Neuroinvasive disease incidence (per 100,000)
Collin	781,907	41	(8)	22	(6)	2.8
Dallas	2,366,302	221	(41)	182	(50)	7.7
Denton	661,808	128	(24)	53	(15)	8.0
Tarrant	1,805,123	152	(28)	105	(29)	5.8
Total	**5,615,140**	**542**	**(100)**	**362**	**(100)**	**6.4**

Figure 1. West Nile virus disease cases in Collin, Dallas, Denton and Tarrant Counties by week of illness onset and clinical syndrome.

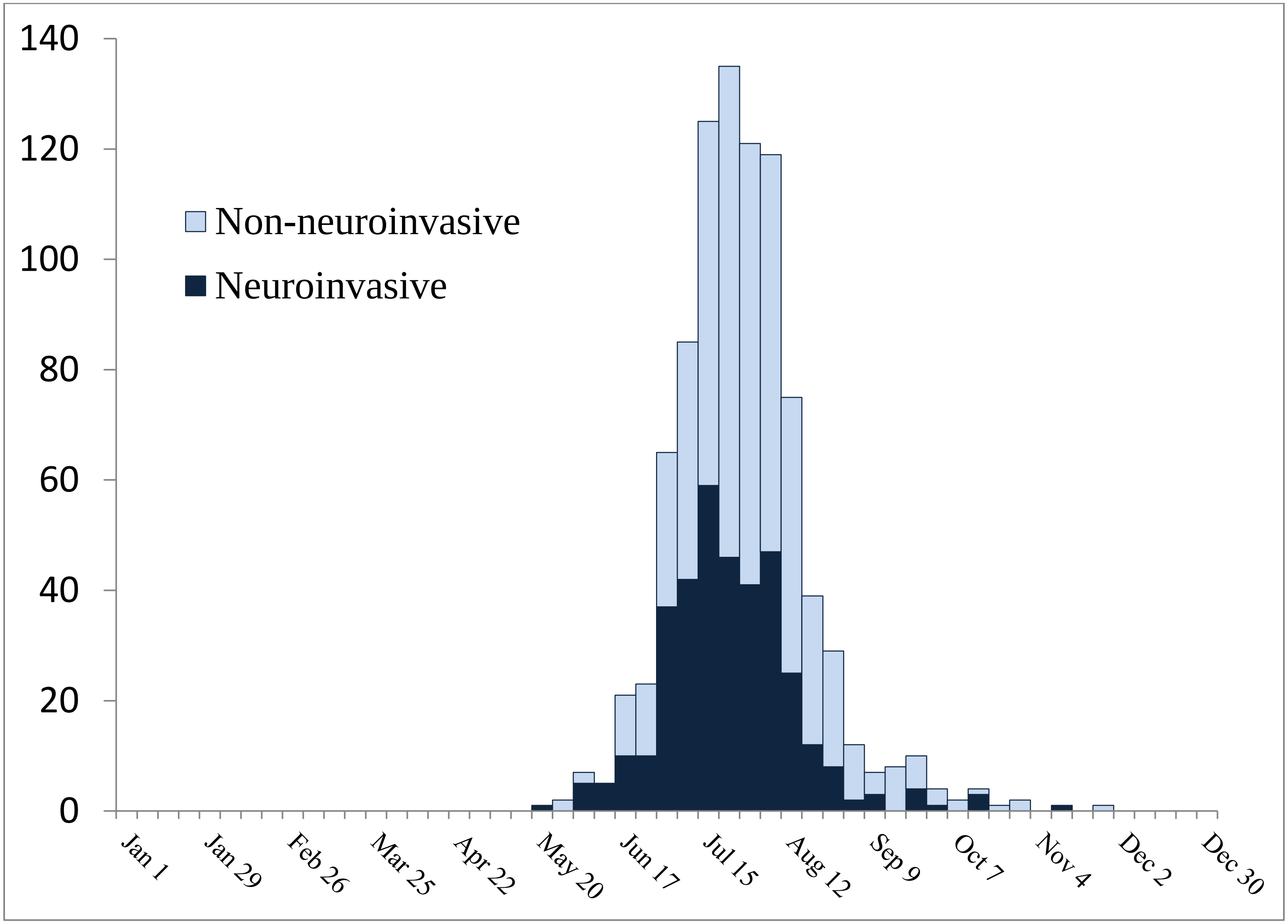


Table 2. Area covered with ground-based insecticide spraying in the 3 weeks before and after the aerial spray applications — Collin, Dallas, Denton, and Tarrant Counties, Texas, 2012

	Total area	**Area covered with ground-based spraying before aerial applications**		**Area covered with ground-based spraying after aerial applications**	
	Square miles	Square miles	(%)	Square miles	(%)
Aerial sprayed areas					
Area 1	559	98	(18)	29	(5)
Area 2	117	16	(14)	62	(53)
Area 3	441	1	(<1)	8	(2)
Total	**1,117**	**115**	**(10)***	**99**	**(9)***
Aerial unsprayed areas					
Collin County	875	31	(4)	49	(6)
Dallas County	292	82	(28)	221	(76)
Denton County	538	12	(2)	4	(1)
Tarrant County	837	4	(<1)	11	(1)
Total	**2,542**	**129**	**(5)†**	**285**	**(11)†**

*$P = 0.3$ for the difference in the proportions covered before and after aerial spraying
†$P < 0.001$ for the difference in the proportions covered before and after aerial spraying

Figure 2. Location of Area 1, Area 2, and Area 3 aerial spray events in northeastern Texas, 2012

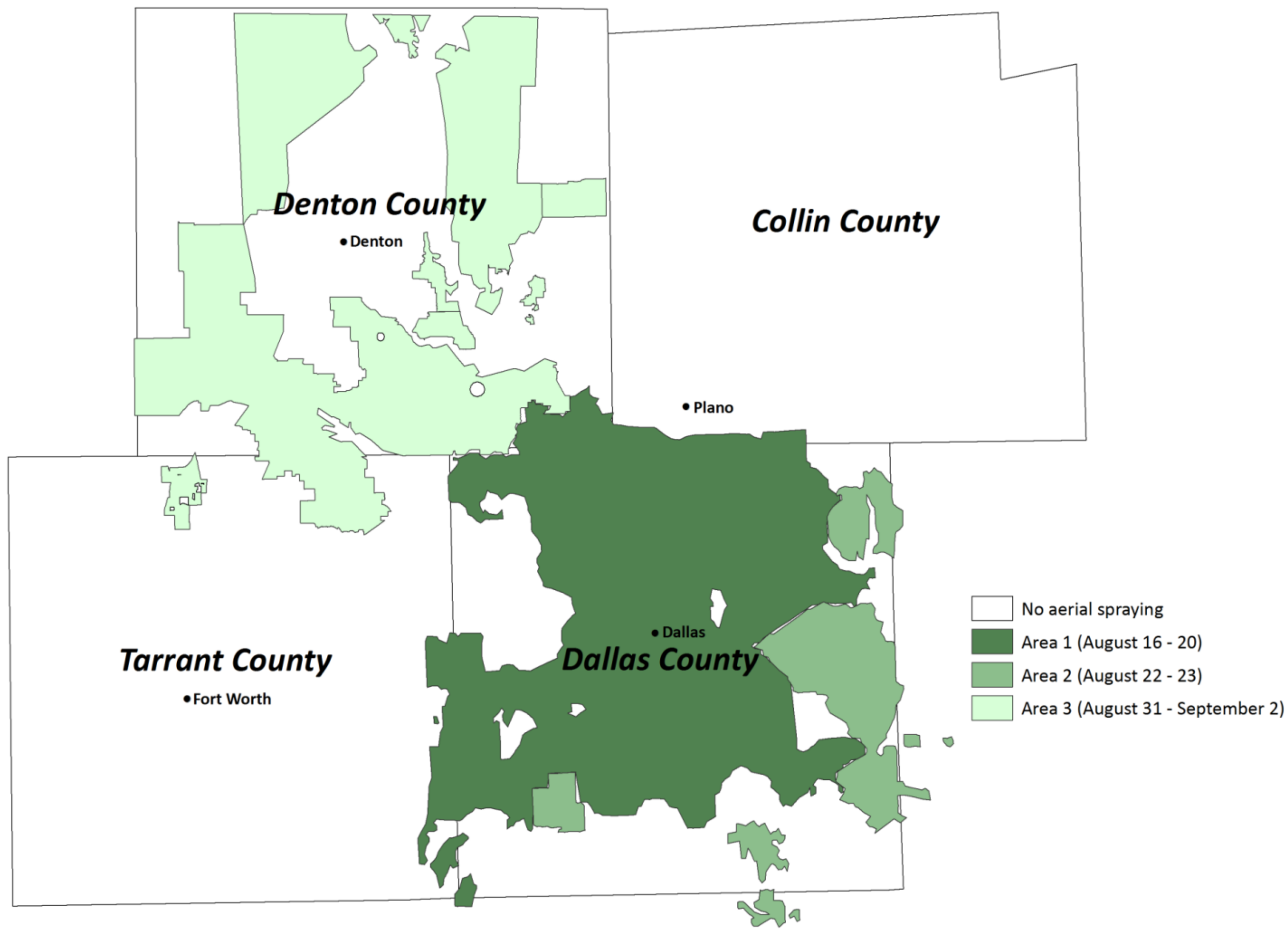


Table 3. West Nile virus neuroinvasive disease cases, incidence rates per 100,000 population, and incidence rate ratios before and after aerial spraying in treated and untreated areas — Collin, Dallas, Denton, and Tarrant Counties, Texas, 2012

	Before aerial spraying			After aerial spraying						
Area	Cases	Population	IR	Cases	Population	IR	IRR*	(95% CI)	Ratio of IRRs†	(95% CI)
Treated	189	2,530,019	7.5	7	2,529,553	0.3	27.0	(12.7-57.4)	**2.55**	**(1.0-6.5)**
Untreated	148	3,085,121	4.8	14	3,084,758	0.5	10.6	(6.1-18.3)		

IR = incidence rates
IRR = Incidence rate ratio
CI = Confidence interval

*Incidence rate before aerial spraying/Incidence rate after aerial spraying
†IRR in treated areas/IRR in untreated areas

Figure 3. Location of Zone 1, Zone 2, and Zone 3 aerial spray events in Dallas County, Texas, 2012

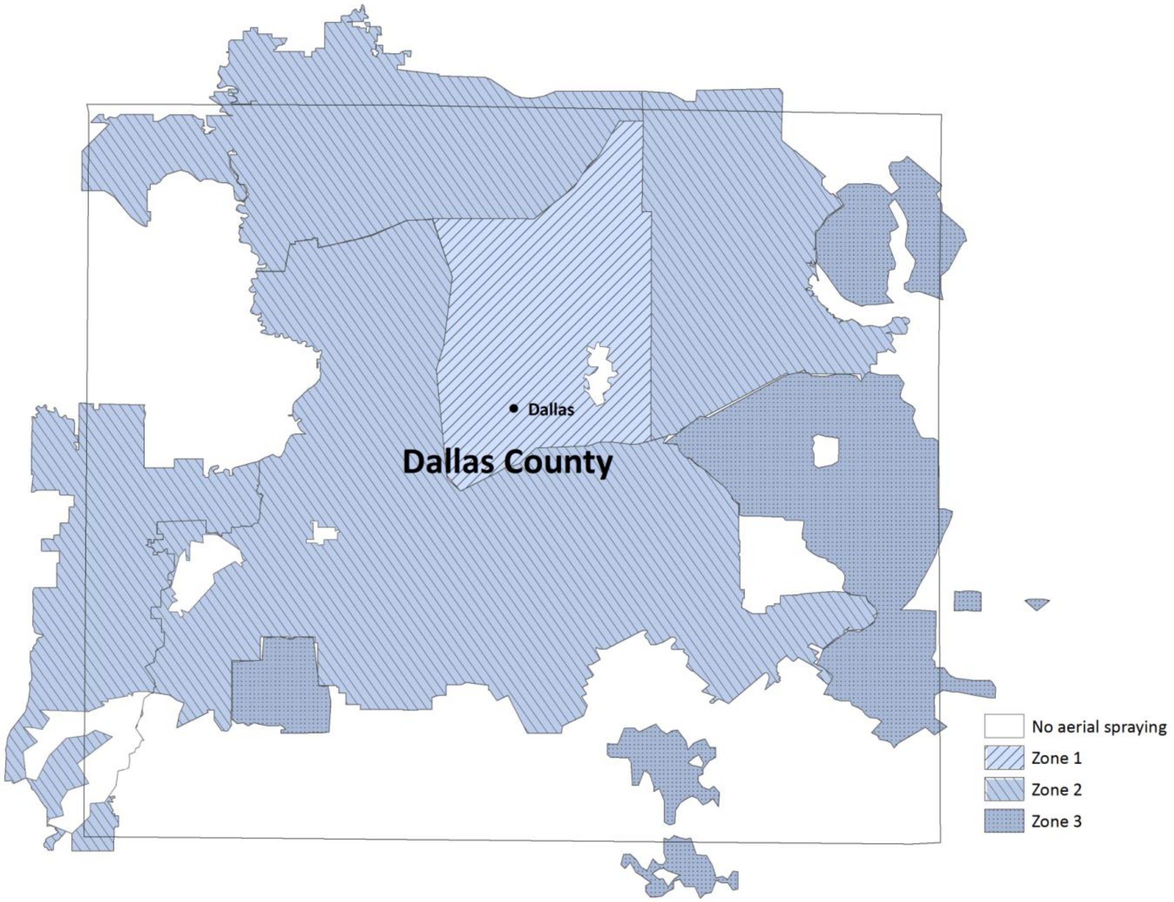


Table 4. Trapping efforts and West Nile virus testing of *Culex quinquefasciatus* mosquitoes collected from stationary gravid traps during the 1 week and 3 weeks before and after aerial insecticide applications — Dallas County, Texas, 2012

		Before aerial spraying				After aerial spraying			
Area	**Cutoff date***	**Trap nights**	**Mosquitoes tested**	**Pools tested**	**Positive pools**	**Trap nights**	**Mosquitoes tested**	**Pools tested**	**Positive pools**
1 week before and after spraying									
Zone 1	Aug 20	73	78	25	1	17	97	11	1
Zone 2	Aug 20	5	46	5	1	4	25	3	0
Zone 3	Aug 23	11	144	11	1	11	110	9	0
Untreated	Aug 20	3	258	18	2	36	186	29	2
	Aug 23	22	190	22	2	17	193	22	1
3 weeks before and after spraying									
Zone 1	Aug 20	74	184	39	2	17	96	11	1
Zone 2	Aug 20	21	221	20	6	23	334	24	0
Zone 3	Aug 23	31	340	26	6	24	331	21	0
Untreated	Aug 20	31	313	29	5	43	298	31	1
	Aug 23	35	342	37	5	32	285	32	1

*Date used to define the time period before and after aerial spray; for the untreated area, the before and after period was calculated using a cut-off date of both August 20 (first row) and August 23 (second row).

Table 5. Abundance of *Culex quinquefasciatus* mosquitoes collected from stationary gravid traps during the 1 week before and after aerial insecticide applications — Dallas County, Texas, 2012

		Observed average mosquitoes per trap night		Model estimated rate of change†		Abundance rate ratio‡	
Area	**Cut-off date***	**Before aerial spraying**	**After aerial spraying**	No.	(95% CI)	No.	(95% CI)
Zone 1	Aug 20	2.4	5.9	2.3	(1.8, 2.9)	1.5	(0.9, 2.3)
Zone 2	Aug 20	9.2	12.5	1.3	(0.8, 1.9)	0.8	(0.5, 1.4)
Zone 3	Aug 23	11.6	15.0	1.8	(1.4, 2.2)	1.9	(1.4, 2.6)
Untreated	Aug 20	10.1	11.0	1.5	(1.1, 2.3)	--	--
	Aug 23	10.9	9.4	0.9	(0.7, 1.1)	--	--

CI = confidence interval

*Date used to define the time period before and after aerial spray; for the untreated area, the before and after period was calculated using a cut-off date of both August 20 (first row) and August 23 (second row).

†Rate of change in *Cx. quinquefasciatus* mosquito abundance for 1 week before and after aerial spraying for each area using a model developed in R software. A value >1.0 signifies an increase in abundance from before to after aerial spraying.

‡Ratio of the rate of change in each treated area compared to the untreated area. For Zones 1 and 2, the rate of change was compared to the untreated area using a before and after cut-off date of August 20; for Zone 3, the rate of change was compared to the untreated area using a before and after cut-off date of August 23.

Table 6. West Nile virus infection rate for *Culex quinquefasciatus* mosquitoes collected from stationary gravid traps during the 1 week and 3 weeks before and after aerial insecticide applications — Dallas County, Texas, 2012

		Infection rate per 1,000 mosquitoes					
		Before aerial spraying		After aerial spraying		Difference before and after†	
Area	**Cut-off date***	Rate	(95% CI)	Rate	(95% CI)	No.	(95% CI)
1 week before and after spraying							
Zone 1	Aug 20	12.6	(0.7, 59.4)	10.4	(0.6, 51.3)	-2.3	(-50.8, 41.9)
Zone 2	Aug 20	24.1	(1.4, 144.0)	0	(0, 88.6)	-24.1	(-216.0, 67.6)
Zone 3	Aug 23	7.0	(0.4, 34.8)	0	(0, 23.2)	-7.0	(-73.2, 17.5)
Untreated	Aug 20	8.0	(0.5, 39.9)	10.9	(1.4, 24.5)	2.9	(-18.4, 28.6)
	Aug 23	10.8	(2.0, 35.5)	5.0	(0.3, 22.3)	-5.9	(-31.4, 15.4)
3 weeks before and after spraying							
Zone 1	Aug 20	10.8	(2.0, 34.8)	10.5	(0.6, 52.5)	-0.3	(-28.0, 42.9)
Zone 2	Aug 20	37.4	(15.0, 83.3)	0	(0, 10.2)	-37.4	(-129.9, -12.3)
Zone 3	Aug 23	20.8	(8.8, 43.4)	0	(0, 9.9)	-20.8	(-69.2, -9.0)
Untreated	Aug 20	18.1	(6.4, 38.6)	3.3	(1.0, 17.2)	-14.8	(-37.8, 1.9)
	Aug 23	15.9	(6.5, 38.3)	3.4	(0.2, 16.5)	-12.4	(-32.3, 4.0)

CI = confidence interval

*Date used to define the time period before and after aerial spray; for the untreated area, the before and after period was calculated using a cut-off date of both August 20 (first row) and August 23 (second row).
†A negative value signifies a drop in infection rate from before to after aerial spraying.

Table 7. West Nile virus Vector Index for *Culex quinquefasciatus* mosquitoes collected from stationary gravid traps during the 1 week and 3 weeks before and after aerial insecticide applications — Dallas County, Texas, 2012

Area	Cut-off date*	Vector Index: Before aerial spraying	Vector Index: After aerial spraying	Difference before and after†
1 week before and after spraying				
Zone 1	Aug 20	0.03	0.06	0.03
Zone 2	Aug 20	0.22	0	-0.22
Zone 3	Aug 23	0.89	0	-0.89
Untreated	Aug 20	0.10	0.10	0
	Aug 23	0.12	0.05	-0.07
3 weeks before and after spraying				
Zone 1	Aug 20	0.04	0.10	0.06
Zone 2	Aug 20	0.43	0	-0.43
Zone 3	Aug 23	0.56	0	-0.56
Untreated	Aug 20	0.15	0.03	-0.12
	Aug 23	0.14	0.03	-0.11

*Date used to define the time period before and after aerial spray; for the untreated area, the before and after period was calculated using a cut-off date of both August 20 (first row) and August 23 (second row).
†A negative value signifies a drop in infection rate from before to after aerial spraying.

Appendix 1. Integrated vector management and recommendations for improving West Nile virus surveillance and prevention programs

Integrated Vector Management (IVM) is based on an understanding of the underlying biology of the transmission system, and utilizes regular monitoring of mosquito populations and West Nile virus (WNV) activity levels to determine if, when and where interventions are needed to keep mosquito numbers below levels which produce risk of human disease, and to respond appropriately to reduce risk when it exceeds acceptable levels.

Operationally, IVM is anchored by a monitoring program providing data that describe:

- Conditions and habitats that produce vector mosquitoes,
- Abundance of those mosquitoes over the course of a season,
- WNV transmission activity levels expressed as WNV infection rate in mosquito vectors
- Parameters that influence local mosquito populations and WNV transmission.

These data inform decisions about implementing mosquito control activities appropriate to the situation, such as:

- Source reduction through habitat modification where feasible,
- Larval mosquito control using the appropriate method for the habitat,
- Adult mosquito control using pesticides applied from trucks or aircraft
- Community education efforts related to WNV risk levels and intervention activities.

Monitoring also provides quality control for the program, allowing evaluation of:

- Effectiveness of larval control efforts,
- Effectiveness of adult control efforts, and
- Causes of control failures (e.g., undetected larval sources, pesticide resistance, equipment failure etc.)

Surveillance programs in IVM: Effective IVM for WNV prevention relies on a sustained, consistent surveillance program that targets vector species. The objectives are to identify and map larval production sites by season, monitor adult mosquito abundance, monitor vector infection rates, document the need for control based on established thresholds, and monitor control efficacy. Surveillance can be subdivided into three categories based on the objective of the surveillance effort. However, the surveillance elements are complementary, and in combination provide the information required for IVM decisions.

A. Larval mosquito surveillance – source mapping.
    a. Larval surveillance involves identifying and sampling a wide range of aquatic habitats for the presence of vector species, maintaining a database of these locations, and a record of larval control measures applied to each. This requires trained inspectors to identify larval production sites, collect larval specimens on a regular basis from known larval habitats, and to perform systematic surveillance for new sources. This information is used to determine where source reduction or larval control efforts should be implemented.

B. Adult mosquito surveillance – vector abundance.

   a. Adult mosquito surveillance is used to describe the spatial distribution and quantify relative abundance of adult vector mosquitoes. This process also provides specimens for evaluating the incidence of WNV infection in vector mosquitoes (see below). Adult mosquito surveillance programs require standardized and consistent surveillance efforts in order to provide data appropriate for monitoring trends in vector activity, for setting action thresholds, and evaluating control efforts. Various methods are available for monitoring adult mosquitoes. Most frequently used in WNV surveillance are the CO_2-baited CDC miniature light traps for monitoring host-seeking *Culex tarsalis* (and potential bridge vector species) and gravid traps to monitor *Cx. quinquefasciatus, Cx. pipiens* and *Cx. restuans* populations. Adult mosquito surveillance should consist of a series of collecting sites at which mosquitoes are sampled using both gravid and light traps on a regular schedule. Fixed trap sites allow monitoring of trends in mosquito abundance and virus activity over time and are essential for obtaining information to evaluate WNV risk and to guide control efforts. Additional trap sites can be utilized on an *ad hoc* basis to provide additional information about the extent of virus transmission activity and effectiveness of control efforts.

C. WNV transmission surveillance – incidence of virus in the environment.

   a. WNV transmission activity can be monitored by tracking the WNV infection rate in vector mosquito populations, WNV-related avian mortality, seroconversion to WNV in sentinel chickens, seroprevalence/seroconversion in wild birds, and WNV veterinary (primarily horse) cases. Mosquito-based surveillance is the most commonly used method to monitor WNV activity since mosquito populations sampled to obtain information about vector abundance can be tested for WNV. This is the surveillance procedure used in Dallas and surrounding areas over previous years, which could provide the historical database against which to evaluate vector abundance and infection rates in the future. Surveillance based on dead birds and sentinel chickens is frequently coupled with mosquito-based monitoring.

   b. Mosquito-based WNV surveillance

      i. The same sampling system used to obtain mosquitoes to evaluate adult mosquito abundance provides mosquitoes for determining WNV incidence in the vector population. Adult mosquitoes are identified to species and sex, counted, and tested for the presence of WNV using either RT-PCR to detect viral RNA or antigen detection tests to detect viral proteins in the mosquitoes. Pooling and testing protocols are detailed elsewhere and will not be covered here.

      ii. Estimates of WNV activity derived from mosquito-based surveillance should provide information that is comparable over time to facilitate detection of trends indicating increasing risk. For this reason, indices such as the number of positive pools or the proportion of positive pools provide limited information because they don't reflect the number of specimens tested in a comparable format. Estimating the Infection Rate (usually reflected as the number of positive mosquitoes per 1000 tested) provides a better index for comparing WNV activity levels, but it doesn't incorporate information about the abundance of infected mosquitoes. A more informative measure of WNV transmission activity is the Vector Index, which is the product of the infection rate in the mosquitoes and the abundance (number collected per trap night). The Vector Index provides an estimate of the number of infected mosquitoes collected per trap night, and is reported to be a useful tool for estimating risk and setting action thresholds. Guidelines for calculating and applying the Vector Index are provided in Appendix 2.

c. Dead bird-based surveillance.
   i. Detection of morbidity/mortality in bird populations appears to be a very sensitive early detection system for WNV activity, particularly in metropolitan areas where there is a sufficient number of people to regularly provide dead-bird reports and there is a community of susceptible bird species. Many communities use dead-bird surveillance to complement mosquito-based surveillance. Dead bird surveillance programs range from those designed to collect/test the birds to verify WNV infection to those using dead bird clusters as indicators of where WNV activity is likely and additional mosquito testing/control is needed.

Utilizing WNV indicators in a response plan: Indices of WNV activity based on mosquitoes, dead birds, or other measures of virus prevalence have value only if they convey information useful in making public health decisions. For this reason, the system that produces the indices must be representative of the area (with adequate temporal and spatial resolution), consistent (comparable over time), and timely (provide sufficient lead time to implement effective interventions when necessary). In addition, the indices must be interpreted with consideration of how they relate to historical epidemiological patterns, and preferably with specific actions and interventions when indices exceed set thresholds. Once WNV risk has been identified, prompt, aggressive action should be taken to mitigate that risk to prevent human cases. Delay in action can allow focal WNV activity to quickly spread.

Mosquito control activities in IVM: Guided by the surveillance described above, integrated efforts to control mosquitoes are implemented to maintain vector populations below thresholds that would facilitate WNV amplification and increase human risk. Failing that, efforts to reduce the abundance of WNV-infected biting adult mosquitoes must be quickly implemented to prevent risk levels from increasing to the point of a human disease outbreak. Properly implemented, a program monitoring WNV activities in the vector mosquito population will provide a warning of when risk levels are increasing. Because of delays in onset of disease following infection, detection, diagnosis, and reporting of human disease, WNV surveillance based on human case reports lags behind increases in risk and is not sufficiently sensitive to allow implementation of outbreak control measures.

A. Source reduction and larval control – managing mosquitoes before they emerge as adults.
   a. Source reduction is simply the elimination or removal of habitats that produce vector mosquitoes. This can range from draining roadside ditches to properly disposing of discarded tires and other trash containers. Only through a thorough surveillance program will sources that are compatible with elimination be identified and appropriately mitigated. In order to effectively control vector mosquito populations through source reduction, all sites capable of producing vector mosquitoes must be identified and routinely inspected for production. This is difficult to accomplish with

a species such as *Cx. quinquefasciatus* that readily utilizes cryptic sites such as storm drainage systems, grey water storage cisterns and run off impoundments. Vacant housing with unmaintained swimming pools, ponds and similar water features are difficult to identify and contribute a significant number of adult mosquitoes that may overwhelm other larval control measures.

b. To manage mosquitoes produced in habitats that are not conducive to source reduction or elimination, pesticides registered for larval mosquito control are applied to the habitats when larvae are detected. There are a variety of larval mosquito control pesticides available and a detailed discussion of their attributes and limitations is beyond the scope of this overview. There is no single larvicide product that will work effectively in every habitat where WNV vectors are found.

c. An adequate field staff with proper training is required to properly identify larval production sites and implement the appropriate management tools.

B. Adult mosquito control – managing vector abundance and reducing risk of human infection during an outbreak.

   a. Source reduction and larvicide treatments frequently are inadequate to maintain vector populations at levels sufficiently low to limit virus amplification. In situations where vector abundance levels are increasing, targeted adulticide applications using pesticides registered by EPA for this purpose can assist in maintaining vector abundance below threshold levels.

   b. In situations where the abundance of infected vectors is approaching or exceeding thresholds related to increased human risk, efforts to reduce infected mosquito abundance must be implemented quickly to reduce the likelihood of human infections and development of outbreak conditions.

   c. Pesticides for adult mosquito control can be applied by truck or aircraft. Truck-based applications may be useful to manage small areas, but are limited in their capacity to treat large areas quickly during an outbreak. Aerial application of mosquito control adulticides are required when large areas must be treated quickly, and can be particularly valuable because controlling WNV vectors such as *Cx. quinquefasciatus* often requires multiple, closely spaced treatments.

C. Legal Control - using public health regulations to facilitate access for surveillance, source reduction or treatment.

   a. Because individually-owned private property may be a major source of mosquito production, larval control on municipal and surrounding private property may be rendered moot by a single neglected property. Local public health statutes or regulations may permit legal action to be taken to address the problem. Often this is a prolonged process during which adult mosquitoes are continuously produced. Knowing the local ordinances and property trespass laws then working to streamline the process can, over time, reduce the likelihood that adulticiding is required.

Quality Control in IVM: All products used to control mosquitoes may be used over a range of application rates. Both larvicides and adulticides should periodically be evaluated to ensure an effective rate of application is being used and that the desired degree of control is obtained. Application procedures should be evaluated regularly (minimally once each season) to make sure equipment is functioning properly to deliver the correct dosages and droplet parameters. Finally, mosquito populations should routinely be evaluated to ensure insecticide resistance is not emerging.

Record Keeping in IVM: Surveillance data describing vector sources, abundance and infection rates, records of control efforts (e.g., source reduction, larvicide applications, adulticide applications), and quality control data must be maintained and used to evaluate IVM needs and performance. Long-term data is essential to track trends and to evaluate levels of risk.

Appendix 2. Calculation and application of a Vector Index reflecting the number of West Nile virus infected mosquitoes in a population with an example using data from Fort Collins, Colorado.

BACKGROUND

The establishment of West Nile virus (WNV) across North America has been accompanied by expanded efforts to monitor WNV transmission activity in many communities. Surveillance programs use various indicators to demonstrate virus activity. These include detecting evidence of virus in dead birds, dead horses, and mosquitoes; and detection of antibody against WNV in sentinel birds, wild birds, or horses (Reisen & Brault 2007). While all of these surveillance practices can demonstrate the presence of WNV in an area, few provide reliable, quantitative indices that may be useful in predictive surveillance programs. Only indices derived from a known and quantifiable surveillance effort conducted over time in an area will provide information that adequately reflects trends in virus transmission activity that may be related to human risk. Of the practices listed above, surveillance efforts are controlled and quantifiable only in mosquito and sentinel-chicken based programs. In these programs, the number of sentinel chicken flocks/ number of chickens, and the number of mosquito traps set per week is known and allows calculation of meaningful infection rates that reflect virus transmission activity.

PREMISE BEHIND DEVELOPING THE VECTOR INDEX.

Mosquito-based arbovirus surveillance provides three pieces of information: 1) The variety of species comprising of the mosquito community; 2) Density of each species population (in terms of the number collected in each trap unit of a given trap type); and 3) if the specimens are tested for the presence of arboviruses, the incidence of the agent in the mosquito population. Taken individually, each parameter describes one aspect of the vector community that may affect human risk, but the individual elements don't give a comprehensive estimate of the number of potentially infectious vectors seeking hosts at a given time in the surveillance area.

Parameter	Information Provided	Value in Surveillance Program
Mosquito Community Composition	Diversity of species in the area	Documents the presence of competent vector species in the area
Mosquito Population Density	Relative abundance of mosquito species in terms of trapping effort	Quantifies the number of individuals of each mosquito species at a given point in time, particularly important for key vector species.
Infection Rate of Virus in Mosquito Population	Proportion of the mosquito population carrying evidence of the disease agent	Quantifies incidence of infected and potentially infectious mosquitoes in the key vector population. Demonstrates if important bridge vectors are involved

VECTOR INDEX

To express the arbovirus transmission risk posed by a vector population adequately, information from all three parameters (vector species presence, vector species density, vector species infection rate) must be considered. The Vector Index (VI) combines all three of the parameters quantified through standard mosquito surveillance procedures in a single value (Jones et al. 2011, Kwan et al, 2012). The VI is simply the estimated **average number of infected mosquitoes collected per trap night** summed for the key vector species in the area. Summing the VI for the key vector species incorporates the contribution of more than one species and recognizes the fact that WNV transmission may involve one or more primary vectors and several accessory or bridge vectors in an area.

Deriving the VI from routine mosquito surveillance data

The VI is expressed as:

$$\text{Vector Index} = \sum_{i=species} \overline{\text{N}}_i \hat{\text{P}}_i$$

Where: $\overline{\text{N}} = \text{Average Density}$ (number per trap night for a given species)

$\hat{\text{P}} = \text{Estimated Infection Rate}$ (proportion of the mosquito population WNV positive)

Calculating the VI in an area where two primary WNV vector species occur:

Step 1: Calculate mosquito density

Trap Site	*Cx. tarsalis*	*Cx. pipiens*
1	68	21
2	42	63
3	139	49
4	120	31
5	42	12
6	31	57
Total	442	233
Average per Trap Night	74	39
Standard Deviation	41	21

Step 2: Calculate the WNV infection rate for each species (as a proportion)

Pools Tested for Virus			
Pool Number	Species	Number in pool	Positives
1	*Cx. tarsalis*	50	0
2	*Cx. tarsalis*	50	0
3	*Cx. tarsalis*	50	1
4	*Cx. tarsalis*	50	0
5	*Cx. tarsalis*	50	0
6	*Cx. tarsalis*	50	0
7	*Cx. pipiens*	50	1
8	*Cx. pipiens*	50	0
9	*Cx. pipiens*	50	0
10	*Cx. pipiens*	50	0
11	*Cx. pipiens*	50	0
Cx. tarsalis			
Infection Rate	Lower limit	Upper limit	Confidence interval
0.0033	0.0002	0.0169	0.95
Cx. pipiens			
Infection Rate	Lower limit	Upper limit	Confidence interval
0.0040	0.0002	0.0206	0.95

Step 3. Calculate individual species VI values, multiplying the average number per trap night by the proportion infected. Calculate combined VI value by summing the individual species VIs.

Vector Index Calculation	*Cx. tarsalis*	*Cx. pipiens*
Avg / trap night	74	39
Proportion infected	0.0033	0.004
VI (individual species)	0.24	0.16
VI (Combined)	**0.40**	

APPLYING THE VI TO SURVEILLANCE DATA

Mosquitoes in Fort Collins, Colorado were surveyed using CO_2-baited CDC miniature light traps set at approximately 35 collecting sites each week during the primary arbovirus transmission period (June through September) of 2003 through 2008. During 2004, the light traps were supplemented with gravid traps to obtain more specimens for infection rate estimation, but only the light trap data were used to determine density per trap night. This was done in order to keep the density units consistent over the years (number collected per CO_2-baited CDC light trap) Samples collected in each trap were identified to species and counted. The *Cx. tarsalis* and *Cx. pipiens* specimens were tested in pools of up to 50 for WNV RNA using a TaqMan RT-PCR and WNV-specific primers/probes.

For each week, *Cx. tarsalis* and *Cx. pipiens* abundance (number per trap night) was determined with light trapping (Figure 1), and the infection rate was determined by testing a sample of the collected mosquitoes for the presence of WNV (Figure 2).

Fig. 1a. Weekly abundance of *Cx. tarsalis* populations in Ft. Collins, Colorado 2003-2008.

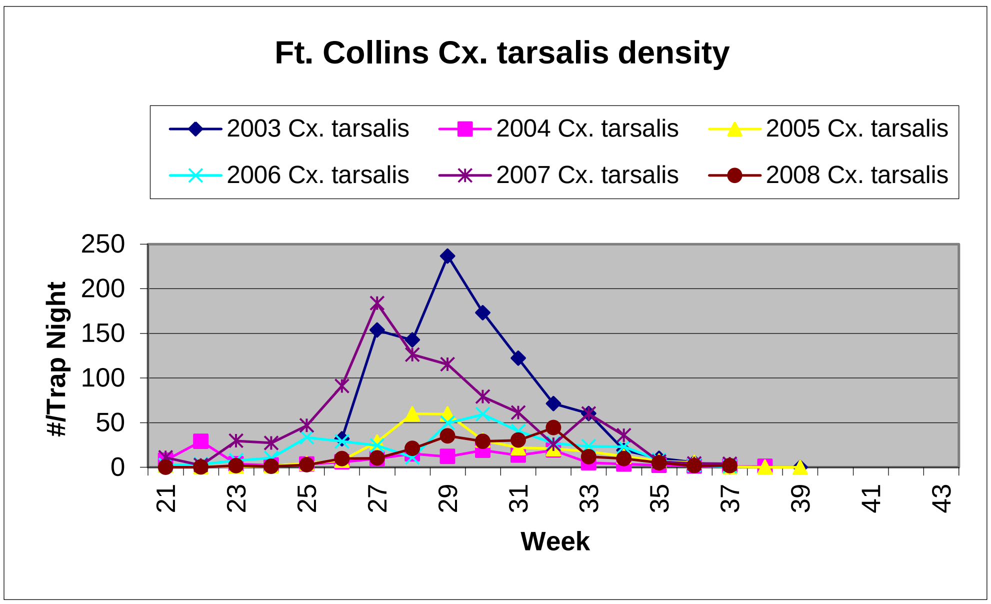


Fig. 1b. Weekly abundance of *Cx. pipiens* populations in Ft. Collins, Colorado 2003-2008.

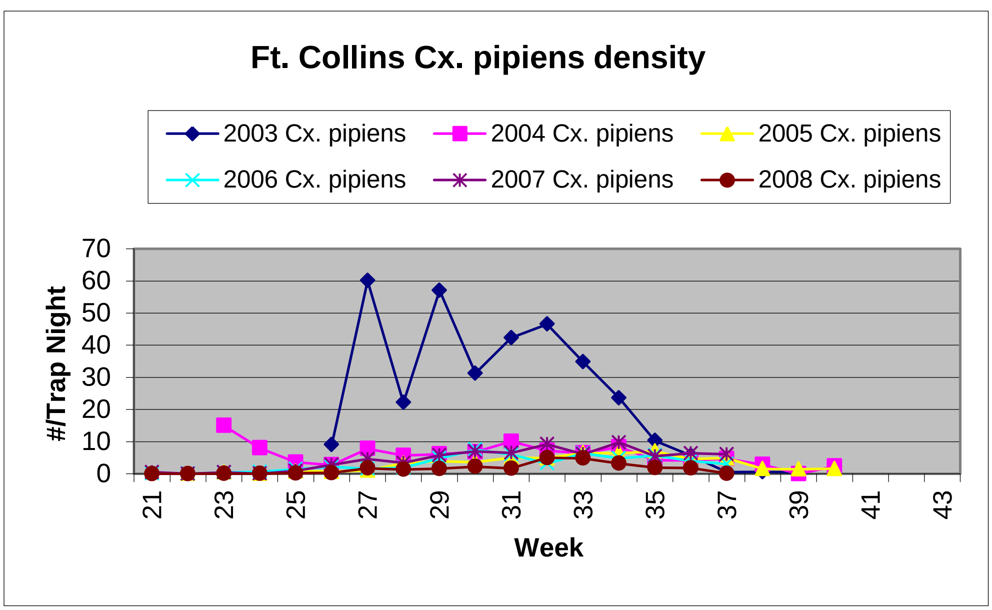


Fig. 2a. Weekly WNV infection rate of *Cx. tarsalis* populations in Ft. Collins, Colorado 2003-2008.

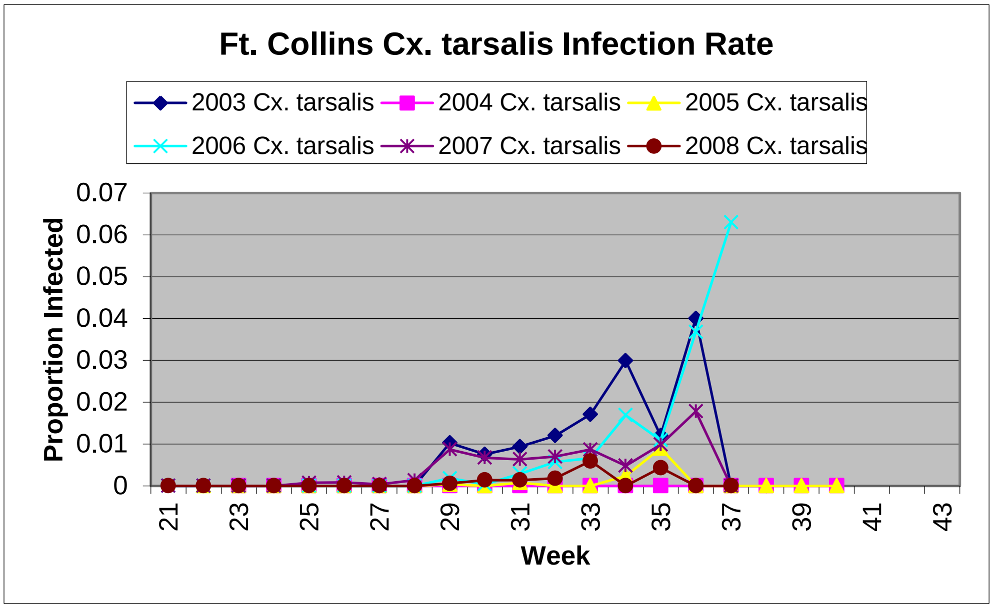
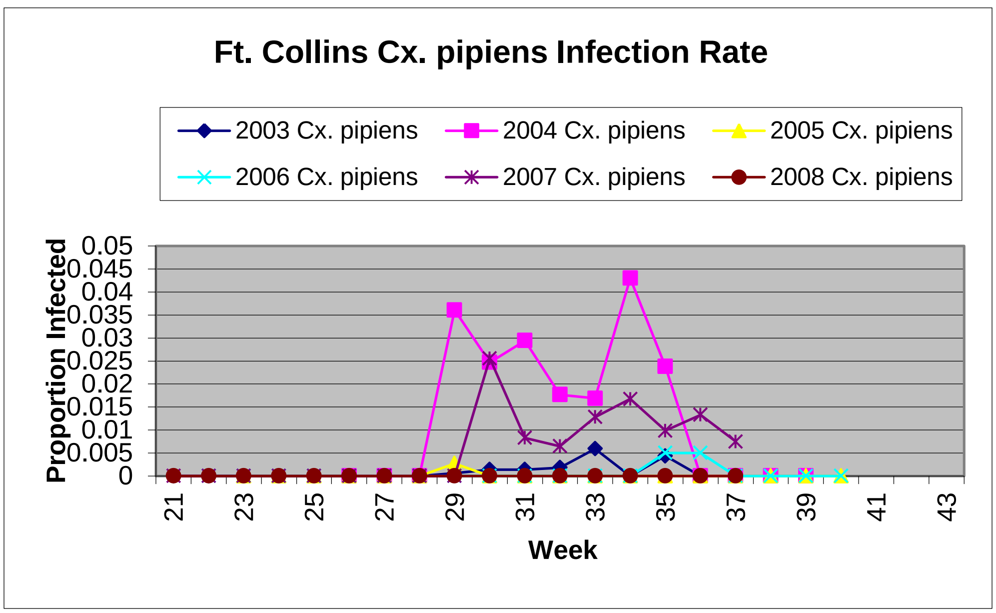


Fig. 2b. Weekly WNV infection rate of *Cx. pipiens* populations in Ft. Collins, Colorado 2003-2008.



A VI for each species was calculated each week by multiplying the number collected per trap night by the proportion WNV infected (Figure 3) and the combined VI was calculated by combining the individual *Cx. tarsalis* and *Cx. pipiens* VI values (Figure 4).

Fig. 3a. Weekly Vector Index for *Cx. tarsalis* in Ft. Collins, Colorado 2003-2008.

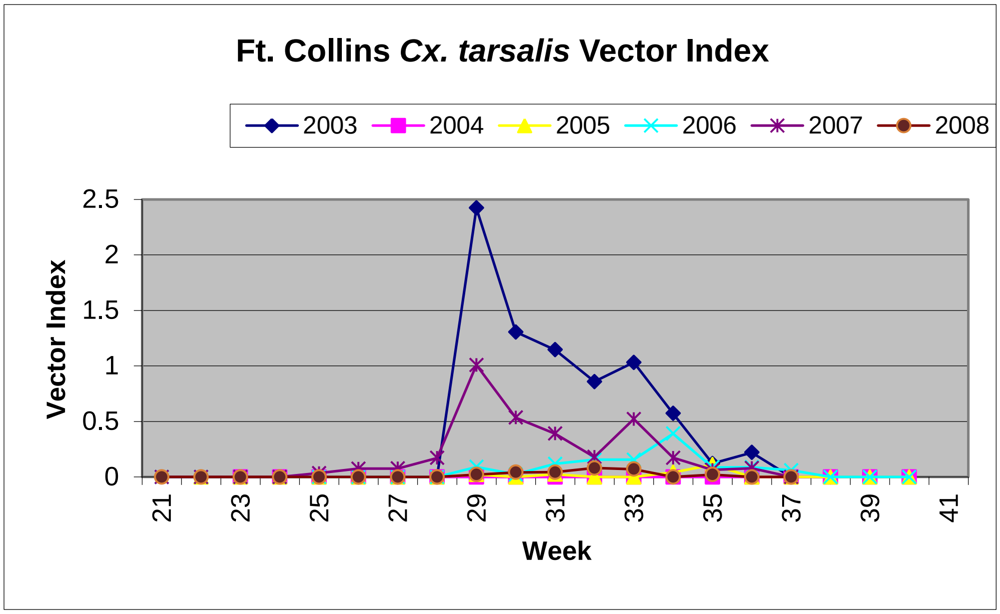


Fig. 3b. Weekly Vector Index for *Cx. pipiens* in Ft. Collins, Colorado 2003-2008.

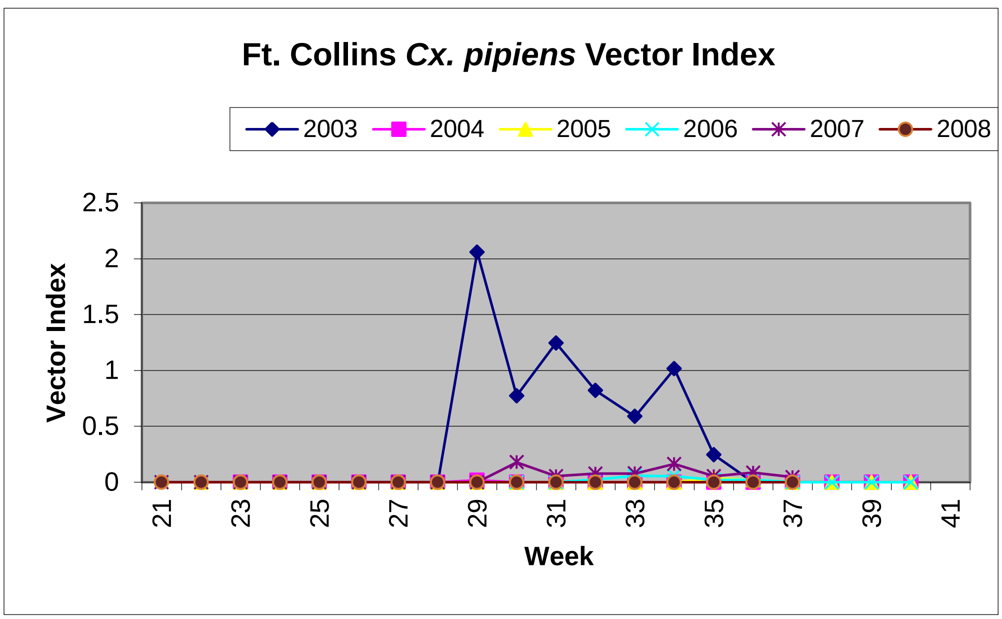
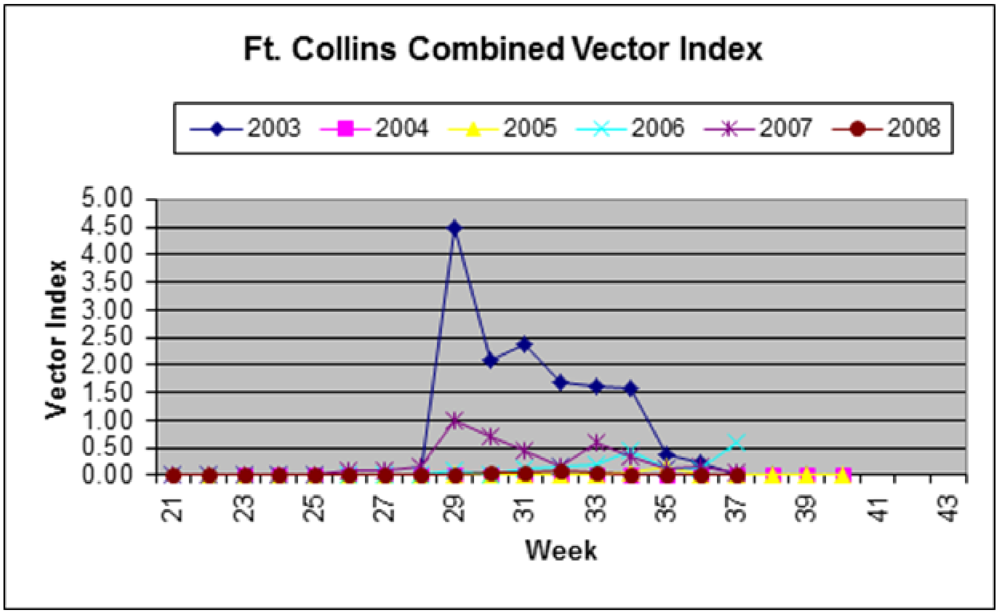
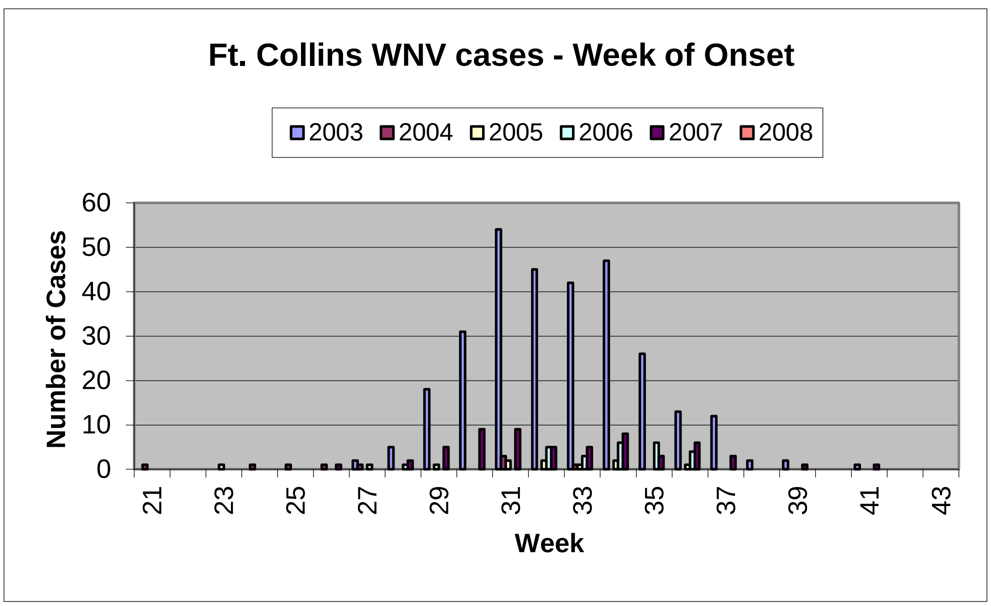


Fig. 4. Combined weekly VI (sum of the weekly VI for *Cx. tarsalis* and *Cx. pipiens*) in Ft. Collins, Colorado 2003-2008.



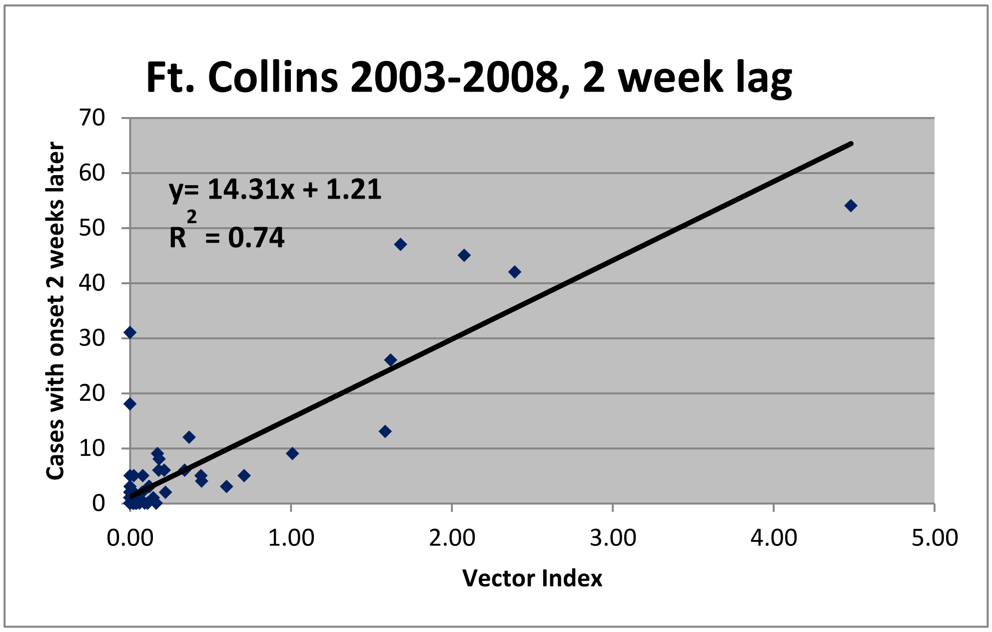
The number of cases in the city with onset each week was obtained from the local health department (Figure 5)

Fig. 5. Number of human WNV cases with onsets each week in Ft. Collins, Colorado 2003-2008.



The association of the VI to the number of human cases with onsets 2 weeks later was evaluated (Figure 6). The results demonstrate that the VI is a good predictor of the number of cases with onset 2 weeks following collection of the mosquitoes. This information can be used to set thresholds for maintaining low mosquito abundance levels as part of an Integrated Vector Management program, and to determine action levels for implementing intensified adulticide operations (aerial or expanded ground spraying) to reduce human risk of infection and to prevent development of risk levels leading to outbreak conditions.

Fig. 6. Combined weekly VI and number of human WNV cases with onsets 2 weeks later for Ft. Collins, Colorado 2003-2008.



It is important to note that the VI values derived from the Ft. Collins data and the thresholds derived from the association between VI and human cases presented here are specific to the Ft. Collins surveillance procedures and the values should not be extrapolated to other areas using different surveillance procedures or involving different vector species. The VI is sensitive to the local surveillance practices and must be developed and interpreted using local surveillance data.

References

Jones RC, Weaver KN, Smith S, et al. Use of the vector index and geographic information system to prospectively inform West Nile virus interventions. 2011; Am Mosq Control Assoc.; 27(3):315-9.

Kwan JL, Park BK, Carpenter TE, Ngo V, Civen R, Reisen WK. Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010.2012; Emerg Infect Dis.18(8):1298-306.

Reisen W, Brault AC. West Nile virus in North America: perspectives on epidemiology and intervention. 2007; Pest Manag Sci.;63(7):641-6.